

**UNIVERSIDADE ESTADUAL PAULISTA- UNESP CÂMPUS DE
JABOTICABAL**

**INFECÇÃO PELO CIRCOVÍRUS SUÍNO TIPO 2 E TIPO 3 EM
LESÕES DE PLEURISIAS DE SUÍNOS AO ABATE**

Eduarda Ribeiro Braga

Médica Veterinária

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Orientador: Prof. Dr. Luis Guilherme de Oliveira

Dissertação apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Mestre em Medicina Veterinária, área: Clínica Médica Veterinária



UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal



CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: PREVALENCE OF PORCINE CIRCOVIRUSES (PCV2 AND PCV3) IN SLAUGHTERED PIGS WITH DIFFERENT PLEURISY LESIONS SCORE: COINFECTIONS WITH *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, AND *Pasteurella multocida*

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Aprovada como parte das exigências para obtenção do Título de Mestra em Ciências Veterinárias, área: Saúde Animal pela Comissão Examinadora:

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Jaboticabal, 05 de maio de 2025

DADOS CURRICULARES DO AUTOR

EDUARDA RIBEIRO BRAGA –Nascida em 28 de fevereiro de 1997, em Coromandel, Minas Gerais – Brasil, é médica veterinária formada pela Universidade Federal de Uberlândia (UFU) em 2021. Em 2022, atuou como trainee na empresa Livingston Enterprises Inc., em Fairbury, Nebraska – Estados Unidos. No mesmo ano concluiu três pós-graduações *Lato Sensu* nas áreas de Produção de Suínos, Reprodução de Suínos e Defesa Sanitária Animal pela Faculdade Iguaçu. Atualmente, é pós-graduanda no Programa de Medicina Veterinária da UNESP – Câmpus de Jaboticabal, na área de Clínica Médica Veterinária, onde desenvolve pesquisas como bolsista FAPESP sob a orientação do Prof. Dr. Luis Guilherme de Oliveira.

“Comece fazendo o que é necessário, depois o que é possível, e de repente você estará fazendo o impossível.”

São Francisco de Assis

Dedico

À minha mãe, Doralice Braga, minha rocha
inabalável e luz que nunca se apaga.

AGRADECIMENTOS

Agradeço, primeiramente, a Deus, por guiar meus passos e colocar em meu caminho não apenas conquistas, mas também os obstáculos — pois até mesmo as dificuldades podem ser dádivas disfarçadas, moldando quem somos e nos preparando para o que está por vir.

À minha família, meu porto seguro, deixo minha eterna gratidão. Em especial à minha mãe, Doralice Braga, e à minha irmã, Lailla Millany, por nunca medirem esforços para me apoiar, por cada palavra de incentivo, cada gesto de amor e cada presença silenciosa nos momentos em que mais precisei.

Sou grata aos colegas do Laboratório de Medicina de Suínos, que caminharam ao meu lado durante essa jornada. A Ana Karolina, em especial, que foi além de uma parceira de mestrado — tornou-se uma irmã de coração, presença constante e insubstituível nos dias bons e nos desafiadores.

Aos amigos de Jaboticabal e aos de infância, espalhados pelo Brasil, meu carinho e reconhecimento. Seria impossível citar todos os nomes, mas saibam que cada mensagem, cada conversa e cada lembrança fizeram toda a diferença. Sem vocês, essa caminhada não teria sido tão leve e significativa.

Agradeço à Iowa State University e à Zoetis – pelo apoio e confiança depositados neste projeto. Minha sincera gratidão ao Dr. Pablo Piñeyro, à Dra. Caroline Pissetti e ao Mestre Igor Savoldi, por toda a assistência técnica, atenção e dedicação ao longo do processo.

À Faculdade de Ciências Agrárias e Veterinárias, Câmpus de Jaboticabal pela oportunidade de cursar Mestrado em uma instituição renomada. À FAPESP por me conceder a bolsa de mestrado.

O presente trabalho contou com o apoio da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), processo nº 2023/01748-0

Por fim, agradeço ao Prof. Dr. Luís Guilherme pelos dois anos de orientação, pela paciência, pelos ensinamentos e pela confiança em meu trabalho.

Levo comigo tudo que aprendi, com gratidão e respeito.

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- B813i Braga, Eduarda Ribeiro
Infecção pelo circovírus suíno tipo 2 e tipo 3 em lesões de
pleurisias de suínos ao abate / Eduarda Ribeiro Braga. --
Jaboticabal, 2025
37 p. : il., tabs., fotos
- Dissertação (mestrado) - Universidade Estadual Paulista
(UNESP), Faculdade de Ciências Agrárias e Veterinárias,
Jaboticabal
- Orientadora: Luís Guilherme de Oliveira
1. Circovírus. 2. Pleurisias. 3. CDRS. 4. Coinfecções. I.
Título.

CERTIFICADO COMISSÃO DE ÉTICA PARA EXPERIMENTAÇÃO ANIMAL

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

**CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS****C E R T I F I C A D O**

Certificamos que o projeto de pesquisa intitulado **"Infecção pelo Circovírus Suíno tipo 2 e tipo 3 em lesões de pleurisias de suínos ao abate"**, protocolo nº 1112/2023, sob a responsabilidade do Prof. Dr. Luís Guilherme de Oliveira, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 15 de março de 2023.

Vigência do Projeto	16/03/2023 a 01/03/2025
Espécie / Linhagem	Sus scrofa domesticus – Linhagem comercial (Landrace x Large White)
Nº de animais	125 (250 amostras de tecido)
Peso / Idade	120 kg / 150 dias
Sexo	Machos e fêmeas
Origem	Abatedouro – frigorífico localizado em Guariba-SP

Jaboticabal, 15 de março de 2023.


Profa. Dra. Paola Castro Moraes
Vice coordenadora – CEUA FCAV

INFECÇÃO PELO CIRCOVÍRUS SUÍNO TIPO 2 E TIPO 3 EM LESÕES DE PLEURISIAS DE SUÍNOS AO ABATE

RESUMO

Dentre as principais lesões encontradas durante a inspeção das carcaças destacam-se as pleurisias, que podem ter como causa diversos agentes infecciosos, e ocasionar condenações parciais ou totais. O objetivo deste trabalho foi detectar PCV2 e PCV3, possíveis patógenos envolvidos nas lesões de pleurisias no abate de suínos. Para tal, foi selecionado um frigorífico localizado no estado de São Paulo, com fluxo de abate constante, onde foram coletadas e analisadas um total de 130 amostras de pulmão e pleura utilizando o Sistema de Avaliação de Pleurisia em Abatedouro (SPES). Os resultados da qPCR mostraram que 63,9% das amostras de pulmão foram positivas para PCV2, enquanto 29,2% foram positivas para PCV3. Nas amostras de pleura, 30,7% foram positivas para PCV2 e 37,7% para PCV3. Além disso, outros agentes patogênicos, incluindo *Mycoplasma hyopneumoniae* (Mhyo), *Actinobacillus pleuropneumoniae* (APP) e *Pasteurella multocida* (PM), também foram detectados em amostras de pulmão e pleura. Coinfecções foram frequentemente observadas em ambos os tecidos. Todas as amostras testadas por imuno-histoquímica para PCV2 foram negativas, assim como todas as testadas por RNAscope para PCV3. Os achados histopatológicos não revelaram lesões robustas ou significativas. O sequenciamento revelou que a variante PCV2d foi a mais prevalente; no entanto, também foram identificadas amostras positivas para PCV2c, um achado raro no Brasil. Nosso estudo destaca as interações complexas no CDRS, bem como a presença de PCV2 e PCV3 nesse contexto.

Palavras - Chave: circovirus, pleura, pulmão, CDRS, coinfecções.

PORCINE CIRCOVIRUS TYPE 2 AND TYPE 3 INFECTION IN PLEURISY LESIONS OF PIGS AT SLAUGHTER

ABSTRACT

Among the main lesions observed during carcass inspection, pleurisy stands out, which can be caused by various infectious agents and may lead to partial or total condemnations. The aim of this study was to detect PCV2 and PCV3, potential pathogens involved in pleurisy lesions in slaughtered pigs. For this purpose, a slaughterhouse located in the state of São Paulo, with a constant slaughter flow, was selected. A total of 130 lung and pleural samples were collected and analyzed using the Slaughterhouse Pleurisy Evaluation System (SPES). qPCR results showed that 63.9% of lung samples tested positive for PCV2, while 29.2% were positive for PCV3. In pleural samples, 30.7% were positive for PCV2 and 37.7% for PCV3. In addition, other pathogens, including *Mycoplasma hyopneumoniae* (Mhyo), *Actinobacillus pleuropneumoniae* (APP), and *Pasteurella multocida* (PM), were also detected in both lung and pleural samples. Coinfections were frequently observed in both tissues. All samples tested by immunohistochemistry for PCV2 were negative, as were those tested by RNAscope for PCV3. Histopathological findings did not reveal robust or significant lesions. Sequencing revealed that the PCV2d variant was the most prevalent; however, positive samples for PCV2c were also identified—a rare finding in Brazil. Our study highlights the complex interactions involved in PRDC, as well as the presence of PCV2 and PCV3 in this context.

Keywords: circovirus, pleura, lung, PRDC, coinfections.

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CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1. INTRODUÇÃO

A intensificação da produção suína em escala global e as características dos sistemas de criação, nos quais os animais são mantidos em alta densidade e provenientes de diversas origens, aumentam o estresse e os tornam mais suscetíveis a surtos de doenças. Dentre essas enfermidades, as doenças respiratórias representam um dos principais desafios sanitários da suinocultura (Morés et al., 2015). No sistema intensivo de produção de suínos, as enfermidades respiratórias apresentam alta incidência e são frequentemente agrupadas sob o termo Complexo de Doenças Respiratórias Suínas (CDRS), condição que gera significativas perdas econômicas (Goecke et al., 2020a, Goecke et al., 2020b).

Embora um único patógeno possa ser capaz de desencadear a doença, na maioria dos casos, há a interação de múltiplos agentes infecciosos, além da influência de fatores ambientais, genéticos e de manejo (Goecke et al., 2020a; Merialdi et al., 2012). O impacto econômico dessas enfermidades é expressivo, refletindo-se na piora das taxas de conversão alimentar, na redução do ganho de peso dos animais, no aumento da morbidade e mortalidade, nos elevados custos com medicamentos e na perda da qualidade da carcaça, que pode ser parcial ou totalmente condenada devido à presença de lesões pulmonares (Chen et al., 2024; Szeredi et al., 2015; Qiu et al., 2024).

A avaliação de lesões pulmonares em abatedouros constitui uma ferramenta valiosa para estimar a prevalência e a gravidade das doenças respiratórias nos rebanhos. Além disso, essa abordagem permite a identificação de fatores de risco nas granjas e viabiliza a vigilância sanitária contínua, possibilitando a adoção de medidas de controle e prevenção (Merialdi et al., 2012). Considerando o impacto econômico das pleurisias e a consequente redução da produtividade, torna-se essencial identificar os principais patógenos envolvidos nesse quadro e compreender seu efeito sobre a produção e a economia.

Dentre os agentes envolvidos nas doenças respiratórias dos suínos, destacam-

se os circovírus suíno (PCVs), amplamente disseminados no mundo (Afolabi et al., 2017). Esse pequeno vírus, pertencente à família *Circoviridae*, é classificado em quatro espécies: PCV1, PCV2, PCV3 e PCV4. Pode manifestar-se de forma clínica ou subclínica, sendo responsável por expressivas perdas econômicas e frequentemente associado a lesões observadas no abate de suínos (Arenales et al., 2022; Opriessnig et al., 2020). Diante da relevância das lesões pulmonares na cadeia produtiva suína e da complexidade etiológica envolvida, torna-se essencial a detecção de PCV2 e PCV3, bem como a correlação desses agentes com as lesões macroscópicas de pleurisas observadas em abatedouros.

2. REVISÃO DE LITERATURA

Os PCVs pertencem à família *Circoviridae*, a qual é dividida em dois gêneros: *Gyrovirus* e *Circovirus*. O gênero *Circovirus* está associado aos suínos e é classificado em quatro espécies: PCV1, PCV2, PCV3 e PCV4 (Rosario et al., 2017; Opriessnig et al., 2020). O PCV é um vírus não envelopado, de simetria icosaédrica, sendo o menor vírus animal descrito, com dimensões variando entre 1670 e 2380 nanômetros (Castro et al., 2007; Fang et al., 2024; Rosario et al., 2017; Varsani et al., 2024). Esses vírus codificam, no mínimo, duas proteínas essenciais: a proteína do capsídeo (Cap) e a proteína associada à replicação (Rep). A expressão gênica ocorre em uma orientação *ambissens*, com os quadros de leitura aberta (*open reading frames* - ORFs) distribuídos entre as fitas viral e complementar da forma replicativa do genoma (Chung et al., 2021; Klaumann, 2018; Rosario et al., 2017; Varsani et al., 2024).

O PCV1 é considerado não patogênico, enquanto o PCV2 está relacionado às doenças associadas ao circovírus suíno (*porcine circovirus associated diseases* - PCVADs), incluindo doenças cardíacas, síndrome da dermatite e nefropatia suína (PDNS), doenças respiratórias e falhas reprodutivas (Opriessnig et al., 2020; Xu et al., 2021). Este vírus é considerado um dos mais impactantes economicamente para a indústria suinícola, sendo capaz de infectar e replicar-se em células epiteliais, monocíticas, endoteliais e fibroblásticas, incluindo células epiteliais intestinais e macrófagos alveolares suínos (Niu et al., 2022). Identificado no Canadá em 1998, o PCV2 sofreu diversas mutações que originaram novas variantes, resultando na subclassificação em genótipos (a-i) (Vargas-Bermudez, Mogollón e Jaime, 2022; Zou et al., 2022). Os genótipos PCV2a, PCV2b e PCV2d estão amplamente disseminados em populações suínas ao redor do mundo. Em contraste, o PCV2c foi identificado exclusivamente em amostras provenientes do Brasil e da Dinamarca, enquanto o PCV2e ocorre predominantemente nos Estados Unidos e no México (Zhang et al., 2024).

O PCV3 foi identificado nos Estados Unidos em 2016, através de métodos de sequenciamento em suínos afetados por sinais respiratórios e neurológicos, inflamação cardíaca e multissistêmica, falha reprodutiva e PDNS (Hung et al., 2021; Saporiti et al., 2021). Desde então, o patógeno foi detectado em diversos países da Ásia, Europa e América do Sul, tanto em animais saudáveis quanto doentes,

consolidando sua relevância global como um potencial patógeno suíno (Klaumann, 2018; Saporiti et al., 2021). No Brasil, um estudo retrospectivo revelou a presença do vírus desde 1967 (Hung et al., 2024; Rodriguez et al., 2020).

Diferentemente do PCV2, o PCV3 apresenta estabilidade genômica significativa, sem mutações relevantes registradas. Inicialmente classificado em três grupos (PCV3a, PCV3b e PCV3c), foi posteriormente reorganizado em dois grandes grupos (a e b) com base nas sequências das ORFs 1, 2 e 3. A classificação mais recente, baseada no genoma completo, divide o PCV3 em dois clados: Clado 1 (incluindo PCV3a, PCV3b, PCV3c e os grupos a e b) e Clado 2, que contém sequências isoladas da China (Vargas-Bermudez, Mogollón e Jaime, 2022). Intimamente associados devido às suas manifestações clínicas semelhantes e presença frequente em coinfeção, estudos apontam que infecções por PCV2 e PCV3 estão associadas a doenças graves, comprometendo a sanidade suína e impactando negativamente a eficiência econômica da produção (Chen et al., 2024; Qiu et al., 2024).

Recentemente, o PCV4 tem atraído atenção devido à sua relação com doenças respiratórias severas e PDNS, embora sua patogênese ainda não esteja completamente elucidada (Kroeger et al., 2024; Qiu et al., 2024). Estudos retrospectivos sugerem sua presença na China desde 2012, com amostras sorológicas de 2008 também positivas (Ge et al., 2021). Detectado pela primeira vez em Hunan, China, em 2019, o PCV4 tem sido identificado em diferentes faixas etárias, incluindo leitões lactentes, animais em fase de creche e terminação, porcas e fetos (Ha et al., 2021; Wang et al., 2022). O PCV4 possui um genoma de aproximadamente 1,77 kb, com estrutura genômica semelhante aos demais circovírus, com duas ORFs principais (Fang et al., 2024; Kroeger et al., 2024). As manifestações clínicas associadas ao PCV-4 incluem PDNS, síndrome de emagrecimento multissistêmico pós-desmame, sinais neurológicos, diarreia, enterite, encefalite, doenças respiratórias, distúrbios reprodutivos e infecções subclínicas (Kroeger et al., 2024; Nguyen et al., 2022).

A infecção pelos circovírus está frequentemente associada a coinfeções com outros patógenos, como *Mycoplasma hyopneumoniae*, parvovírus suíno, vírus da síndrome reprodutiva e respiratória suína, vírus da gripe suína e *Salmonella* spp. (Castro et al., 2007; Maes et al., 2023; Ouyang, 2019). As coinfeções resultam em sinais clínicos exacerbados, incluindo febre, depressão, tosse, anorexia, corrimento

nasal e ocular, hiperpneia e pneumonia fatal (Opriessnig, Giménez-Lirola e Halbur, 2011; Segalés et al., 2004).

As doenças respiratórias suínas têm um impacto econômico significativo. É estimado um custo de R\$ 216 milhões por ano para a suinocultura brasileira em perdas econômicas por pleurisias, pneumonias e aderências (Nascimento et al., 2018). Segundo Alberton e Morés (2008), no abatedouro aproximadamente 50% dos animais são desviados ao Serviço de Inspeção Final (SIF) por apresentarem algum tipo de lesão pulmonar, e tais lesões correspondem à aproximadamente 50% das condenações de carcaças. Os animais que durante a linha de inspeção são identificados com lesões de pleurisias, são desviados ao SIF para avaliação, podendo ser destinados para o aproveitamento condicional ou condenação total da carcaça (Maes et al., 2023; Nascimento et al., 2018). No Brasil, a prevalência de desvio de carcaças ao abate ao SIF, por aderências de pleura é de 3,72% (Coldebella et al., 2018).

A pleurisia ou pleurite como também chamada, são aderências fibrinóticas presentes entre as membranas parietal e visceral do saco pleural (Maes et al., 2023; Nascimento et al., 2018). É um achado comum em suínos e é frequentemente visto na inspeção post mortem de carcaças (Maes et al., 2023; Marruchella et al., 2019). A pleurisia fibrinótica se caracteriza por um processo inflamatório crônico, sendo esta a apresentação mais comumente encontrada em suínos nos frigoríficos (Jager et al., 2012). Em rebanhos brasileiros, estudos anteriores relataram casos de pleurisia com prevalências variando de 9% a 14%. O diagnóstico da pleurisia continua sendo um desafio, pois a doença é frequentemente assintomática, com lesões identificadas apenas no momento do abate (Andreasen; Mousing; Thomsen, 2001).

É importante destacar que nem todas as lesões pulmonares crônicas resultam de infecções bacterianas; infecções virais e fatores ambientais também desempenham um papel significativo em seu desenvolvimento (Arruda et al., 2024; Petri et al., 2023). Estudos observacionais têm identificado associação entre a prevalência de pleurisia e a soroprevalência de patógenos que, embora não sejam conhecidos por causar pleurisia, incluem *Mycoplasma hyopneumoniae* e PCV2. Essas observações são provavelmente atribuídas à natureza multietiológica dos problemas respiratórios clínicos na maioria das granjas, em vez de sugerirem que esses patógenos sejam agentes causadores da pleurisia (Maes et al., 2023). Embora a capacidade de um único patógeno causar doença seja reconhecida, interações entre

múltiplos agentes simultâneos são frequentemente observadas. Essa complexidade é ainda mais exacerbada por influências ambientais, fatores genéticos e práticas de manejo.

Com base na literatura disponível, conclui-se que as lesões pulmonares são altamente prevalentes na cadeia produtiva de suínos, resultando, em grande parte, da interação entre múltiplos agentes etiológicos, incluindo o possível envolvimento dos circovírus suíno. O controle rigoroso de novos genótipos de PCVs é essencial para mitigar sua disseminação e minimizar impactos futuros na suinocultura. No entanto, ainda persistem lacunas significativas no conhecimento sobre a estrutura e a patogênese desses vírus, tornando indispensáveis a vigilância contínua e estudos moleculares e epidemiológicos mais aprofundados. Diante desse cenário, este estudo teve como objetivo principal detectar a presença de PCV2 e PCV3 em suínos abatidos por meio de qPCR, imuno-histoquímica e RNAscope, correlacionando sua ocorrência aos achados macroscópicos de pleurisia e às alterações histopatológicas. Além disso, buscou-se estabelecer associações entre a presença desses vírus e a detecção de *Mycoplasma hyopneumoniae*, *Pasteurella multocida* e *Actinobacillus pleuropneumoniae*, visando aprofundar a compreensão da interação desses patógenos no contexto do CDRS.

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CAPÍTULO 2 – Prevalence of porcine circoviruses (PCV2 and PCV3) in slaughtered pigs with different pleurisy lesions score: coinfections with *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, and *Pasteurella multocida*¹

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Abstract

Circovirus porcine (PCV) is a widespread pathogen in swine, consisting of four species: PCV1, PCV2, PCV3, and PCV4. Coinfection with other pathogens exacerbates the severity of Porcine Respiratory Disease Complex (PRDC), leading to significant economic losses. In Brazil, pleurisy lesions in pigs, often due to chronic inflammation from bacterial, viral, and environmental factors, are a major economic concern. This study aimed to detect PCV2 and PCV3 in PRDC by identifying these viruses in lung and pleural samples from pigs with varying degrees of pleurisy's lesions. A total of 130 lung and pleural samples were collected from a slaughterhouse and analyzed using the Slaughterhouse Pleurisy Evaluation System (SPES). The qPCR results showed that 63.9% of lung samples tested positive for PCV2, while 29.2% were positive for PCV3. In pleural samples, 30.7% were positive for PCV2 and 37.7% for PCV3. Additionally, other pathogens, including *Mycoplasma hyopneumoniae* (Mhyo), *Actinobacillus pleuropneumoniae* (APP), and *Pasteurella multocida* (PM), were also detected in both lung and pleural samples. Coinfections were frequently observed in both tissues. All samples tested by immunohistochemistry for PCV2 were negative, as were all those tested by RNAscope for PCV3. The histopathological findings did not reveal robust or significant lesions. Sequencing revealed that the PCV2d variant was the most prevalent; however, we also identified positive samples for PCV2c, a rare finding in Brazil. Our study highlights the complex interactions in PRDC, as well as the presence of PCV2 and PCV3 in this context.

Keywords: circovirus, pleura, lung, PRDC, coinfections.

¹ Este capítulo corresponde ao artigo científico submetido à revista Research in Veterinary Science (22/04/2025) e encontra-se em avaliação para publicação.

1. Introduction

Circovirus porcine (PCV) is the smallest identified animal virus, omnipresent pathogen in swine production, belonging to the Circoviridae family, and is subdivided into four species: PCV1, PCV2, PCV3, and PCV4 (Maity et al, 2023). While PCV-1 is considered non-pathogenic for swine, the other three genotypes possess the ability to infect immune cells, leading to inflammation, immune complex formation, and immunological dysregulation (Kroeger et al., 2024; Rakibuzzaman, 2021). The severity of the disease is intrinsically linked to the presence of pathogens involved in coinfections, which can exacerbate the infection and the lesions (Goecke et al., 2020; Ouyang, 2019).

Recent studies have highlighted the association of PCV with other pathogens responsible for the development of the Porcine Respiratory Disease Complex (PRDC), such as *Actinobacillus pleuropneumoniae* (APP), *Pasteurella multocida* (PM) and *Mycoplasma hyopneumoniae* (Mhyo) (Arruda et al., 2024; Castro et al., 2007; Ouyang, 2019; Saade et al., 2020). When this complex is established, the clinical signs observed include fever, depression, cough, anorexia, nasal and ocular discharge, hyperpnea, and pneumonia (Opriessnig; Giménez-Lirola; Halbur, 2011; Segalés et al., 2004). This respiratory disease incurs significant economic losses and may be associated with lesions identified during the slaughter of pigs (Arenales et al., 2022; Opriessnig et al., 2020).

It is estimated that the annual cost of the Brazilian swine industry amounts to BRL 216 million due to economic losses caused by pleurisy, pneumonia, and adhesions (Nascimento et al., 2018). According to Alberton and Morés (2008), approximately 50% of the animals at the slaughterhouse are redirected to the Final Inspection Service (DIF) due to some form of pulmonary lesion, and these lesions account for approximately 50% of carcass condemnations. Animals identified with pleurisy lesions during the inspection line are diverted to the DIF for evaluation, where they may either be approved for conditional use or condemned in total (Maes et al., 2023; Nascimento et al., 2018). In Brazil, the prevalence of carcass redirection to the Federal Inspection Service (SIF) at slaughter due to pleural adhesions is 3.72% (Coldebella et al., 2018).

Pleurisy consists of fibrinous adhesions present between the parietal and visceral membranes of the pleural sac (Maes et al., 2023; Nascimento et al., 2018). It is a common finding in swine and is frequently observed during post-mortem inspection

of carcasses (Maes et al., 2023; Marruchella et al., 2019). In Brazilian herds, previous studies have reported cases of pleurisy with prevalences ranging from 9% to 14%. It is important to note that not all chronic pulmonary lesions result from bacterial infections; viral infections and environmental factors also play a significant role in their development (Arruda et al., 2024; Petri et al., 2023). The diagnosis of pleurisy continues to pose a challenge, as the disease is often asymptomatic, with lesions identified only at the time of slaughter (Andreasen; Mousing; Thomsen, 2001).

Our study aimed to detect PCV2 and PCV3 using qPCR in lung and pleura samples from pigs, classified according to the macroscopic findings of pleurisy observed at the slaughterhouse. Additionally, we investigated the prevalence of Mhyo, APP, and PM, key pathogens in the SRDC. To further characterize the lesions and gain a more precise understanding of the pathological processes, we performed histopathological, immunohistochemical, and RNAscope analyses.

2. Material and methods

2.1 Sample collection

A total of 130 lung samples and 130 pleural samples were collected from pigs of different origins over a period of 30 days at a slaughterhouse in Guariba, São Paulo - Brazil. The project was approved by the Animal Ethics Committee (CEUA) of the Faculty of Agricultural and Veterinary Sciences (FCAV/UNESP), Jaboticabal, São Paulo - Brazil (Protocol nº. 001112). The collected samples originated from different geographical locations, specifically from the municipalities of Iaras (São Paulo), São Gabriel do Oeste, and Campo Grande (both in Mato Grosso do Sul).

After identifying the carcasses, the lungs were classified according to the score of pleurisy using the Slaughterhouse Pleurisy Evaluation System (SPES) proposed by Dottori et al. (2007), which employs scores ranging from 0 to 4, with 0 indicating no pleurisy lesions and 4 representing the most severe level of pleurisy. Briefly, a score of 0 indicates the absence of lesions. Score 1 describes pleurisy affecting the cranio-ventral portion of the lung, with interlobular adhesion. In score 2, the pleurisy is unilateral and mild, located in the diaphragmatic lobe. Score 3 characterizes mild bilateral pleurisy, affecting both diaphragmatic lobes, with large unilateral adhesion in the diaphragmatic lobe. Finally, score 4 represents the most severe condition, with large bilateral adhesions between the diaphragmatic lobes on one side and the

thoracic wall on the other.

An assessment of each pulmonary lobe was conducted utilizing the scoring system developed by Madec and Kobisch (Madec F, Kobisch M, 1982; Ostanello et al., 2007). It classifies severity based on the percentage of the lobe's surface affected. Score final 0 indicates a normal lobe, without any pulmonary consolidation. Score final 1 refers to up to 25% of the lobe's surface being affected. Score final 2 covers a consolidation range of 26 to 50%, while score final 3 refers to an area affected between 51 and 75%. Finally, score final 4 represents the most severe stage, with more than 76% of the lobe's surface compromised by pulmonary consolidation. The pulmonary consolidation was calculated with the assistance of the Ceva Lung Program® software (Ceva Animal Health, France).

The collection of biological samples occurred during the evisceration phase of the slaughter line, fragments of approximately 5 cm were collected from pleura on the carcasses and from the apical-cardiac-diaphragmatic lobes of their respective lungs (Thacker et al., 2006). Ultimately, the conveniently selected groups for sampling were organized into five sets, each consisting of 26 lung samples and 26 samples of their respective pleura.

The samples collected for qPCR were stored in RNase and DNase-free cryogenic microtubes (Axygen, USA) and preserved in liquid nitrogen during transport to the Swine Medicine Laboratory at UNESP Jaboticabal, where they were maintained in a freezer at -20°C until analysis. For histopathological and immunohistochemical analysis, lung samples measuring 3x3 cm were immediately fixed in 10% buffered formalin and maintained in this fixative for 48 hours prior to histological processing.

2.2 Laboratory analysis

2.2.1 DNA extraction and quality control

DNA extraction from the samples was performed using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The concentration of DNA in the samples was measured using spectrophotometry, with the NanoDrop One 2000 Spectrophotometer (Thermo Fisher Scientific®, Wilmington, United States). To eliminate the possibility of inhibitors in the extracted DNA samples and to avoid false negatives in the qPCR analysis, all samples were subjected to conventional PCR targeting the endogenous gene glyceraldehyde-3-phosphate

dehydrogenase (GAPDH), following a previously published protocol (Birkenheuer et al., 2003).

2.2.2 qPCR assay for PCV2, PCV3, Mhyo, APP, and PM

All reactions were conducted using the CFX96™ Thermal Cycler (Bio-Rad®, Marnes-la-Coquette, France). The samples were tested in duplicate, with a ΔC_q of less than or equal to 0.5 and an efficiency ranging from 90% to 105%. Amplification efficiency was calculated based on the slope of the standard curve for each run, adhering to the standards established by Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) (Bustin et al., 2009). The negative control consisted of ultrapure sterile water (Nuclease-Free Water, Promega®, Madison, Wisconsin, USA). For the quantification of DNA from clinical samples, serial dilutions were performed to determine the standard curve generated with different concentrations of synthetic DNA (GBlock®, IDT, USA) containing the target sequences of the pathogens (10^7 copies/ μ L to 10^1 copies/ μ L).

The primers and probes used for the molecular detection of each pathogen are detailed in Table 1. For the assays of PCV2 and PCV3, the qPCR protocol previously described by Goecke et al. (2019) was utilized, with some modifications. For PCV2, the reaction had a final volume of 10 μ L, composed of 5.0 μ L of master mix (2x qPCRBIO Probe Mix No-Rox), 10 μ M of each primer, 30 μ M of probe, 2.35 μ L of ultrapure sterile water (Nuclease-Free Water, Promega®, Madison, Wisconsin, USA), and 1.5 μ L of DNA. All amplifications were conducted under the same cycling conditions: 95°C for 2 minutes, followed by 40 cycles of 94°C for 15 seconds and 60°C for 60 seconds.

While PCV3, the reaction also had a final volume of 10 μ L, consisting of 5.0 μ L of master mix (2x qPCRBIO Probe Mix No-Rox), 10 μ M of each primer, 10 μ M of probe, 2.25 μ L of ultrapure sterile water (Nuclease-Free Water, Promega®, Madison, Wisconsin, USA), and 1.5 μ L of DNA. Again, all amplifications followed the same cycling conditions: 95°C for 2 minutes, followed by 40 cycles of 94°C for 15 seconds and 59°C for 60 seconds. For the detection of the agents Mhyo, APP, and PM (independent of the capsule type), a multiplex assay previously described by Petri et al. (2023) was performed.

Table 1. Pathogens, assay names, sequences of primers and probes, gene name and fragment size used for detection of the pathogens.

Pathogen	Primers and Probes	Sequence (5'-3')	Gene	Fragment Size	Reference
PCV2	QF QR Probe	GATGATCTACTGAGACTGTGTGA AGAGCTTCTACAGCTGGGGACA FAM TCAGACCCCGTTGGAATGGTACTCCTC- BHQ1	- Cap	152	Goecke et. al., 2019
PCV3	QF QR Probe	AGTGCTCCCATTTGAACG ACACAGCCGTTACTTCAC FAM ACCCCATGGCTCAACACATATGACC- BHQ1	- Cap	135	Goecke et. al., 2019
Mhyo	QF QR Probe	TAAGGGTCAAAGTCAAAGTC AAATTAAAAGCTGTTCAAATGC FAM -AACCAGTTTCCACTTCATCGCC- BHQ1	p102	150	Petri et al., 2023
PM	QF QR Probe	GGGCTTGTCGGTAGTCTTT CGGCAAATAACAATAAGCTGAGTA TexRd CGGCGCAACTGATTGGACGTTATT3- IB®RQ	- kmt1	148	Petri et al., 2023
APP	QF QR Probe	AGTGCTTACCGCATGTAGTGGC TTGGTGCGGACATATCAACCTTA Cy5 -CGATGAACCGATGAGCCGCC- IB®RQ	OmlA	153	Petri et al., 2023

2.2.3 Histopathology

Out of the 130 lung samples, 127 fragments were routinely processed for histopathological examination and stained with hematoxylin and eosin (HE). The slides were examined under an optical microscope, and the lesions were evaluated for the presence or absence of pleurisy, pneumonia, multinucleated cells, and bronchitis.

Lesions of the Bronchus-Associated Lymphoid Tissue (BALT) were classified into five categories according to the methodology of Woolley LK et. al (2012): absent (0); mild diffuse increase of lymphocytes in the peribranchial, peribronchiolar, and perivascular tissues (+); moderate and diffuse increase of lymphocytes and/or presence of some lymphoid nodules (++); pronounced number of lymphoid nodules (+++); and many lymphoid nodules affecting most of the assessed lung section (++++).

Interstitial pneumonia was classified based on four criteria, considering the

assessment of interstitial inflammation, the amount of lung parenchyma affected and thickening of the alveolar wall. A score of 0 indicated the absence of lesions, a score of 1 represented a lesion affecting 1% and 25% of the lung parenchyma and minimal thickening of the alveolar wall, a score of 2 indicated lesion affecting 25% and 50% of the lung parenchyma and mild thickening of the alveolar wall, a score of 3 indicated a lesion affecting 50% and 75% of the lung parenchyma and moderate thickening of the alveolar wall, and finally, a score of 4 indicated a lesion affecting 75% and 100% of the lung parenchyma and severe thickening of the alveolar wall.

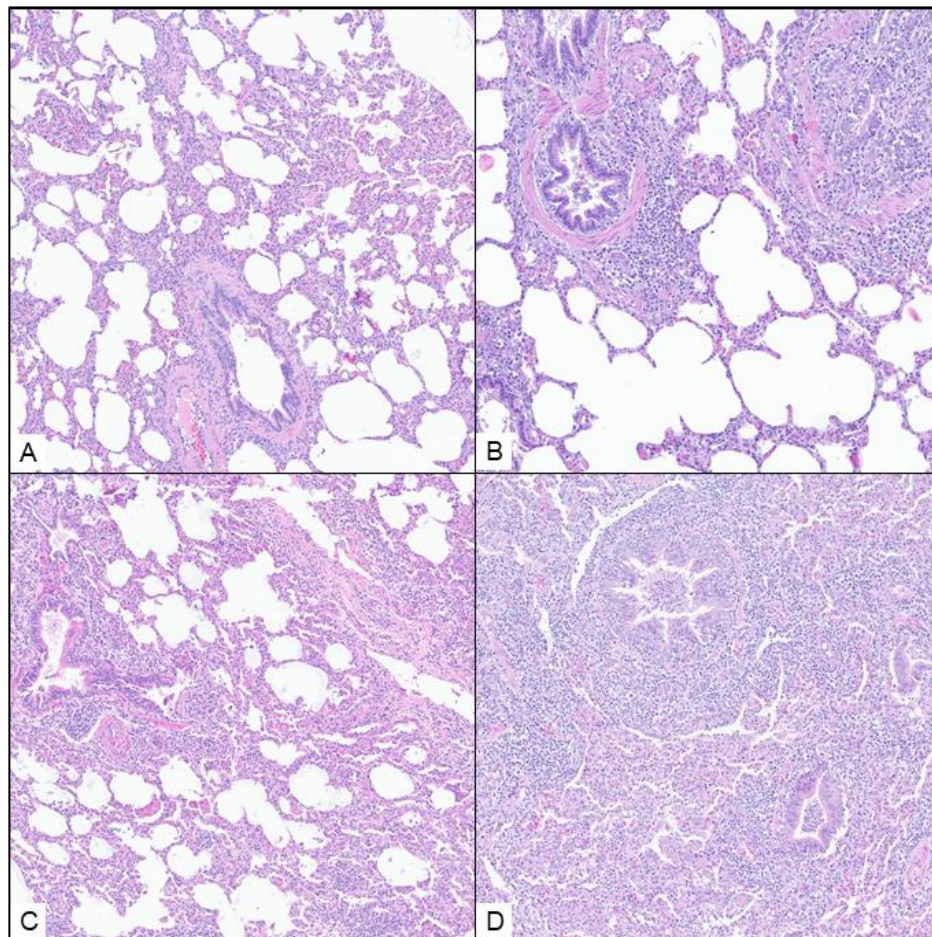


Figure 1. Score of interstitial pneumonia. A: score 1, 10%, and 25% of the lung parenchyma affected and minimal thickening of the alveolar wall. B: score 2, 25% and 50% of the lung parenchyma affected and mild thickening of the alveolar wall. C: score 3, 50% and 75% of the lung parenchyma affected and moderate thickening of the alveolar wall. D: score 4, 75% to 100% of the lung parenchyma affected and severe thickening of the alveolar wall.

2.2.4 Immunohistochemistry for PCV2

The immunohistochemical protocol for the detection of PCV2 was conducted using the Leica BOND RX platform. The antibody used was a rabbit polyclonal anti-

ORF-2, diluted to 1:1000, developed by Dr. Opriessnig from ISU VDL. The procedure began with a peroxide block for 5 minutes, followed by incubation with the primary antibody for 15 minutes. After this step, a polymer was applied for 8 minutes, followed by staining with DAB Refine for 10 minutes. The staining was complemented with hematoxylin for 5 minutes, concluding with dehydration and mounting of the slides. All steps of the protocol were performed using products from the BOND Polymer Refine Detection kit from Leica, ensuring efficacy and precision in antigen detection. All lung samples that presented a cycle threshold (Ct) value of < 30 were tested.

2.2.5 RNA Scope for PCV3

All lung samples previously tested positive for PCV3, with a Ct value below 30, were subjected to RNAscope analysis. Lung tissue samples fixed in paraffin were pretreated according to the RNAscope 2.5 assay user manual from Advanced Cell Diagnostics (ACD) (UM 322,452), with target retrieval for 15 minutes at 98-102 °C and incubation with protease plus for 30 minutes at 40 °C. PCV3 detection by in situ hybridization was performed as follows: specific probes targeting the reverse complementary nucleotide sequence of the PCV3 viral mRNA (region 2–1049 of the ORF1 gene, GenBank: HQ839721.1) (ACD, Newark, CA, USA) were used for viral confirmation. The RNAscope positive control probe ScPPIB (catalog number 428591), targeting the eukaryotic PPIB gene, and the RNAscope negative control probe DapB (catalog number 310043) were designed and synthesized by ACD company. The paraffin-embedded tissue sections were deparaffinized and treated with hydrogen peroxide at room temperature for 10 minutes. The slides were then hybridized using a hybridization buffer, and sequence amplifiers were added. The red colorimetric staining detected the PCV3 hybridization signal, while counterstaining was performed with hematoxylin.

2.2.6 Sequencing of PCV2

The sequencing was performed using the Sanger method, and the molecular characterization of the samples, as well as the phylogenetic comparisons of PCV2, were based on the polymorphism of the *Cap* gene (ORF2). Sample qualification was by nested-PCR, and also the amplification of the *Cap* gene (ORF2). The methodology and the sequence of the primers used are confidential, in accordance with the policies

of the third-party laboratory responsible for the analyses.

The phylogenetic classification of the samples was conducted following the criteria established by Sato et al. (2017) and Yang et al. (2017). To ensure a representative analysis, we selected samples from three different cities where the animals originated, considering both sample quality and the extracted DNA. The goal was to balance the distribution of collected samples over six sampling days. Thus, on D1, samples were collected from pigs in Iaras-SP; on D2, D3, D4, and D5, from São Gabriel do Oeste-MS; and on D6, from Campo Grande-MS. Given the high cost associated with the analyses, it was possible to sequence 29 out of the 83 positive samples, with 21 originating from São Gabriel do Oeste, 6 from Campo Grande, and 2 from Iaras.

2.3 Statistical analysis

The results were analyzed and graphically represented using GraphPad Prism 8 and Python. A normality test was conducted using the Shapiro-Wilk test, and the data were deemed non-parametric. Based on this outcome, Dunn's test was applied to assess statistical differences between the variables. The pathogen quantification values and pulmonary lesions among the groups were subjected to correlation analysis using Spearman's test, aiming to measure the association between the variables through the correlation coefficient. A P-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Lung consolidation

In Figure 2, a heatmap can be observed that relates the scores of pleurisies and consolidation. The number of animals is represented in relation to the scores of consolidations and pleurisies. Spearman correlation was conducted, yielding a coefficient of 0.70 and a p-value of 0.001.

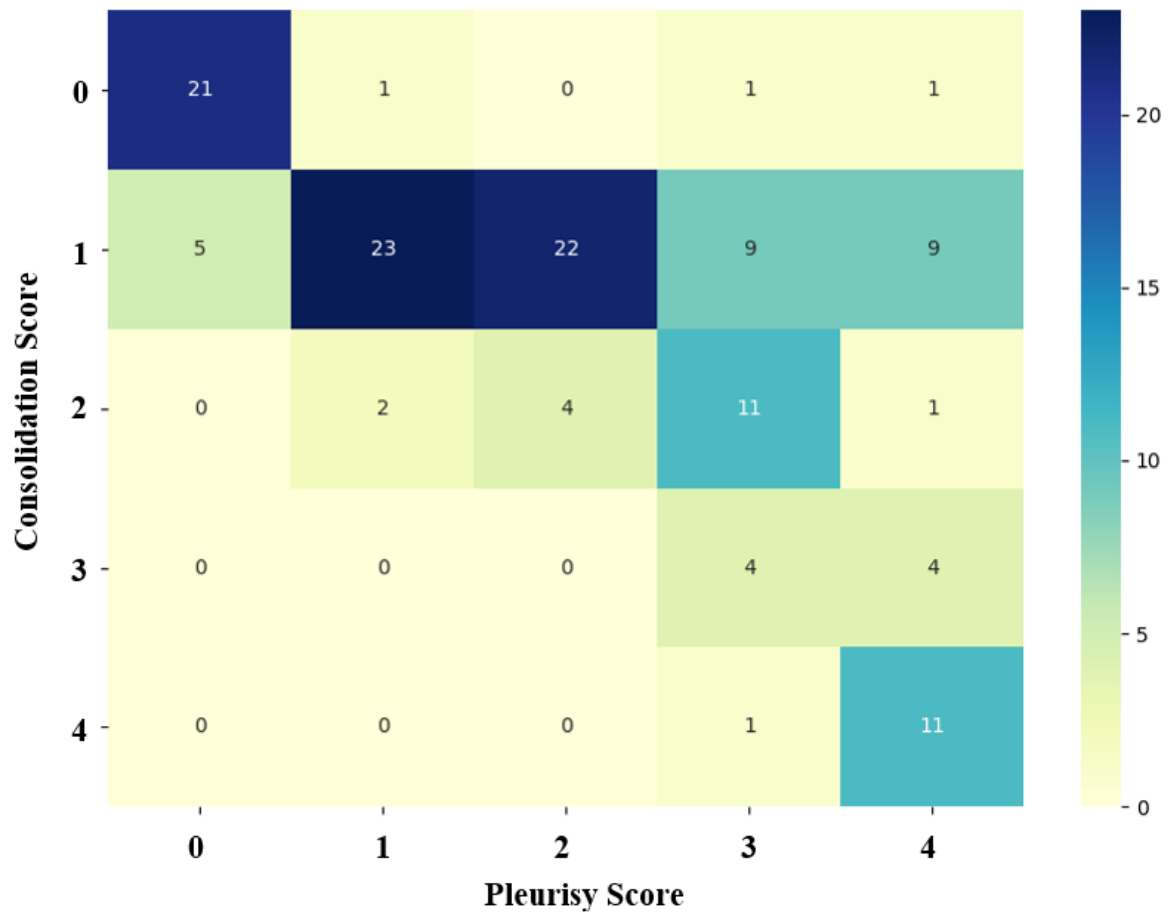


Figure 2. Heatmap of consolidation by pleurisy score.

3.2 Detection of pathogens

In the molecular analysis of lung samples, PCV2 was present in 63.9% (83/130) of the samples, while PCV3 was identified in 29.2% (38/130). The positivity rate for Mhyo reached 90.8% (118/130), and for APP, it was 60% (78/130). Regarding PM, the positivity rate was 26.1% (34/130). The analysis of the pleura also yielded positive results: PCV2 was detected in 30.7% (40/130) of the tested samples, while PCV3 was identified in 36.15% (47/130). For Mhyo, the positivity rate was 63% (82/130) and for APP, it was 36.9% (48/130), while PM showed a positivity rate of 11.5% (15/130). All pathogens were detected across all scores of pleurisies, both in the lung and in the pleura, according to figures 3 and 4. In figure 5 it represents absolute quantification of researched pathogens, details about the molecular analysis are available in the supplementary materials.

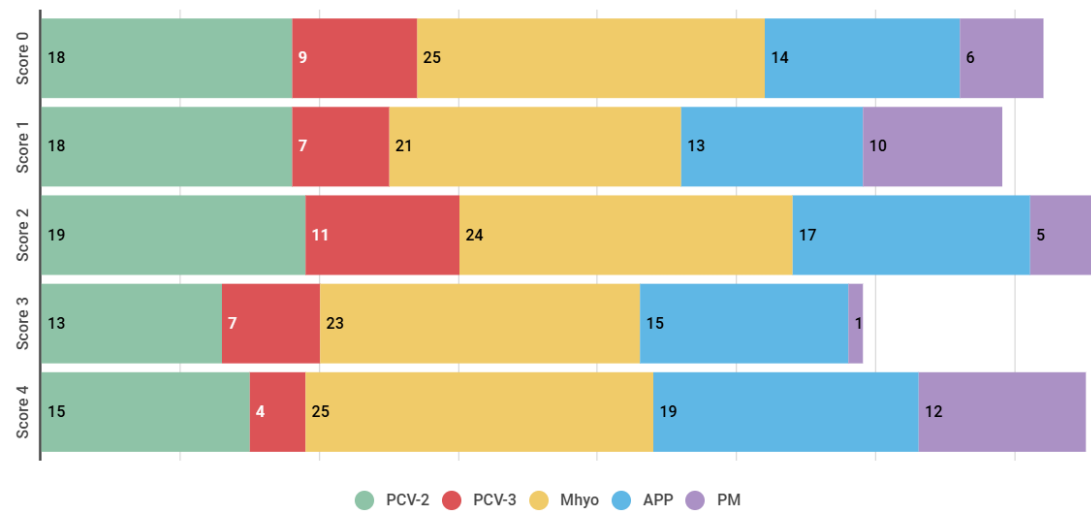


Figure 3. Number of positive lung samples in qPCR according to pleurisy scores.

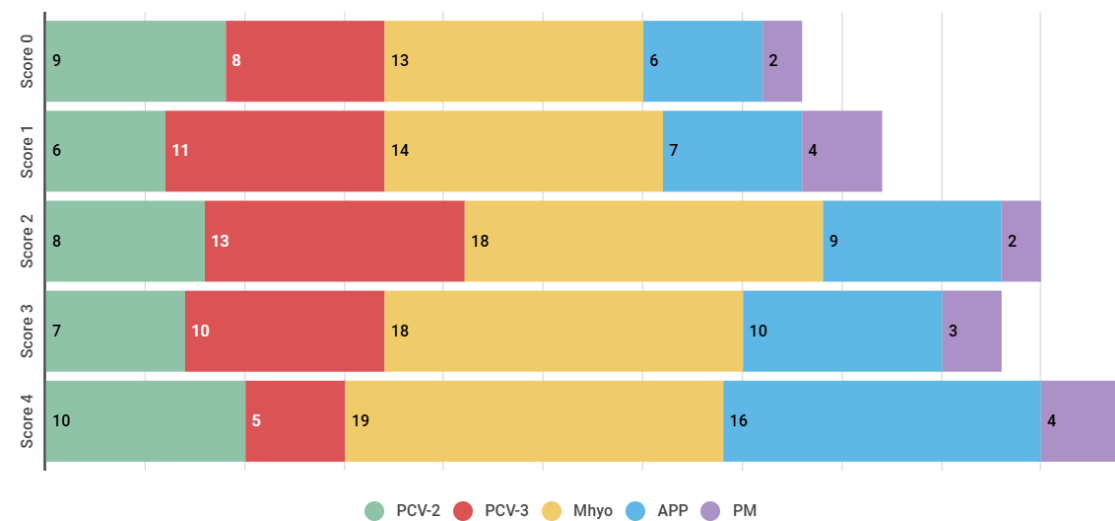


Figure 4. Number of positive pleura samples in qPCR according to pleurisy scores.

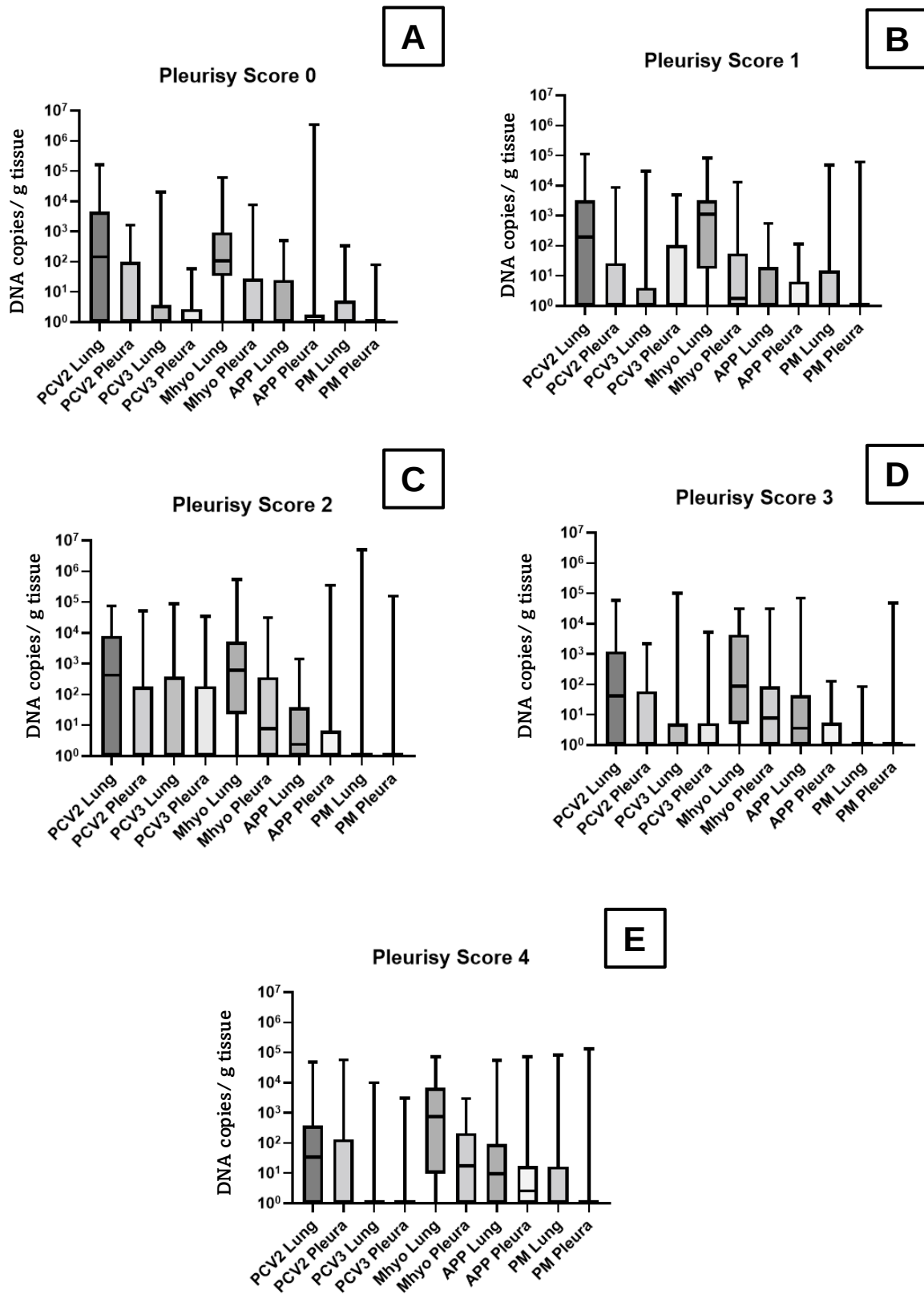


Figure 5. Absolute quantification of PVC2, PVC3, Mhyo, APP and PM according to the organ collected, pleurisy score and categories maximum, median and minimum. A: Pleurisy score 0. B: Pleurisy score 1. C: Pleurisy score 2. D: Pleurisy score 3. E: Pleurisy score 4.

3.3 Coinfections

As illustrated in Figure 6, the joint frequency of PCV2, Mhyo, and APP detected in the lungs was 15.4% (20/130). Coinfection between PCV2 and Mhyo in the lungs was observed in 14.6% (19/130) of the samples. Furthermore, both the coinfection between Mhyo and APP and the coinfection involving PCV2, PCV3, Mhyo, and APP were identified in 10.7% (14/130) of the lungs assessed. The simultaneous presence of PCV3, Mhyo, APP, and PM also demonstrated a value of 9.2% (12/130).

As illustrated in Figure 7, the joint frequency of Mhyo and APP detected in the pleurae was 11.5% (15/130). Coinfection between PCV2 and Mhyo in the pleurae was observed in 6.9% (9/130) of the samples. Additionally, both the coinfection between PCV3, Mhyo, and APP and the coinfection involving PCV2, PCV3, Mhyo, and APP were identified in 8.4% (11/130) of the assessed pleurae.

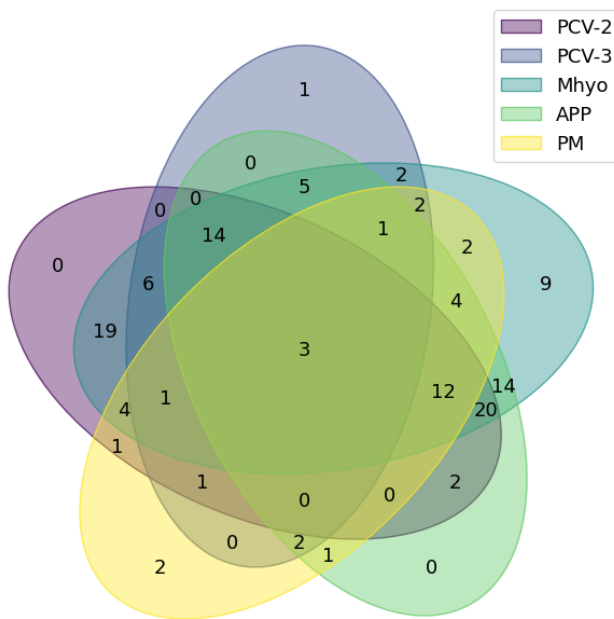


Figure 6. Number of animals tested positive for pathogens in lung samples via qPCR.

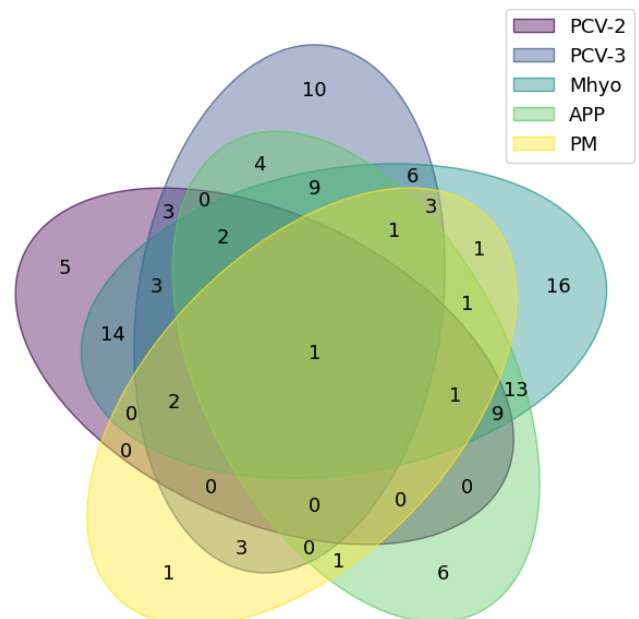


Figure 7. Number of animals tested positive for pathogens in pleura samples via qPCR.

3.4 Sperman correlation matrix

Table 2 presents the significant correlations identified in the data analysis. Positive values indicate a positive correlation, meaning that as one variable increases, the other also tends to increase. In contrast, negative values indicate a negative correlation, indicating that as one variable increases, the other tends to decrease. Values close to zero reflect a weak or nonexistent correlation between the variables. The t-test conducted for evaluation yielded simulated p-values greater than 0.05.

Table 2. Spearman correlation analysis results.

Pleurisy Score	Organ	Pathogens		Correlation Value	Correlation Direction	Correlation Intensity	P Value	Confidence Interval
0	Pleura	PCV2 x PM		0.431	Positive	Moderate	0.03	0.03 to 0.71
1	Pleura	PVC3 APP	x	0.399	Positive	Moderate	0.04	-0.001 to 0.69
2	Pleura	PCV2 Mhyo	x	0.488	Positive	Moderate	0.01	0.08 to 0.75
3	Lung	PCV2 Mhyo	x	0.557	Positive	High	0.001	0.20 to 0.78
4	Pleura	PCV2 Mhyo	x	0.500	Positive	High	0.01	0.10 to 0.75

3.5 Histopathology

Only 9.4% (12/127) of the samples evaluated by histopathology exhibited multinucleated cells. It is worth noting that all animals with this cell type were positive for PCV2. Regarding the 118 animals positive for Mhyo, 40.1% (51/127) displayed BALT hyperplasia. In conducting the Spearman correlation analysis between the variables "macroscopic pleurisy score" and "microscopic pleurisy", no significant correlation was found ($r = -0.000361$; $p\text{-value} = 0.996$). A summary of the histopathological results is presented in Table 3, and the complete data is

available in the supplementary materials.

Table 3. Number and percentage of samples classified by different lesion types, including no lesion, moderate pneumonia, severe pneumonia, and other conditions.

Type of Lesion	Percentage (%)
No lesions	42.5 (54/127)
Interstitial Pneumonia	51.1 (65/127)
Mild Pneumonia	15.7 (20/127)
Moderate Pneumonia	7.8 (10/127)
Severe Pneumonia	1.5 (2/127)

3.6 Immunohistochemistry and RNA Scope

All lung samples tested by immunohistochemistry for PCV2 and RNAscope for PCV3 yielded negative results. Representative images of negative samples used in each technique are available in the supplementary material.

3.7 Sequencing

Sequencing was performed on 29 PCV2 positive lung samples, and the results are detailed in Table 4. The phylogenetic tree from the analysis is available in the supplementary material.

Table 4. Distribution of PCV2 variants across different locations and the total number of samples sequenced.

Sample origin	PCV2b	PCV2d	PCV2c	Total Samples
São Gabriel do Oeste	4	17	0	21
Campo Grande	0	6	0	6
Iaras	0	0	2	2
Total	4	23	2	29

4. Discussion

Respiratory diseases in intensive pig production systems demonstrate a high incidence and prevalence of pulmonary lesions globally, leading to significant economic losses and underscoring the need for effective health monitoring and identification of these conditions (Arruda et al., 2024; Goecke et al., 2020). According to the assessment of consolidation and pleurisy scores, we

found a strong positive correlation, as per Cohen (1988). The p-value obtained was 0.001, which practically indicates that the results are statistically significant, reflecting a robust effect. These lesions compromise not only the health of the animals but also negatively affect various carcasses and meat quality parameters (Maes et al., 2023).

As in the present study, Pagot et al. (2007) observed that cranioventral consolidation and pleurisy exert a synergistic effect. Cranioventral pulmonary consolidation, characteristics of Mhyo infections, and pleurisy in slaughter pigs associated with APP stand out as major pathological manifestations (Enøe et al., 2002; Pieters, Maes, 2019). The lesions of cranioventral pulmonary consolidation and pleurisy are not exclusive to these two pathogens, as other infectious agents may cause similar lesions and be involved in a polymicrobial disease complex. Additionally, lesions of cranioventral pulmonary consolidation may also be attributed to viral infections (Maes et al., 2023).

The relevance of PCV2 and PCV3 in the PRDC has been emphasized, although gaps remain in the understanding of the underlying processes (Burrai et al., 2023). Our results indicate a widespread presence of PCV2 in the evaluated animals, alongside the presence of PCV3. Observational studies have identified an association between the prevalence of pleurisy and the seroprevalence of pathogens that, while not known to cause pleurisy, include Mhyo and PCV2. These observations are likely attributable to the multi-etiological nature of clinical respiratory issues on most farms, rather than suggesting that these pathogens are causative agents of pleurisy (Maes et al., 2023). While the ability of a single pathogen to cause disease is acknowledged, interactions among multiple concurrent agents are frequently observed. This complexity is further exacerbated by environmental influences, genetic factors, and management practices (Goecke et al., 2020; Merialdi et al., 2012).

Our analysis revealed a significant presence of PCVs in coinfections. As highlighted by Ouyang et al. (2019), this pathogen is known to induce immunosuppression in pigs, thereby increasing the opportunity for opportunistic agents to invade and subsequently proliferate. Both PCV2 and PCV3 are primarily replicated in lymphoid tissues, resulting in the destruction of lymphoid follicles. This process, in turn, leads to immunosuppression, creating a favorable environment for the emergence of other opportunistic pathogens (Xu et al., 2021).

Boeters et al. (2023) overviewed the median economic impact of one or several co-existing PRDC pathogens existing in literature, where it ranged from €2.30 to €15.35 per finished pig.

Additional studies suggest that co-infection by bacteria and virus may exacerbate pulmonary lesions (Saade et al., 2020). The coexistence of these two types of pathogens presents significant challenges for the prevention and control of circovirus disease. In the correlation analysis related to pleurisy scores, we observed moderate to high intensity correlations, all with positive orientation, suggesting synergy in the quantification of these pathogens. However, upon performing a t-test to assess the statistical significance of the results, we obtained simulated p-values exceeding 0.05, suggesting causality in these findings.

Pulmonary histopathological evaluation did not reveal significant findings directly associated with PCV2, such as the presence of multinucleated giant cells, a finding frequently linked to viral infections (Toyama et al., 2022). However, morphological analysis indicated that 56.6% (47/83) of PCV2-positive animals exhibited interstitial pneumonia in histopathological examinations. For PCV3, 44.7% (17/38) of virus-positive animals also presented this lesion. Although interstitial pneumonia cannot be exclusively attributed to PCV2 or PCV3, both viruses are known to be closely associated with this condition, often in conjunction with other pathogens (Chen et al., 2021; De Conti et al., 2021).

Most Brazilian farms vaccinate against PCV2, Mhyo, APP, and PM. Considering that the slaughterhouse animals in this study were vaccinated, we suggest that commercial vaccines are effective in reducing lesions, as histopathological findings did not reveal significant exacerbations. According to Segalés and Sibila (2022), the threshold for diagnosing subclinical PCV2 infection has not yet been established, but it is suggested that viral quantification should be below 10^7 viral copies/g. Our findings, with all quantification results below 10^5 viral copies/g, classify the PCV2-positive animals as subclinical, despite being viremic at slaughter. This raises concerns about the durability of the immune response, as these animals may act as viral reservoirs, complicating infection control and eradication efforts.

The Spearman correlation analysis between microscopic and macroscopic pleurisy evaluation revealed a lack of correlation between the variables, a finding that may reflect the chronic nature of the process. At the time of slaughter, it is

expected that pigs present either active chronic conditions or lesions in advanced stages of healing, which can hinder the precise identification of pleural lesions (Hälli et al., 2020; Jäger et al., 2012; Petri et al., 2023). In the study by Malcher et al. (2024), the data revealed that severe pleuritis lesions reduce the average daily gain and carcass weight, resulting in an additional cost per kg (US\$ 1.29 compared to US\$ 1.32 for milder cases). Furthermore, these lesions decrease total revenue by 1.36% and reduce the return on investment from 5.33% to 3.90%. Given the economic significance of these lesions and the findings presented in this study, the inherent challenges in diagnosing pleurisy become evident, reinforcing the need for further research on the subject to improve detection and evaluation methods for this condition.

In the immunohistochemical evaluation, all lungs that tested positive for PCV2 with a cycle threshold (Ct) below 30 were selected for analysis. The same criterion was applied to PCV3 using the RNAscope technique. However, no PCV2 viral proteins were detected in the analyzed tissue, and all slides were negative for PCV3. These results may suggest that there is no active infection by these viruses in the specific tissue or that the viral loads are too low to be detected. However, it is important to note that a negative result does not completely rule out the possibility of subclinical infection or the presence of the virus in other parts of the body.

Overall, our sequencing revealed a predominance of the PCV2d variant; however, despite sequencing only two samples from Iaras (SP), we found a rare genotype in both samples, PCV2c, which has only one available description made by Franzo et al. (2015), who detected it in the state of Mato Grosso. According to a study conducted in Brazil by Miotto et al. (2024), in the analysis of 333 clinical PCV2-positive samples from different stages of pig production, 79.87% (266/333) were identified as PCV2d, which corroborates our findings, as we obtained 79.31% (23/29). Our genotype aligns with international studies that identify the PCV2d and PCV2b genotypes as the most prevalent today (Dei et al., 2023; Lv et al., 2023; Yuan et al., 2024).

The data obtained from the slaughterhouses provide unique and valuable feedback for pig producers and their herd veterinarians, as well as serving as a crucial source for epidemiological and efficacy studies. The lesions also hold economic relevance, as they are associated with increased marketing time.

Furthermore, they may generate complications in slaughterhouses, given that the carcasses require trimming due to partial condemnations. This negative effect elevates the risk of issues during processing and storage of carcasses, underscoring the importance of implementing effective strategies for controlling and preventing these respiratory infections in pigs destined for slaughter.

Our results are of significant epidemiological importance for guiding future actions related to the control and treatment of PCV2 and PCV3 infections, as well as in managing PRDC. Further research is needed to identify potential improvements and restructuring in the field. In this context, the data obtained from slaughterhouses can complement the information collected on farms, integrating a monitoring system for animal health.

5. Conclusion

Therefore, it can be concluded that lung lesions, particularly pleuritis, are common in the swine production chain, usually resulting from the interaction of multiple etiological agents. Although our study has shown that Circovirus Porcine (PCV2 and PCV3) is not the primary cause of these lesions, its presence, along with other pathogens, contributes to the severity of the clinical condition.

Funding

The authors would like to thank São Paulo Research Foundation (FAPESP) [Grants 01748-0/2023 and 01391-8/2024] and National Council for Scientific and Technological Development (CNPq) for the productivity grant to Luís G. de Oliveira [Grant 316447/2021-8] for funding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Iowa State University and Zoetis - Global Animal Health Company for their support of the project. They also express their gratitude to Dr. Pablo Piñeyro, Dr. Caroline Pissetti and MSc. Igor Savoldi for

their valuable technical assistance. We also thank Dr. Anderson Gris and Dr. Emanoelly Silva for their assistance in the histopathological analysis.

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