UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS
SETOR DE PATOLOGIA VETERINÁRIA

ASPECTOS PATOLÓGICOS E ETIOLÓGICOS DAS CAUSAS DE MORTE DE SUÍNOS DE RECRIA E TERMINAÇÃO NO SUL DO BRASIL

Tese de doutorado

Aluna: Me. Manoela Marchezan Piva
Orientador: Prof. Dr. Saulo Petinatti Pavarini

Porto Alegre, Rio Grande do Sul
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O presente trabalho foi relizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001 e Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

CIP - Catalogação na Publicação

Piva, Manoela Marchezan
Aspectos patológicos e etiológicos das causas de morte de suínos de recria e terminação no sul do Brasil / Manoela Marchezan Piva. -- 2023.
83 f.
Orientador: Saulo Petinatti Pavarini.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Veterinária, Programa de Pós-Graduação em Ciências Veterinárias, Porto Alegre, BR-RS, 2023.

1. Patologia suína. 2. Artrite bacteriana. 3. Toxoplasma gondii. 4. Polisserosite. I. Pavarini, Saulo Petinatti, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

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ASPECTOS PATOLÓGICOS E ETIOLÓGICOS DAS CAUSAS DE MORTE DE SUÍNOS DE RECRIA E TERMINAÇÃO NO SUL DO BRASIL

Aprovada em 08 de dezembro de 2023.

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RESUMO

O Brasil é o quarto maior produtor e exportador de carne suína mundial e é de extrema importância o desenvolvimento de trabalhos sobre a sanidade do rebanho suíno brasileiro. O objetivo desse projeto é elucidar algumas patologias ainda pouco estudadas que afetam suínos de crescimento e terminação. Inicialmente, descrever os aspectos clínicos, etiológicos e patológicos de 76 casos de suínos caídos em duas granjas tecnificadas de crescimento e terminação no sul do Brasil. Desses casos, as artrites e as espondilites supurativas foram as principais causas de suínos caídos nas baias (29/76 e 10/76 respectivamente), seguidos pela circovirose (8/76), fraturas ósseas (7/76), meningoencefalite supurativas (6/76), meningoencefalomielite não supurativas (4/76), embolismo fibrocartilaginoso (3/76), epifisiolise (3/76), mielite bacteriana ascendente (3/76), e outras condições (3/76). Posteriormente, caracterizou-se aspectos clínicos, patológicos, bacteriológicos e moleculares da polisserosite por *Pasteurella multocida* em suínos de crescimento e terminação. Essa condição demonstrou que cepas de alta patogenicidade circulam e geram alta mortalidade em rebanhos no Sul do Brasil. Foi possível identificar que as cepas eram do tipo capsular A e positivas para o gene de virulência *pfhA*. E por fim, apresentou-se aspectos patológicos e moleculares de um surto de toxoplasmose fatal em um rebanho de suínos de crescimento e terminação, no estado de Santa Catarina, Brasil. Nesse caso, a doença ocorreu de maneira aguda e levou a morte cerca de 4,2% do lote. Clinicamente manifestou-se como uma doença febril e com lesões evidentes de necrose muscular, linfadenite e hepatite granulomatosa e necrotizante, além de pneumonia intersticial linfoplasmocítica. Foi identificado um genótipo semelhante a um anteriormente conhecido, TgCkBrSC4, que já havia sido isolado em galinhas de vida livre no estado de Santa Catarina. Adicionalmente, a produção de um material técnico teve objetivo de difundir uma informação atualizada acerca de diagnóstico de doenças dos suínos, e servir como uma ferramenta adicional de consulta para estudantes, médicos veterinários e patologistas veterinários que trabalham no diagnóstico. O livro inclui tópicos importantes relacionados a técnica de necropsia e envio de amostras para diagnóstico laboratorial, alterações pós-mortais, não lesões e lesões sem significados clínicos. Além disso, tópicos sobre as principais doenças que acometem os suínos na maternidade, creche, crescimento/terminação e matrizes, com fotos e informações detalhadas de conduta diagnóstica.

Palavras-chave: Patologia suína; Artrite bacteriana; *Toxoplasma gondii*; Polisserosite.

ABSTRACT

Brazil is the fourth largest producer and exporter of pork globally, and the development of studies on the health of the Brazilian swine herd is of utmost importance. The aim of this project is to elucidate some less-studied pathologies that affect growing and finishing pigs. Initially, the project will describe the clinical, etiological, and pathological aspects of 76 cases of fallen pigs in two technified growing and finishing farms in southern Brazil. Among these cases, arthritis and suppurative spondylitis were the main causes of fallen pigs in the pens (29/76 and 10/76, respectively), followed by circovirus infection (8/76), bone fractures (7/76), suppurative meningoencephalitis (6/76), non-suppurative meningoencephalomyelitis (4/76), fibrocartilaginous embolism (3/76), epiphysiolysis (3/76), ascending bacterial myelitis (3/76), and other conditions (3/76). Subsequently, the project characterized clinical, pathological, bacteriological, and molecular aspects of polyserritis caused by *Pasteurella multocida* in growing and finishing pigs. This condition demonstrated that highly pathogenic strains circulate and cause high mortality in herds in southern Brazil. It was possible to identify that the strains were of capsular type A and positive for the virulence gene pfhA. Finally, the project presented pathological and molecular aspects of a fatal toxoplasmosis outbreak in a herd of growing and finishing pigs in the state of Santa Catarina, Brazil. In this case, the disease occurred acutely and led to the death of approximately 4.2% of the herd. Clinically, it manifested as a febrile disease with evident lesions of muscular necrosis, lymphadenitis, and granulomatous and necrotizing hepatitis, in addition to lymphoplasmacytic interstitial pneumonia. A genotype similar to a previously known one, TgCkBrSC4, which had been isolated in free-range chickens in the state of Santa Catarina, was identified. Additionally, the production of technical material aimed to disseminate updated information on the diagnosis of swine diseases and serve as an additional reference tool for students, veterinarians, and veterinary pathologists working in diagnosis. The book includes important topics related to necropsy technique and sample submission for laboratory diagnosis, post-mortem changes, non-lesions, and lesions without clinical significance. Moreover, it covers topics on the main diseases affecting pigs in maternity, nursery, growth/finishing, and breeding, with photos and detailed information on diagnostic procedures.

Keywords: Swine Pathology; Bacterial Arthritis; *Toxoplasma gondii*; Polyserritis.

SUMÁRIO

1 INTRODUÇÃO

A carne suína é a mais consumida no mundo e o Brasil é o 4º maior produtor e exportador mundial de suínos, o que correspondeu a uma produção de 4,44 milhões de toneladas de carne em 2020 (ABPA, 2021). A região sul do Brasil é a principal produtora e é responsável por cerca de 66,5% do abate nacional de suíno (IBGE, 2020). Atualmente no Brasil, há cerca de 3100 granjas tecnificadas de reprodução e aproximadamente 15000 granjas de engorda (crechários, terminações e "*wean to finish*") (ABCS, 2016). O aumento da demanda pela carne suína levou à intensificação da produção, com granjas alojando milhares de animais em densidades, muitas vezes, propícias à rápida transmissão de patógenos (MARTINEZ, 2002).

Uma variedade de doenças, de causas infecciosas e não infecciosas, pode afetar os suínos e causar mortalidade e/ou perdas produtivas. Altos índices de mortalidade refletem a saúde do plantel ou falhas de manejo e, antes de determinar medidas preventivas, é importante avaliar as principais causas e os fatores de risco para a mortalidade dos suínos em fase de crescimento e terminação (MOREAU *et al*., 2001).

Dentre as principais causas de morte em suínos de crescimento e terminação, destacam-se as pneumonias. Com taxas que variam de 33% (PIVA *et al.*, 2020), 17,24% (LIPPKE *et al*., 2007), 31,5% (GARDEN, 1985) e 24,7% (STRAW *et al.*, 1983). Dentre os principais agentes etiológicos envolvidos nas pneumonias em suínos, destacam-se o Vírus da Influenza A, *Mycoplasma hyopneumoniae* e *Pasteurella multocida*, geralmente em associação (MORÉS *et al*., 2015; PIVA *et al*., 2020; DE CONTI *et al*., 2021). Nesse caso Influenza A e *M. hyopneumoniae* são responsáveis pela lesão primária do epitélio respiratório, permitindo uma porta de entrada ideal para bactérias secundárias, como *P. multocida*. (MORÉS *et al*., 2015)

Pasteurella multocida é o principal agente bacteriano isolado em pneumonias de suínos no Brasil (MORÉS *et al*., 2015; PIVA *et al*., 2020; DE CONTI *et al*., 2021). Porém, também tem potencial septicêmico, e podem produzirlesões caracterizadas por pneumonia hemorrágica e polisserosite em suínos de terminação (CAPPUCCIO *et al*., 2004, OLIVEIRA FILHO *et al.*, 2015). Essas bactérias já foram demonstradas como causa de doença clínica em estudos experimentais, geralmente associadas às cepas de alta patogenicidade. O gene responsável por essa alteração é denominado *PfhA* (hemaglutinina filamentosa), um gene de aderência que é expresso em todas as

cepas de alta patogenicidade de *P. multocida* já identificadas (OLIVEIRA FILHO *et al.*, 2015; SAHOO *et al.*, 2020).

Curiosamente, a polisserosite causada por *P. multocida* em suínos não é ainda muito bem documentada em casos de ocorrência natural da doença. Na literatura, há apenas um artigo de polisserosite em suínos naturalmente infectados por *P. multocida*, que apresenta a descrição da doença na Índia e identificação de genes de patogenicidade (SAHOO *et al.*, 2020). Nesse trabalho, os autores trazem contribuições sobre a invasão no sistema nervoso central por *P. multocida*, que leva a lesões discretas de meningoencefalite não supurativa (SAHOO *et al.*, 2020).

Outra causa de morte que abordaremos nesse projeto é a toxoplasmose, uma zoonose causada por *Toxoplasma gondii*, um protozoário intracelular obrigatório (VESCO *et al*., 2007). Gatos domésticos e selvagens são os hospedeiros definitivos que excretam oocistos no ambiente, e outras espécies de mamíferos, que incluem suínos e humanos, são hospedeiros intermediários (TENTER *et al*., 2000). As infecções ocorrem após a ingestão de alimentos ou água contaminados com oocistos, ou através do consumo de carne crua ou com baixo tempo de cocção que contém cistos teciduais, o que geralmente resulta em doença subclínica (DUBEY; JONES, 2008; DUBEY, 2008; DUBEY; LAPPIN, 2015). Além disso, fatores relacionados ao manejo, como a presença de lâmina d'água nas pocilgas, bebedouro tipo canaleta e a presença de áreas alagadiças nas propriedades, foram associados à maior prevalência da infecção (TSUTSUI *et al.*, 2003).

Os suínos podem ser uma importante fonte de infecção por *T. gondii* para humanos (FEITOSA *et al.*, 2014). No Brasil, muitos estudos sorológicos recentes têm demonstrado infecção por *T. gondii* em suínos em diferentes regiões do país. As prevalências giram em torno de 22,5% (CORREA *et al.*, 1978) e 29,72% (BARCI *et al.*, 1998) em São Paulo; 37,84% (VIDOTTO *et al.*, 1990) e 24% (GARCIA *et al.*, 1999) no Paraná; e 33,75% no Rio Grande do Sul (FIALHO; ARAÚJO, 2003). No nordeste do Brasil, FEITOSA *et al.*, (2014) relataram soroprevalência de 19,5%. Em alguns estados a prevalência é um pouco menor, em torno de 1,11% no Rio de Janeiro (SOUZA, 1995) e 15,35% no Paraná (TSUTSUI *et al.*, 2003).

Há raros relatos de toxoplasmose em espécies de produção, e geralmente estão associados a abortamentos em suínos e ruminantes (GABARDO *et al.*, 2013), assim como mortalidade em cães, frequentemente, associado a coinfecção pelo vírus da cinomose canina (MORETTI *et al.*, 2002). No Brasil, há um relato de mortalidade de galinhas comerciais e criadas soltas, associado a toxoplasmose sistêmica (VIELMO *et al.*, 2019), e há poucos relatos da doença clínica em suínos.

Olinda *et al.* (2016) relatam dois casos de toxoplasmose sistêmica fatal em suínos de subsistência, com idade de um e quatro meses de vida. Os suínos tiveram curso clínicos de 4-5 dias, e apresentaram apatia, anorexia, tremor muscular, diarreia, dispneia e perda de peso. Histologicamente, demonstraram pneumonia intersticial linfo-histiocítica associada a necrose multifocal alveolar e bronquiolar. Havia, também, hepatite necrótica e granulomatosa multifocal. Associado as lesões, era possível observar os taquizoítos livres e cistos de *T. gondii*, que foram identificados pela imuno-histoquímica e reação em cadeia de polimerase (PCR), posteriormente (OLINDA et al., 2019).

Outro tema que abordaremos nesse trabalho são as enfermidades relacionadas aos suínos caídos nas granjas de crescimento e terminação. Esses suínos são aqueles que se apresentam em decúbito lateral ou esternal, não conseguem se levantar, com dificuldade de locomoção e, também, os que têm sinais clínicos neurológicos centrais e medulares. Esses animais são comumente chamados de porcos “downer pigs” ou “downed pigs” (SEGURA-CORREA *et al.,* 2011). Suínos caídos ou não ambulatórios podem ser observados como resultado de uma infinidade de doenças que afetam principalmente o Sistema Nervoso Central (SNC) e o sistema musculoesquelético, incluindo condições infecciosas, nutricionais, traumáticas e degenerativas (SEGURA-CORREA *et al.,* 2011).

Este tipo de manifestação clínica é frequentemente observado em porcas, que representa causa de morte de 13% até 23,9% dos animais desta categoria (D'ALLAIRE *et al.,* 1987; SEGURA-CORREA *et al.,* 2011; SCHWERTZ *et al.,* 2021). Essas doenças são comuns na rotina das granjas de suínos, geralmente de difícil tratamento, que leva a um prognóstico ruim, onde a eutanásia é um desfecho comum nesses casos. O diagnóstico clínico dessas condições é desafiador; entretanto, o diagnóstico patológico é uma importante ferramenta para elucidar as principais causas e prevenir mortes e descartes por lesões no abatedouro (BRAGA *et al.,* 2006; MARQUES *et al.,* 2012).

Muitos desses casos envolvem lesões embólicas, decorrentes de lesões de cauda, por mordedura. Essas lesões são comumente observadas em granjas, associadas a diversos fatores, como a superlotação, desafios com temperatura e unidade, disputa por comedouro, deficiências nutricionais e outros fatores estressantes (HARLEY *et al.,* 2012). Alguns trabalhos têm sido realizados em abatedouro, pois essas lesões embólicas levam a abscessos e processos infeciosos sistêmicos, que gera condenação de carcaça (BRAGA *et al.,* 2006; HARLEY *et al.,* 2012; MARQUES *et al.,* 2012). Essas lesões, geram inúmeras perdas produtivas, tanto no abatedouro,

quanto no aumento de mortalidade na granja, que pode chegar a 5,4%, associadas a lesões tromboembólicas bacterianas sistêmicas (PIVA *et al.*, 2020).

Portanto, o objetivo dessa tese foi elucidar algumas patologias ainda pouco estudadas na suinocultura brasileira. Inicialmente, descreveu-se os aspectos clínicos, etiológicos e patológicos de 76 casos de suínos caídos em duas granjas tecnificadas de crescimento e terminação no sul do Brasil. Posteriormente, caracterizou-se os aspectos clínicos, patológicos, bacteriológicos e moleculares da polisserosite por *Pasteurella multocida* em suínos em crescimento e terminação. Também, apresentou-se aspectos patológicos e moleculares de um surto de toxoplasmose fatal em um rebanho de suínos de crescimento e terminação, no estado de Santa Catarina, Brasil. E, por fim, apresentar em forma de livro, informações práticas acerca de diagnóstico de doenças dos suínos, com objetivo de difundir as informações geradas por essa tese e demais trabalhos realizados no Setor de Patologia Veterinária.

2 RESULTADOS DA TESE

Os resultados da tese serão apresentados na forma de artigos científicos.

2.1 Artigos e livro

Neste item serão apresentados os artigos/livro intitulados:

- **ARTIGO 1.** "**Non-ambulatory pigs in two Brazilian growing-finishing farms: a clinic, etiological and pathological perspective on 76 cases"**
- **ARTIGO 2.** "***Pasteurella multocida* polyserositis in growing-finishing pigs**"
- **ARTIGO 3.** "**Outbreak of toxoplasmosis associated with muscular lesions in finishing pigs caused by an atypical *Toxoplasma gondii* genotype"**
- **LIVRO. "Guia de necropsia e patologia de suínos"**

ARTIGO 1

“**Non-ambulatory pigs in two Brazilian growing-finishing farms: a clinic, etiological and pathological perspective on 76 cases”,** o qual foi publicado na revista *Porcine Health Management*, https://doi.org/10.1186/s40813-022-00279-6.

Non-ambulatory pigs in two Brazilian growing-finishing farms: a clinic, etiological and pathological perspective on 76 cases

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ABSTRACT

Background: Non-ambulatory pigs, colloquially known as downers or downed pigs, are animals presented with limited to no mobility, usually as a result of pre-existing neurologic or musculoskeletal conditions. Impaired ambulation is a major cause of euthanasia in pigs, leading to economic losses and animal welfare concerns. Additionally, reaching the underlying diagnosis of impaired ambulation in pigs is commonly a challenging task for swine practitioners. The aim of this necropsy-based study was to report the clinical, etiological, and pathological findings of 76 non-ambulatory grower-finisher pigs, and to correlate tail-biting lesions with the causes of

death/reason for euthanasia in non-ambulatory pigs. Necropsies of downed pigs were performed during on-site visits to two pig farms in southern Brazil.

Results: The diagnosis of the conditions was based on the clinical, macroscopic, histopathological, bacteriological, immunohistochemical, and molecular findings. The diseases diagnosed in non-ambulatory pigs in this study were suppurative arthritis (29/76), suppurative spondylitis (10/76), PVC-2 associated diseases (8/76), bone fracture (7/76), non-suppurative meningoencephalomyelitis (4/76), suppurative meningoencephalitis (6/76), fibrocartilaginous thromboembolism (3/76), epiphysiolysis (3/76), ascending bacterial myelitis (3/76), and other conditions (3/76). The frequency of suppurative arthritis, suppurative spondylitis, and ascending bacterial myelitis/meningitis was higher in pigs with tail biting lesions than controls ($P<0.001$).

Conclusions: Non-ambulatory pigs were observed during the entire rearing period, however, the occurrence of non-ambulatory pigs increased in animals aged ≥ 150 days. Infectious diseases were the most common cause of downed pigs, mainly associated with chronic bacterial infections. Tail biting lesions were an important predisposing factor to suppurative arthritis, suppurative spondylitis, and ascending bacterial myelitis/meningitis.

Keywords: Swine pathology; locomotor disorders; neurological diseases; tail biting lesion.

BACKGROUND

Pigs presenting significant locomotion deficits, including difficulty to stand up and inability to walk are known as non-ambulatory pigs (Ritter et al 2009). Non-ambulatory pigs may be colloquially referred to as "downers" or "downed" pigs and represent a recurring problem in commercial pig farms worldwide [1]. Non-ambulatory pigs may be observed as a result of a

plethora of diseases affecting primarily the Central Nervous System (CNS) and the musculoskeletal system, including infectious, nutritional, traumatic, toxic, and degenerative conditions [2].

Non-ambulatory pigs frequently die or are euthanized in the farms due to poor prognosis. Additionally, partial or total carcass condemnation due to disseminated lesions are common outcomes at the instances where these pigs arrive at the slaughterhouse [3]. These lesions are reported to be largely associated with tail lesions in swine [4]. Furthermore, non-ambulatory pigs are estimated to cost 46 million dollars annually to the U.S. swine industry [3]. This problem had an estimated cost of $54.91 per non-ambulatory pig in the US between 2012 and 2015 and affected 0.63% of pigs slaughtered between those years [5].

Besides economic losses, non-ambulatory pigs represent a major concern regarding animal welfare standards in pig farms. Impaired ambulation may lead to insufficient food and water consumption and may favor injuries occurring as a result of negative interactions with other pigs. Additionally, several diseases leading to impaired ambulation are painful and impact negatively normal pig behavior [3].

This type of clinical manifestation bas been frequently reported in sows, comprising the cause of death/main reason for euthanasia of 13% - 23.9% of animals in this category in previous studies [6, 1, 7]. Diseases leading to impaired ambulation and lameness are also common in rearing pigs, however, comprehensive studies documenting the etiologic and pathologic findings observed in these cases are limited.

Investigating the underlying causes for impaired ambulation may be challenging for swine practitioners due to the great variety of possible causes, the manifestation of nonspecific clinical signs and the need for a systematic and detailed assessment of the CNS and the musculoskeletal system in these cases. Thus, it is plausible to assume that the underlying cause for impaired

ambulation in pigs remains undetermined in many, if not most cases occurring in the farms. In this scenario, the necropsy examination represents an important tool to investigate and elucidate the main causes of impaired ambulation in pigs, yielding crucial information to measure and monitor continuous improvements for animal welfare in the swine industry and mitigate economic losses.

In a previous work, we assessed the causes of mortality in the growing-finishing phase in two pig farms in Southern Brazil [8]. In this necropsy-based study, 610 pigs were evaluated, 76 (12.5%) of which were non-ambulatory pigs. In the current study, the aim was to describe in greater depth and with visual resources the clinical, etiological, and pathological aspects of this subset of cases. In addition, analysis to correlate tail-biting lesions with the causes of death/reason for euthanasia in non-ambulatory pigs were conducted.

RESULTS

The underlying cause for impaired ambulation was infectious in 61/76 pigs (80.2%) and non-infectious in 15/76 (19.8%). Fourteen different pathological entities were diagnosed based on the necropsy findings. Information on the frequency of diagnosis and distribution according to sex, Body Condition Score (BCS) and frequency of tail-biting lesions are shown in Table 1. The distribution of diseases is depicted according to age group categories, and it is possible to observe a greater involvement of animals at the end of the finishing phase (≥ 150 days).

The proportion of pigs with tail-biting associated diseases (suppurative arthritis, suppurative spondylitis and ascending bacterial myelitis/meningitis) differed (Pearson's Chi-square test P value<0.001) between pigs with (67.74%) and without tail-biting lesions (0.04%). Pigs with tail-biting lesions were 56.7 times (95% CI: 12.5 to 256.3) more likely to have suppurative arthritis, suppurative spondylitis, or ascending bacterial myelitis/meningitis than controls (Logistic regression P value<0.01).

Despite the high frequency of barrows among affected pigs, the proportion of tail-biting lesions did not differ (Fisher Exact P value=0.720) between barrows (51.51%) and females (65.50%). Information for individual entities diagnosed during this study is described in the following subsections.

Table 1: Postmortem diagnoses of 76 non-ambulatory pigs in the growing-finishing phase.

Diagnosis	Incidence				Tail-biting lesion		EBC	N/E
	%	N	barrows	females	%	n		
Suppurative arthritis	38.2	29/76	24	5	44.82	13/29	2	7/22
Suppurative spondylitis	13.2	10/76	7	3	60	6/10	2	2/8
PCV-2 associated diseases	10.5	8/76	1	7	0	0/8	3	2/6
Bone fracture	9.2	7/76	3	4	14.2	1/7	3	0/7
Suppurative meningoencephalitis	8	6/76	3	3	16.6	1/6	3	4/2
Non-suppurative encephalomyelitis	5.3	4/76	2	2	0	0/4	3	1/3
Fibrocartilaginous embolism	3.9	3/76	1	2	0	0/3	4	2/1
Epiphysiolisis	3.9	3/76	2	1	0	0/3	3,5	1/2
Ascending bacterial myelitis/meningitis	3.9	3/76	2	1	100	3/3	2,5	1/2
Others	3.9	3/76	2	1	0	0/3	3	2/1
Total	**100**	**76**	**47**	**29**	**31.8**	**24/76**		

n: number; %: percentage; BCS: Body Condition Score; N/E: natural death / euthanasia.

Suppurative arthritis and spondylitis

Pigs diagnosed with suppurative arthritis presented non-weight-bearing lameness, enlarged joints, and reluctance or impossibility to stand up and walk. The clinical course was predominantly chronic. Grossly, affected joints were enlarged and filled with purulent material. In many cases, the joint was surrounded fibrous connective tissue, with the formation of periarticular abscesses. The most commonly affected joint in this condition was the femoral-tibia-patellar (16/29), followed by the tarsal joint (8/29), humerus-radio-ulnar (6/29), coxofemoral (5/29), costochondral (4/29), scapula-humerus (3/29), metacarpal-phalanx, interphalangeal and tibiotarsal (2/29 each), and carpal joint (1/29). The lesion was restricted to a single joint in 11/29 pigs, and the involvement of two or more joints was seen in 18/29.

Microscopically, there was marked necrosis of the synovial membrane, accompanied by fibrinosuppurative exudate, aggregates of bacterial cocci, and proliferation of fibrous connective tissue. Bacterial culture was obtained in thirteen cases (13/29), with identification of *Pasteurella multocida* type D (4/29), *Trueperella pyogenes* (4/29), *Staphylococcus* spp. (2/29), and *Streptococcus suis* (2/29).

Cases of spondylitis affected pigs in the final period of the finishing phase. The main clinical signs observed were paresis/paralysis of the pelvic limbs and inability to stand up. Lesions were observed in the lumbosacral (5/10) and thoracic (5/10) segments of the vertebral column. Concomitant suppurative lesions found in pigs with spondylitis included arthritis (5/10) and embolic pneumonia (3/10).

Gross lesions in cases of spondylitis consisted of a single focally extensive area of enlargement in the vertebral column, surrounded by a thick white and firm capsule, containing pus that involved and infiltrated the affected vertebrae. Microscopically, these lesions were

characterized by osteonecrosis, accompanied by fibrinosuppurative inflammation, coccoid bacterial aggregates, and proliferation of fibrovascular tissue in the surrounding area. This diagnostic group was characterized by chronic clinical course. Bacterial culture was obtained in two of the ten samples with isolation of *Staphylococcus* spp. and *Pasteurella multocida* (one case each).

Porcine Circovirus type 2 (PCV-2) associated disease

PCV-2 associated disease was diagnosed as the underlying cause for impaired ambulation in eight pigs (8/76), with the presence of two classic clinical syndromes, Porcine Dermatitis and Nephropathy Syndrome (PDNS) (6/8), and Post-weaning Multisystemic Wasting Syndrome (PMWS) (2/8). Affected pigs had subacute to chronic clinical signs, with prolonged lateral recumbency (4/8), muscle tremors (2/8), and ataxia (2/8).

Affected pigs had different combinations of lesions that are typical of the syndromes associated with PCV-2 infection, including granulomatous lymphadenitis, interstitial nephritis, and pneumonia. Additionally, in pigs with PCV-2 associated disease that were recumbent, inflammatory lesions were observed in the central nervous system (CNS) (8/8) and skeletal musculature (5/8). Neurologic lesions were characterized by non-suppurative meningoencephalomyelitis (4/8), and vasculitis involving mainly blood vessels of the neuropil and leptomeninges (8/8). The CNS inflammatory component in these cases was comprised by macrophages, lymphocytes, fewer multinucleated giant cells (3/8) eosinophils (1/8), and multifocal areas of gliosis (2/8). The inflammatory infiltrate often formed small nodular aggregates surrounding blood vessels.

Muscle changes in cases of PCV-2 associated disease were grossly characterized by pale areas interspersed with petechiae predominantly in muscle fascias (3/5), mainly in the pelvic (4/5) and thoracic limbs (3/5). Histologically, affected muscles had biphasic muscle necrosis of varying degrees, with intense vasculitis, hemorrhage, and inflammatory infiltrate of neutrophils, lymphocytes, macrophages, and multinucleated giant cells. Six samples sent to PCR for PCV-2 were positive (6/6). IHC for PCV-2 carried out using sections of CNS of affected pigs detected positive immunolabeling in only one case (positive immunolabeling in lymphocytes and endothelial cells) (1/8). IHC for PCV-2 conducted using sections of the muscle of affected pigs was negative in all cases (5/5). However, all cases of PCV-2 associated disease had positive results for IHC for PCV-2 in sections of lymph nodes (8/8).

Bone fractures

Pigs with bone fractures had clinical signs of acute or peracute onset, mainly characterized by paralysis of the pelvic limbs and inability to stand up. Pigs in this study had vertebral fractures (6/7), distributed in the lumbosacral segment (L5, L6, and sacrum) (3/6), followed by the thoracic segment (T7-T8 and T10) (2/6), and the lumbar segment (L2-L3) (1/6). In one case the fracture was observed in the humerus (1/7). Macroscopically, vertebral fractures were associated with marked subdural hemorrhage. Bone repair changes and hemorrhage were seen on histology. Additionally, the spinal cord had microscopic findings consistent with Wallerian degeneration in two cases, compatible with spinal cord compression secondary to bone fracture and subdural hemorrhage. Only subdural hemorrhage was observed in the remaining cases.

Suppurative Meningoencephalitis

Six pigs with suppurative meningoencephalitis of probable bacterial origin were assessed. The main clinical signs were motor incoordination, lateral recumbency, and convulsions, predominantly with acute evolution. Gross findings included leptomeningeal hyperemia and fibrin deposition in the brain (2/6), and the remaining organs were unremarkable. The main microscopic finding was fibrinosuppurative leptomeningitis with occasional coccoid bacterial aggregates. Bacterial isolation from CNS sample was successful in only one case, with the identification of *Streptococcus suis*.

Valvular endocarditis caused by *Streptococcus suis* was observed concomitantly with PCV-2 associated systemic disease in two cases of pigs diagnosticated with suppurative meningoencephalitis. One of these cases also presented a marked non-communicating hydrocephalus as a result of a bacterial fibrinosuppurative meningo-chorio-ependymitis.

Non-suppurative encephalomyelitis

Non-suppurative encephalomyelitis (NEM), with distinct lesions from cases of PCV2-associated neurologic disease was diagnosed in four cases. Pigs in this category had clinical signs of acute onset, and subacute to chronic clinical course, with manifestation of lateral decubitus, paralysis or tetraparesis, ataxia and convulsions. Gross lesions were absent. Microscopic findings included moderate lymphoplasmacytic perivascular infiltrate in several sections of the brain and meninges (4/4), in all segments of spinal cord (3/4) and choroid plexus (1/4), accompanied by multifocal gliosis (3/4) and neuronophagia (2/4). Lymphoplasmacytic ganglioneuritis with Wallerian degeneration and occasional neuronophagia were also observed in cases with spinal cord lesions. In the brain, the lesions were more prominent in the brainstem, while in the spinal cord, they were more prominent in the gray matter. Swabs of the meninges and/or samples of

cerebrospinal fluid were collected in all four cases, and no significant bacterial growth was cultured.

Fibrocartilaginous embolism

Three cases of CNS fibrocartilaginous embolism (FCE) were diagnosed. The first pig (pig 1) was found in lateral decubitus, with tetraparesis, and had severe lesions in the cervical segment of the spinal cord. The second pig (pig 2) with FCE was found dead but had significant skin abrasions, indicating recumbency prior to death. This pig presented uroperitoneum due to urinary bladder rupture, in addition to a focally extensive area of malacia in the lumbosacral region of the spinal cord. In the third case (pig 3), emboli were found in blood vessels of the brain and cerebellum. This pig had a previous history of fighting with other pigs the day before, and the animal developed persistent lateral decubitus, pedaling movements, convulsive episodes, and opisthotonus. All pigs diagnosed with this condition showed clinical signs of superacute or acute onset. Histopathology of the CNS revealed focal or multifocal areas of necrosis in segments of the spinal cord, especially affecting the gray matter (pigs 1 and 2) and in the cerebellum (pig 3). Wallerian degeneration was observed in the white matter (pigs 1 and 2). In all cases, light basophilic solid material was observed within the lumen of arterioles adjacent to affected areas, especially in the meninges. This material was paucicellular and resembled the nucleus pulposus of the intervertebral discs. This material was evidenced in Alcian Blue (AB) stain.

Epiphysiolysis

The diagnosis of epiphysiolysis (fracture of the proximal epiphysis of the femur) was made in three pigs. Fractures occurred in the femoral head bilaterally (2/3) and unilaterally (1/3) and the

pigs had severe lameness and persistent recumbency. The clinical course was subacute. Macroscopically, fracture and separation of the femoral head were observed, accompanied by thickening of the adjacent joint capsule by a white and firm tissue (fibrosis). Microscopically, a linear fracture separating the epiphyseal plate from the metaphysis was observed, accompanied by infiltration of neutrophils and macrophages.

Ascending bacterial myelitis/meningitis

Ascending bacterial myelitis/meningitis affected three pigs. The animals in this condition presented disease of subacute to chronic clinical course, always associated with tail-biting lesions and clinical signs of paresis/paralysis of the pelvic limbs. Macroscopically, the lesions in the tail were characterized by loss of the distal portion of tail, with crust formation and purulent content on the cut surface. The intradural space was filled with purulent material, which covered the leptomeninges and effaced partially the spinal cord parenchyma on a cut surface of the lumbosacral segment. Spinal cord histology revealed focally extensive areas of marked liquefactive necrosis affecting white and gray matter with suppurative myelitis, numerous aggregates of bacteria and proliferation of fibrous connective tissue in surrounding areas. From these cases, two swabs from the spinal cords were sent to bacteriological culture, but there was no bacterial growth.

Others

Diagnoses with two or fewer cases were grouped into the category "others". This group was comprised by one case of brain abscess, one case of bilateral symmetrical polioencephalitis of undetermined origin, and one case of eosinophilic meningoencephalitis compatible with water deprivation. No bacterial growth was observed in brain samples of these cases.

DISCUSSION

The term "downer pig" derives from "downer or downed sow syndrome". Non-ambulatory pigs, colloquially known as “downed pigs” or “downers”, occur as the clinical manifestation of numerous musculoskeletal and CNS conditions [1]. Major economic losses have been attributed to the occurrence of non-ambulatory pigs in the swine industry, with estimated losses of $54.91 per affected pig [5]. Additionally, non-ambulatory pigs represent an important issue regarding welfare standards in modern pig farms [5]. A significant number of pigs from our original study were categorized in the subset of non-ambulatory pigs, indicating the importance of this clinical manifestation as a cause of death/reason for euthanasia in the assessed pig farms.

In this study, fourteen different pathological entities were identified as the underlying disease of non-ambulatory pigs, highlighting the heterogeneity of possible etiologies for this clinical manifestation. Although numerous conditions were identified, only a few pathological entities represented the majority of cases, including chronic bacterial infections possibly occurring secondarily to tail biting lesions, systemic viral infections and primary CNS bacterial infections. The remaining conditions occurred less frequently and were considered sporadic.

Although non-ambulatory pigs are a common problem in pig farms, thorough necropsy-based studies assessing the underlying causes for naturally occurring cases are exceedingly scarce, specially for grower-finishers. Therefore, our results add to the current knowledge on this topic and represent a valuable resource for study. Additionally, the findings of this study support the appropriateness of a necropsy-based approach with targeted ancillary testing to investigate the underlying causes for impaired ambulation in pigs.

The majority of non-ambulatory pigs were submitted for necropsy at the end of the finishing phase (150-175 days), with a predominance of chronic diseases. This age-related predisposition may occur as a result of several factors, including the prolonged time necessary for a lesion to spread from a primary site (tail lesion, for example) to distant sites, culminating in systemic lesions and clinical signs [4]; the predisposition of heavier pigs to become recumbent and succumb due to locomotor lesions [7]; and the delay of farm employees to decide for euthanasia in cases with an unfavorable prognosis.

Tail biting lesions were common and significantly associated with cases of suppurative arthritis, suppurative spondylitis, and ascendant bacterial myelitis/meningitis in this study. Tail lesions occur with greater frequency in older pigs, and are linked with stressors, including overcrowding and competition for food [9], which contributes to the later onset of the diagnosed tail biting-related diseases. In addition to these conditions, tail biting lesions are linked to increased rates of carcass condemnation at the slaughterhouse due to secondary embolic bacterial lesions [10, 4]. Tail biting lesions are also an important indicator of animal welfare in pig farms [5]. Sex predisposition for the occurrence of tail biting lesions has been previously reported for barrows [11], however, no sex predisposition was inferred in this study.

Arthritis was the most common underlying cause for non-ambulatory pigs in this study. Stifle joints are usually the most commonly affected in cases of suppurative arthritis in older pigs [12, 13], which corroborates to findings of this work. *T. pyogenes*, *Staphylococcus* spp. and *Streptococcus* spp. have been reported as the most important bacteria involved in suppurative arthritis in pigs [14, 15]. Interestingly, *Pasteurella multocida* was isolated in four of our cases, which is considered less common. However, studies have pointed out the septicemic involvement of *P. multocida* in cases of fibrinosuppurative arthritis [16].

Pigs with suppurative spondylitis, ascending bacterial myelitis/meningitis, FCE and vertebral fracture had clinical signs consistent with a secondary spinal cord injury, manifested by compressive or direct lesions on lower motor neurons, which can cause bladder distension and paresis/paralysis of the pelvic limbs [17,18,19]. In this work, cases of urinary bladder rupture associated with ascending bacterial myelitis and FCE were observed (one case each). Bacterial culture from cases of suppurative spondylitis and ascending bacterial myelitis/meningitis failed to identify a microorganism in most cases in this study, which may have occurred due to the chronicity of the lesions and frequent treatment attempts using systemic antibiotic therapy [14].

Bone fractures were occasionally seen in this study and cases were likely sporadic, as no epidemiological or morphological evidence of any predisposing factor was found, such as metabolic bone diseases or history of electrical injury. Fights and interactions among pigs may predispose older and heavy pigs to bone fractures [2]. *Streptococcus suis* and *Glaesserella parassuis* are the bacteria most commonly associated with suppurative meningitis in nursing piglets and weaners [20, 21]. In this work, suppurative meningitis was observed in younger, recently housed pigs, which may be related to stressor factors associated with adaptation to a different environment and feeding [22].

PCV-2 associated disease is multifaceted and represent one of the most important conditions in the swine species. In this study, the disease frequently led pigs to recumbency, due to muscular and CNS injuries, which often occurred concomitantly. CNS lesions in cases of PCV-2 associated diseases are classically described in pigs with PDNS and PMWS [23]. Macroscopically, the typical lesion is characterized by multiple petechiae in the cerebellum, however the entire CNS may be affected including the spinal cord [24, 25, 26]. Alternatively, muscle changes are not commonly found [27]. Histologically, muscle lesions detected in our cases

were similar to those previously reported [28]. In this study, in addition to muscle necrosis, intense fibrinoid vascular degeneration was also observed. The lesions found in these cases may indicate a more acute clinical course when compared to previous descriptions [28], due to the large amount of neutrophilic infiltrate and vascular lesions. The absence of immunoreactivity in affected tissues may suggest that these changes could have occurred as a result of other mechanisms, including type III hypersensitivity reaction [29], without direct involvement of the virus in all systemic lesions [25, 28]

Viral diseases affecting the CNS are not common in the swine diagnostic routine, especially in growing-finishing pigs [30]. Possible causes of non-suppurative encephalomyelitis in pigs include Porcine Teschovirus type A (PTV) [31], Porcine Sapelovirus type A (PSV) [32], Porcine Astrovirus type 3 (PoAstV-3) [33], Suid herpesvirus 1 (SHV-1) [34], Porcine Circovirus type 2 (PCV-2) [35], Porcine Hemagglutinating Encephalitis virus (PHEV) [36], and Porcine Reproductive and Respiratory Syndrome virus (PRRSV) [37]. The findings observed in our cases are highly suggestive of infection by a neurotropic virus, such as PSV, PTV and PoAstV-3 [32, 33, 38]. Some of these viruses have been identified in fecal samples in Brazilian pig farms [39, 40], and more recently as a cause of clinical disease in a herd in southern Brazil [38]. In this study, no complementary tests were performed to determine the etiology involved in cases of non-suppurative encephalomyelitis.

Ischemic myelopathy due to FCE is a disease clinically characterized by acute, non-painful, and non-progressive neurological dysfunction. Sporadic reports of FCE in pigs have been documented leading to ischemic infarction in the spinal cord or brain [41, 42, 43, 44]. These present findings suggest that FCE may be more common than previously believed in pigs. The pathogenesis of FCE in animals is unclear, nevertheless, some factors may be related to the

occurrence of this condition, such as degeneration of the dorsal annulus of the intervertebral disc, herniation of the disc or extrusion of the nucleus pulposus, in addition to minor traumas in the region [45], persistence of embryonic remnant vessels within the nucleus pulposus [46], and discospondylitis [47]. At necropsy, no evidence of preceding injuries affecting the vertebral column and intervertebral discs were found in our cases. It is believed that the habit of fighting in pigs, as well as hyperstimulation of pigs during handling or transportation may be associated with small vascular traumas, predisposing pigs to FCE. In one of the cases of this study, FCE affected the cerebellum. This lesion distribution is not commonly observed in cases of FCE, with rare reports in veterinary literature [48]. In this case, it is suggested that the nucleus pulposus may have originated from the cervical segment of the vertebral column, and the material entered small arteries, and occluded the lumen of vertebral arteries retrogradely, similarly to previous descriptions [49, 42].

Epiphysiolysis affected pigs at the end of finishing phase. This disease usually occurs in gilts between four and eight months of age and consists of aseptic fracture of the femoral neck [50]. The cause has been associated with excessive tension in the hip joint due to excessive weight, which leads to fracture of the physeal region. Clinical lameness is usually severe and of sudden onset, which may be unilateral or bilateral as observed in the pigs of this study [51].

CONCLUSIONS

Numerous conditions leading to the clinical manifestation of non-ambulatory pigs were identified in growing-finishing farms in this study. Clinical cases occurred predominantly at the end of the finishing phase. Diagnosed entities included pathologies of the locomotor system (suppurative arthritis, epiphysiolysis, and fractures), CNS (suppurative meningoencephalitis and

non-suppurative encephalomyelitis, ascending bacterial myelitis, fibrocartilaginous embolism, brain abscess, polioencephalitis of non-determined origin, and eosinophilic meningoencephalitis), and also multisystemic pathologies such as PCV-2 associated diseases.

Tail biting lesions were an important predisposing factor and acted as the probable primary site of infection for cases of suppurative arthritis, spondylitis, and ascending bacterial myelitis. Neurologic and muscular lesions were common in cases of PCV-2 associated diseases, reinforcing the importance of this conditions as differential diagnosis in cases of non-ambulatory pigs. The necropsy examination is an essential tool to monitor the underlying causes of non-ambulatory pigs, generating essential information on infectious and non-infectious diseases affecting commercial herds. Information obtained from similar investigations is paramount to improve field diagnostic efforts, mitigate economic losses and improve animal welfare in the swine industry.

MATERIAL AND METHODS

A larger study conducted by our research group has previously assessed the causes of mortality in grower-finishers in two pig farms in Southern Brazil [8]. In this necropsy-based study, we evaluated 610 pigs, 76 (12.5%) of which were non-ambulatory pigs. Now, we further explore the postmortem examination findings of this subset of cases.

Postmortem examinations and sampling were carried out in two commercial pig farms (Farm A and B) located in the western region of the state of Santa Catarina, Brazil. Farm A and B were visited four times, each visit lasted 12 days, with eight visits in total. Both farms housed 70-day-old weaners, and pigs were transferred to sow farms or sent to slaughter by the end of the finishing phase (175 days of age). During the visits, necropsy procedures were carried out in all

pigs that died spontaneously or were euthanized by farm employees. A total of 610 pig necropsies were performed, and results have been published elsewhere [8].

In this study, we focus on the subset of cases of non-ambulatory pigs. All pigs included in this study had a history of impaired ambulation, including difficulty to stand up and walk and prolonged sternal or lateral recumbency, which was the primary cause of death or reason for euthanasia. Information provided by farm employees consisted of age, sex, and clinical history, including disease evolution, administered medications, and response to treatment in affected pigs. Body Condition Score (BCS) was inferred through visual evaluation. BCS was graded from 1 to 5; BCS 1 comprised cachectic pigs and BCS 5 comprised pigs with significant fat coverage [52]. The clinical evolution in each case was classified as peracute (0 to 24 h), acute (24 to 96 h), subacute (4 to 14 days), and chronic (> 14 days) [53].

Postmortem examinations were carried out, gross lesions were recorded, and organ fragments were systematically collected and fixed in 10% formalin solution. Subsequently, tissues were routinely processed for the preparation of histological slides, which were stained by hematoxylin and eosin (HE), in addition to Alcian blue stain in selected cases. When there was a history of decubitus, locomotor deficits, and/or neurological clinical signs, the necropsy procedure included an evaluation focused on the CNS and musculoskeletal systems. This evaluation comprised a detailed inspection of the appendicular and axial skeletons, the skeletal muscles of fore and hindlimbs, the appendicular synovial joints, the brain, and spinal cord, with subsequent collection of samples of these tissues for histology. When infectious agents were suspected to be involved in the lesions, fragments of organs and body fluids were collected, kept refrigerated, and sent within 24 hours for bacteriological, biochemical, and molecular examinations.

Samples sent for bacterial culture were inoculated in blood agar plates (BA) and MacConkey agar (MC) and incubated at 36°C +/- 1°C for 18-48 hours in an aerobic atmosphere [8]. Biochemical characterization of the colonies for the identification of bacteria was performed as previously described [54]. For the detection of Porcine Circovirus type 2 (PCV-2), pool samples of lymph nodes of six cases of PCV-2 associated diseases were submitted to PCR according to the methodology previously described [55]. Additionally, immunohistochemistry (IHC) was performed to detect PCV-2 antigens in selected sections of lymph nodes, skeletal muscle, and CNS in suspect cases, according to the methodology previously described [56].

The proportion of pigs diagnosed with a tail-biting related disease was compared between two categories: pigs with tail-biting lesions (yes) and pigs with no tail-biting lesions (no). Tail-biting related diseases encompassed cases of suppurative arthritis, suppurative spondylitis, and ascending bacterial myelitis/meningitis. The proportion of pigs with tail-biting lesions was also compared between sex categories (barrows or females). The comparison used Pearson's Chi-square test, except if the variables showed an expected number of observations less than 5 in 25% of the cells of the contingency table, then Fisher's Exact test was used instead.

The odds ratio (OR) and confidence interval (95% CI) of tail-biting-related diseases were calculated from the odds that a case (with tail-biting lesions) was exposed to the odds that a control (without tail-biting lesion) was exposed using logistic regression. The significance level chosen was 5% for all hypothesis tests. Statistical analysis was performed using commercial software.

Acknowledgements

We greatly acknowledge the farmers and Seara Alimentos LTDA for sharing their data and allowing the conduction of this study.

Authors' contributions

Manoela Marchezan Piva: Study design, necropsy and histopathological exams, data collection and analysis, manuscript writing. Claiton I. Schwertz, Luan Cleber Henker, Ronaldo Michel Bianchi, Regina Tose Kemper: Necropsy and histopathological exams and manuscript writing. Bruno Albuquerque de Almeida: statistical analysis and writing review. Ricardo Yuiti Nagae, Taís Regina Michaelsen: selecting farm for the conduction of the study, microbiological examinations, writing review. Saulo Petinatti Pavarini: research coordination and writing review. All authors reviewed, edited and approved the final manuscript.

Funding

Financial support was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)—Finance Code 001, Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS), and Pró-reitoria de Pesquisa da Universidade Federal do Rio Grande do Sul (Propesq/UFRGS).

Availability of data and materials

Besides the presented data, raw data can be shared upon reasonable request by contacting the corresponding author and will require prior acceptance from herd owners.

Ethics approval and consent to participate

The research project that gave rise to the present data was approved by the Research Committee (COMPESQ) of the Federal University of Rio Grande do Sul (UFRGS) (Project number 40376). The manuscript does not contain clinical studies or patient data.

Consent for publication

Not applicable.

Competing interest

The authors declared no competing interests.

Abbreviations

Alcian Blue stain (AB)

Blood agar plates (BA)

Body Condition Score (BCS)

Central Nervous System (CNS)

Fibrocartilaginous Embolism (FCE)

Porcine Hemagglutinating Encephalomyelitis Virus (PHEV)

Immunohistochemistry (IHC)

MacConkey agar (MC)

Non-suppurative encephalomyelitis (NEM)

Odds ratio (OR)

Porcine Astrovirus type 3 (PoAstV-3)

Porcine Circovirus type 2 (PCV-2)

Porcine Dermatitis and Nephropathy Syndrome (PDNS)

Porcine Reproductive and Respiratory Syndrome virus (PRRSV)

Porcine Sapelovirus type A (PSV)

Porcine Teschovirus type A (PTV)

Post-weaning Multisystemic Wasting Syndrome (PMWS)

Suid herpesvirus 1 (SHV-1)

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ARTIGO 2

"***Pasteurella multocida* polyserositis in growing-finishing pigs**", o qual foi publicado na revista *Journal of Comparative Pathology*, https://doi.org/10.1016/j.jcpa.2023.03.003.

Infectious disease

Running head: *M.M. Piva, C.I. Schwertz, R.M. Bianchi et al.*

Pasteurella multocida polyserositis in growing–finishing pigs

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ARTICLE INFO

Keywords:

pneumonia

septicaemia

serositis

swine pathology

ABSTRACT

Pasteurella multocida is the main secondary bacterium isolated from cases of swine pneumonia. Although highly pathogenic strains of *P. multocida* have been associated with primary septic lesions and polyserositis in pigs, studies on this pathological presentation in naturally occurring cases are limited. The aim of this work was to characterize the clinical, pathological and molecular findings in cases of *P. multocida* polyserositis in growing–finishing pigs in a commercial farm in Brazil. The mean age of 17 investigated pigs was 120 days. Clinically, the disease was acute (11/17), with clinical signs of dyspnoea and apathy. Sudden death occurred in some animals (6/17). The main gross findings included fibrinous serositis affecting the abdominal and thoracic cavities (17/17), fibrinous pericarditis (15/17), marked cranioventral pulmonary consolidation (17/17) and splenic infarcts (3/17). *P. multocida* was isolated in all cases from systemic sites, including the pericardial sac and abdominal exudate. Molecular typing of genus and species was performed on four isolates, and all were characterized as *P. multocida* type A. Another five isolates were positive

for the pathogenicity marker gene *pfh*A by polymerase chain reaction. This study reinforces the role of *P. multocida* as a cause of polyserositis in growing–finishing pigs.

1. Introduction

Pasteurella multocida is an aerobic, non-motile, non-spore-forming, bipolar, gram-negative coccobacillus of the family Pasteurellaceae that causes disease in several species, including ruminants, rabbits, chickens and pigs. *P. multocida* commonly causes an acute bacterial disease characterized by bronchopneumonia, septicaemia and sudden death, usually associated with stressors and concomitant infection by respiratory viruses or mycoplasma (Caswell and Williams, 2016). In pigs, *P. multocida* is considered to be a major pathogen of the respiratory tract and is the main secondary bacterial agent isolated from cases of swine pneumonia (Morés *et al*, 2015; De Conti *et al*, 2021).

In recent years, highly pathogenic strains of *P. multocida* have been described as possible primary agents causing pneumonia and septicaemia in pigs (Cappuccio *et al*, 2004; Kich *et al*, 2007; Pors *et al*, 2011; Oliveira Filho *et al*, 2015; Paladino *et al*, 2017) and the *pfhA* gene was reported to be a good marker for these strains (Oliveira Filho et al, 2015). However, studies describing naturally occurring cases of swine polyserositis associated with highly pathogenic strains of *P. multocida* are scarce. The aim of this work was to characterize the clinical, pathological, bacteriological and molecular aspects of polyserositis due to *P. multocida* in growing–finishing pigs.

2. Materials and methods

Data from a previous necropsy-based study on causes of death in growing–finishing pigs in Southern Brazil were used. The original work was carried out in two mechanized pig farms in the western region of the State of Santa Catarina, Southern Brazil, in which mortality had been monitored during 2018 and 2019 (Piva *et al*, 2020). Four visits were made to each farm, with a duration of 12 days each visit, for post-mortem examination of all pigs that died during the period of the visits. A subset of 17 cases of polyserositis due to *P. multocida* was further analysed. Cases meeting the following criteria were included: (1) a combination of gross findings of fibrinous pleuritis, pericarditis and peritonitis; and (2) isolation of *P. multocida* from multiple sites, including exudates from different body cavities.

The farm on which cases occurred was a Certified Suidae Breeder Farm focused primarily on raising and selecting replacement gilts. This farm had up to 18,000 pigs housed in eight sheds. Piglets (male and females), originally from a single different pig farm, were received at 70 days of age. Non-selected gilts and all males were sent to slaughter at 175 days of age (125 kg). Selected replacement gilts were sent to destination farms at 140 days of age. Males and females were kept in separate facilities, received water and feed *ad libitum*. Monthly mortality rates during the period of study ranged from 0.9 to 1.54%. All pigs were vaccinated with commercial inactivated vaccines against porcine circovirus type 2 and *Mycoplasma hyopneumoniae* (days 21 and 42 of age) (Porcilis® PCV M Hyo, MSD Animal Health, www.msd-saude-animal.com.br/produto/porcilis-pcv-m-hyo) and with autogenous vaccines against *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica* and *P. multocida* types A and D (days 42 and 63 of age) (Autogenous vaccines, MicroVet, www.microvet.com.br).

At the time of necropsy, information was collected for each animal, including age, sex, clinical history and previous treatments carried out by farm employees. Post-mortem examinations were routinely conducted, and gross changes were recorded. All lungs were evaluated according to the Pneumonia Severity Index (PSI) in order to quantify the pulmonary lesions through a validated methodology (Piffer and Brito, 1991). Tissue samples of the main organs were collected, fixed in 10% buffered formalin solution and routinely processed for the preparation of histological slides, which were stained by haematoxylin and eosin (HE).

Bacterial culture was performed on all cases. Lung samples and, when present, fibrinous exudates from the abdominal, thoracic and pericardial cavities, synovial fluid and cerebrospinal fluid were aseptically collected, kept refrigerated and sent to a support laboratory for bacteriological and biochemical examinations within 24 h. Samples were cultured on blood agar and MacConkey agar and incubated at 36°C ± 1°C for 18–48 h in aerobic atmosphere. They were also cultured on a second blood agar plate striated with a *Staphylococcus hyicus* colony supplemented with nicotinamide adenine dinucleotide (NAD) and incubated microaerophilically at 36°C ± 1°C for 24–48h, enabling the growth of *Glaesserella parasuis* and *A. pleuropneumoniae*. Biochemical characterization of the colonies was performed for the identification of the isolated bacteria (Quinn *et al*, 2011).

For further molecular analysis, lung samples and body cavity exudates were collected and kept frozen at –20°C. Some of these samples (n = 9) were subjected to DNA extraction using the DNeasy Blood and Tissue Kit (Qiagen, www.qiagen.com) and others (n = 8) were extracted with MagMAX CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific, www.thermofisher.com). Of the DNA samples extracted with the Qiagen kit, four were submitted to multiplex polymerase chain

reaction (PCR) (Townsend et al, 2001) to search for *P. multocida kmt1* gene and to detect capsular typing A (*hya*D-*hya*C) and D (*dcb*F) genes and the other five underwent PCR for detection of the *pfh*A pathogenicity gene (Ewers et al, 2006). The remaining samples were not tested by PCR due to lack of viable genetic material or because frozen samples were no longer available.

Lungs samples from all cases were submitted to immunohistochemistry (IHC) for detection of the main primary agents associated with swine pneumonia in Brazil (*M. hyopmneumoniae* and influenza A virus), and lymph node samples from all cases for porcine circovirus type 2 (PCV-2). A polyclonal monospecific recombinant antibody against lactate dehydrogenase protein (p36) (dilution 1:800) was used to detect *M. hyopneumoniae* as described (Castro et al., 2009). A non-commercial antibody (dilution 1:1000) was used for PCV-2, with a modification of a previously described technique (Sorden et al., 1999). Monoclonal antibodies against the nucleoprotein (clones A1, A3 Blend, dilution 1:1000; Sigma-Aldrich, www.sigmaaldrich.com) was used for detection of influenza A virus, using a modification of a previously described method (Vicent et al., 1997). The IHC procedure was standardized by MACH 4 Universal HRP-Polymer Detection System (Biocare Medical, https://biocare.net)). The final labelling was performed with the Romulin AEC Chromogen Kit (BioCare Medical). For positive controls for *M. hyopmneumoniae*, influenza A virus and PCV-2, lung tissue from positive pigs for the respective agents were used. For negative controls, primary antibodies were replaced by Universal Negative Control Serum (BioCare Medical) in the lung sections tested.

3. Results

Seventeen cases with a diagnosis of polyserositis due to *P. multocida* were included. The age of affected pigs ranged from 93 to 150 days (mean of 120 days and median of 125 days) (Table 1). Males (11/17) and females (6/17) were affected. Eleven pigs died acutely with a clinical course from the onset of clinical signs until death ranging from 1–3 days. Clinical signs reported in these pigs included severe dyspnoea (7/17), apathy (4/17), cyanotic skin (2/17) and emaciation (2/17). These pigs were often treated with antibiotic therapy before death (7/17). In 6/17 cases, pigs were found dead, with no reported clinical signs or history of antibiotic treatment in the days preceding death.

Macroscopically, the serous surfaces of multiple organs were covered by deposits of white to yellow fibrinous material, accompanied by variable amounts of free citrus-yellow liquid, which filled the affected body cavities. These changes varied from moderate (11/17) to marked (6/17) and were seen in the thoracic cavity, affecting the parietal and visceral pleural surfaces (17/17), in the peritoneum (17/17) and on the visceral and parietal pericardium (15/17). In some cases, multifocal fibrin adhesions connected the visceral and parietal pleurae and multiple organs within the abdominal cavity.

In the lungs, intense cranioventral consolidation was seen in all cases, sometimes extending to the caudal lobes, corresponding to an average of 41.8% of the total lung area. Consolidated areas were purple–red, firm and the cut surface revealed multifocal to coalescing 0.5–5 cm white areas of necrosis (11/17), which were occasionally surrounded by a small amount of firm, white fibrotic tissue (3/17). The interlobular septa were sometimes expanded by yellowish gelatinous oedema fluid and fibrin. Blackened and reddish areas were seen on the cut surface of the parenchyma, and a large amount of serosanguineous oedema fluid oozed out.

In three cases (cases 2, 6 and 7) there was diffuse splenomegaly, accompanied by focal (cases 2 and 6) or multifocal (case 7), well-demarcated dark-red and firm nodular infarcts (2–3 cm), which protruded from the cut surface. The splenic capsule was covered by fibrin in these areas.

Microscopically, fibrinosuppurative polyserositis was characterized by marked thickening and fibrin deposition, admixed with intense infiltrates of degenerated neutrophils, lymphocytes and fewer macrophages, on the visceral and parietal pleura, pericardium and peritoneum. Basophilic coccobacillary aggregates were sometimes associated with serosal inflammation. Microscopically, lung lesions had two morphological patterns: necrosuppurative (11/17) and bronchosuppurative (6/17). The necrosuppurative pattern corresponded to multifocal to coalescent areas of coagulative necrosis, surrounded by a rim of intense infiltration of elongated degenerated neutrophils (oat cells), lymphocytes and macrophages admixed with fibrin, oedema fluid and haemorrhage and sometimes proliferation of fibrous connective tissue. Basophilic coccobacillary aggregates were sometimes seen within necrotic areas. Within the pulmonary parenchyma, septa and pleura, frequent vascular lesions were observed, including fibrinoid vascular degeneration and thrombosis. The adjacent lung parenchyma was atelectatic, infiltrated with a similar inflammatory component and had alveolar septal thickening due to interstitial inflammation associated with proliferation of type II pneumocytes. Interlobular septa and the pleura were expanded by moderate fibrin deposition and infiltrates of neutrophils, lymphocytes, plasma cells and macrophages. Similar inflammatory and vascular changes were seen in the bronchosuppurative pattern of pueumonia but necrotic areas were not present.

In 13/17 cases, lesions compatible with infection by influenza A virus were also presentand were characterized by multifocal necrotizing bronchiolitis, with associated peribronchiolar

lymphoplasmacytic inflammation. Proliferation of fibrous connective tissue that resulted in obliterating bronchiolitis was considered as chronic sequelae of influenza virus infection. In 5/17 cases, mild multifocal hyperplasia of bronchi-associated lymphoid tissue was also seen.

Splenic nodules corresponded to areas of infarction characterized by coagulative necrosis of the parenchyma, with intense haemorrhage, neutrophilic infiltration, fibrin deposition, multifocal thrombosis and coccobacillary aggregates. In 4/17 cases, lesions of leptomeningitis were present and characterized by mild expansion of the leptomeninges and multifocal perivascular inflammatory cell infiltrates of neutrophils, lymphocytes and rare macrophages.

P. multocida was isolated from all 17 cases, with two or more samples being taken from each pig. The four PCRs for species-specific and capsular typing confirmed *P. mutocida* type A. *PfhA* gene in all five evaluated samples. Bacterial culture of all cases resulted in a single isolate of *P. multocida*, but no growth of other bacteria such as *Streptococcus suis*, which could cause similar lesions (Gottschalk and Segura, 2000). Microaerophilic bacterial isolation striated with *Staphylococcus hyicus* colony supplemented with NAD was negative for griwth of bacteria such as *Glaeserella parasuis* and *A. pleuropneumoniae* in all cases.

Immunohistochemistry (IHC) resulted in labelling of *M. hyopneumoniae* in five cases (5/17): two cases had moderate and multifocal and trhee had discrete multifocal immunolabelling within bronchi and bronchioles and on the surface of the respiratory epithelium. IHC for influenza A virus and PCV 2 was negative in all cases.

4. Discussion

The macroscopic, microscopic and bacteriological findings confirmed the diagnosis of polyserositis due to *P. multocida* in the growing–finishing pigs in this study. The tested isolates were positive for capsular type A and *pfhA* gene, which is characteristic of highly pathogenic strains of *P. multocida. P. multocida* is the most important secondary and opportunistic bacterial pathogen of the respiratory tract of pigs and causes suppurative bronchopneumonia mainly related to co-infections with *M. hyopneumoniae* and influenza A virus (Hansen *et al,* 2010; Morés *et al,* 2015; De Conti *et al,* 2021). Nevertheless, highly pathogenic strains of *P. multocida* type A, as in this study, have been reported to cause primary lesions of necrohaemorrhagic pneumonia, pleuritis and fibrinous pericarditis (Cappuccio *et al,* 2004; Kich *et al,* 2007; Pors *et al,* 201; Oliveira Filho *et al,* 2015; Paladino *et al,* 2017). Experimental infections resulted in production of polyserositis in pigs inoculated with *P. multocida* strains isolated from pleuropneumonia outbreaks in Brazilian farms (Oliveira Filho *et al,* 2015). Interestingly, polyserositis caused by *P. multocida* in pigs has not been well-documented in naturally occurring cases.

In our cases, polyserositis was invariably accompanied by typical *P. multocida* lung lesions, suggesting that serosal and systemic dissemination occured secondarily to pneumonia in these cases. After colonizing the lungs and causing pulmonary lesions, *P. multocida* gains entry to the bloodstream, leading to fibrinous pleuritis, pericarditis and peritonitis. Interestingly, this pathology becomes an important differential diagnosis for other bacteria that cause polyserositis in pigs (eg, *G. parasuis*, *S. suis* and *Mycoplasma hyorhinis*) (Zheng *et al,* 2009; Palzer *et al,* 2015). Generally, these bacteria do not produce the pattern of lung injury observed in this study, and normally affect younger animals. In addition to fibrinous serositis, changes indicative of septicaemia may be observed in other organs (Oliveira Filho *et al,* 2015). In our study, splenic infarctions were identified in three cases, which was most likely caused by bacterial spread via the haematogenous

route, following the intense vascular injury and thrombosis in the lung. When there are splenic infarcts, *P. multocida* is frequently isolated from spleen infarcts fragments (Oliveira Filho *et al,* 2015).

The clinical course was predominantly acute in our cases, which is supported by the acute nature of the histological lesions. The presence of extensive parenchymal necrosis and associated vascular injuries (fibrinoid vascular necrosis and thrombosis) in the lungs resulted in intense oedema and respiratory distress, leading to death. Pulmonary vascular lesions probably facilitated concomitant septicaemia and bacterial colonization in extrapulmonary sites. Since *P. multocida* infection is commonly diagnosed in cases of swine pneumonia, isolation of this organism from samples of various exudates and extrapulmonary tissues is of paramount importance in making an accurate diagnosis of polyserositis/systemic infection caused by this agent (Oliveira Filho *et al,* 2015).

Many species of bacteria in the family Pasteurellaceae (eg, *A. pleuropneumoniae, Actinobacillus suis* and *Mannheimia haemolytica*) can produce lung lesions similar to those described in cases of *P. multocida*-associated pneumonia (Rice *et al,* 2007; Gómez-Laguna *et al,* 2014; Caswell and Williams, 2016). Typically, in pneumonic mannhemiosis in cattle and *A. pleuropneumoniae* infection in pigs, large and irregular areas of coagulative necrosis become surrounded by a rim of elongated degenerated neutrophils (‘oat cells’), mixed with necrotic alveolar macrophages, necrotic vasculitis and thrombosis, as seen in this study and in experimental infections by *P. multocida* (Oliveira Filho *et al,* 2015; Paladino *et al,* 2017). These lesions are possibly related to similar toxins produced by these bacteria, such as leukotoxin and ToxA, which

may be involved in the pathogenesis of the parenchymal necrosis and vascular lesions (Oliveira Filho *et al*, 2015; Sahoo *et al*, 2020).

Although the histological lesions have similarities, macroscopically, the characteristic lesion caused by *A. pleuropneumoniae* infection in pigs is a focal dorsocaudal necrohaemorrhagic area, especially in the caudal lobes, associated with pleuritis (Gómez-Laguna *et al*, 2014). In contrast, infection with *P. multocida* usually results in intense cranioventral consolidation, which may be associated with pleuritis. (Similar lung lesions may occur in ruminants infected with *M. haemolytica*, which does not produce polyserositis). Although these macroscopic differences are generally reliablet the differential diagnosis in such swine cases needs to be confirmed by bacterial isolation, as was done in this study, agent identification by IHC or molecular tests.

The filamentous haemagglutinin-encoding (*pfhA*), haemoglobin-binding (*hgbB*) and dermonecrotic toxin (*toxA*) genes are the most important reported pathogenicity markers of *P. multocida*. These virulence genes are related to the ability of bacteria to colonize and cause lung damage (Ewers *et al*, 2006). *PfhA* encodes an adhesin responsible for bacterial adherence and colonization of the upper respiratory tract. This gene is commonly detected in *P. multocida* strains considered to be of high clinical pathogenicity in Brazil (Harper *et al*, 2006). *PfhA* was identified by PCR in all tested samples in this study, similar to the findings of experimental infection of highly pathogenic *P. multocida* type A in Brazilian pigs (Oliveira Filho *et al*, 2015).

Several studies on *P. multocida*-associated severe pneumonia and experimentally reproduced polyserositis were associated with capsular type A and strains positive for the *pfhA* gene (Ono *et al*, 2003; Paladino *et al*, 2017; Oliveira Filho *et al*, 2018), as in our cases. These genetic alterations may be a consequence of the horizontal transfer of genes or the independent evolution of different

lineages of isolates (Davies *et al,* 2003; Bethe *et al,* 2009). Expression of the *pfhA* gene has been associated with an increased ability of *P. multocida* type A to act as a primary pathogen, causing pneumonia and septicaemia in the absence of predisposing factors. In our study, 13 cases had lesions of necrotizing/obliterating bronchiolitis compatible with influenza A virus infection, and the lungs of five cases were positive for *M. hyopneumoniae*. Therefore, in these cases, it is possible that interactions with these important primary agents have potentiated *P. multocida* infection.

M. hyopneumoniae and influenza A virus probably played important pathogenetic roles as co-factors in this study. Although the immunohistochemical techniques used are highly sensitive for these agents (Castro *et al*, 2009; Vicent *et al*, 1997), the negative immunolabelling for influenza A virus may be related to the rapid passage of the virus in the respiratory tract (up to 96 h after infection), leaving only compatible histological lesions. In the case of *M. hyopneumoniae,* infection is chronic and immunolabelling in finishing pigs tends to be frequent, as found in this study (13/17). In only two cases in this study, was no interaction between *P. multocida* infection and primary porcine respiratory complex agents detected by histology or IHC. As immunolabelling for *M. hyopneumoniae* is usually multifocal in bronchi and bronchioles, false negative results may be obtained when only small sections of lung tissue are evaluated. Therefore, *M. hyopneumoniae* infection cannot be excluded in these animals as they were present in an infected herd. As there were only two cases with no identified co-infections, there is insufficient evidence to conclude that *P. multocida* acted as a primary agent in these cases.

The limitations of this study included the unavailability of information on the capsular type and pathogenicity factors for most of the *P. multocida* isolates identified. Also, specific PCR tests were not performed to carry out a differential diagnosis for *M. hyorhinis*. Successful testing was

precluded due to the conditions in which the samples were stored and prolonged storage prior to testing.

5. Conclusion

This study reinforces the association of highly pathogenic strains of *P. multocida* with mortality in pigs. In cases where identification was possible, all *P. multocida* isolates were positive for capsular type A and the *pfhA* virulence gene. *P. multocida* must, therefore, be included as a differential diagnosis in cases of fibrinous polyserositis with concomitant severe pneumonia in growing–finishing pigs.

Funding

The work was developed and financed with the support of the National Council for Scientific and Technological Development (CNPq) and Seara Alimentos Ltda.

Acknowledgments

The authors thank INATA Produtos Biológicos Ltda for their help with immunohistochemistry.

Declaration of competing interests

The authors declared no conflicts of interest in relation to the research, authorship or publication of this article.

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Table 1

Polymerase chain reaction identification of virulence gene and capsular type of *Pasteurella multocida* isolates from cases of polyserositis in growing–finishing pigs

Case no.	**Sex**	**Age (days)**	***P. multocida* isolation sites (tissues and exudates*)**	**PCR *pfhA***	**PCR capsular type (A or D)**
1	F	100	Lung, thorax abdomen, and CSF	Positive	-
2	M	130	Lung, thorax and abdomen	Positive	-
3	M	150	Lung and thorax	-	Type A
4	M	150	Lung, abdomen and CSF	-	Type A
5	M	150	Lung and thorax	-	-
6	M	100	Lung, thorax, abdomen and pericardium	-	-
7	F	100	Lung, thorax, abdomen and CSF	-	-
8	F	100	Lung, thorax and abdomen	-	-
9	F	100	Lung, thorax, abdomen and pericardium	-	-
10	M	140	Lung, thorax, abdomen and pericardium	-	-
11	M	135	Lung, abdomen and pericardium	-	-
12	M	125	Lung, thorax, abdomen and pericardium	-	-

13	F	113	Lung, thorax, pericardium and spleen	-	Type A
14	M	125	Lung, thorax and abdomen	-	Type A
15	M	93	Thorax and abdomen	Positive	-
16	M	93	Lung, thorax, abdomen, pericardium and meninges	Positive	-
17	F	140	Thorax, abdomen and pericardium	Positive	-

-, not performed; M, male; F, female; CSF, cerebrospinal fluid, PCR, polymerase chain reaction.

*, thoracic, abdominal and pericardial exudates were used for bacterial isolation.

ARTIGO 3

“**Outbreak of toxoplasmosis associated with muscular lesions in finishing pigs caused by an atypical *Toxoplasma gondii* genotype”,** o qual foi publicado na revista *Revista Brasileira de Parasitologia Veterinária*, https://doi.org/10.1590/S1984-29612022055.

Outbreak of toxoplasmosis associated with muscular lesions in finishing pigs caused by an atypical *Toxoplasma gondii* genotype

Surto de toxoplasmose associado a lesões musculares em suínos de terminação causado por um genótipo atípico de *T. gondii*

Short title: Outbreak of toxoplasmosis in finishing pigs

Abstract: *Toxoplasma gondii* infections are usually asymptomatic in pigs, and an acute clinical disease is rare in this host. This study aimed to determine the pathological and molecular aspects of an outbreak of fatal systemic toxoplasmosis in finishing pigs in Brazil. The outbreak occurred on a commercial finishing pig farm in the state of Santa Catarina in southern Brazil. The farm had 1500 pigs and 3.8 % of mortality rate during the outbreak. The pigs had fever, anorexia, apathy, and locomotor deficits. Seven pigs were necropsied. Gross findings included multifocal to coalescent pale areas in skeletal muscles, lymphadenomegaly, hepatosplenomegaly, and non-colapsed lungs. The histological findings included granulomatous lymphadenitis, hepatitis and splenitis, myositis necrotizing, and lymphoplasmacytic interstitial pneumonia. Lung and liver lesions were occasionally accompanied by *T. gondii* parasitic structures. Positive immunolabeling for *T. gondii* tachyzoites and encysted bradyzoites was detected in all examined pigs. PCR-RFLP (11 markers) and microsatellite analysis (15 markers) identified the non-archetypal genotype #278 in pigs. This is the first report of systemic toxoplasmosis in pigs with muscle lesions and additionally shows the diversity of disease-causing *T. gondii* genotypes circulating in animals in Brazil.

Keywords: Swine diseases, *Toxoplasma gondii*, myositis, genotyping, PCR-RFLP, microsatellite analysis.

Resumo: As infecções por *Toxoplasma gondii* são geralmente assintomáticas em suínos, e uma doença clínica aguda é rara nessa espécie. Este estudo teve como objetivo determinar os aspectos patológicos e moleculares de um surto de toxoplasmose sistêmica fatal em suínos em terminação no Brasil. O surto ocorreu em uma granja comercial de suínos em terminação no estado de Santa Catarina, no sul do Brasil. A granja tinha 1500 suínos e a

taxa de mortalidade durante o surto foi de 3.8 %. Os suínos apresentaram febre, anorexia, apatia e déficits locomotores. Sete suínos foram necropsiados. Os achados macroscópicos incluíram áreas pálidas multifocais a coalescentes nos músculos esqueléticos, linfadenomegalia, hepatoesplenomegalia e pulmões não colapsados. Os achados histológicos incluíram linfadenite, hepatite, esplenite granulomatosa e miosite necrosante, assim como pneumonia intersticial linfoplasmocítica. Lesões pulmonares e hepáticas foram ocasionalmente acompanhadas por estruturas parasitárias de *T. gondii.* A imunomarcação positiva para taquizoítos e bradizoítos encistados de *T. gondii* foi observada em todos os suínos examinados. PCR-RFLP (11 marcadores) e análise de microssatélites (15 marcadores) identificaram o genótipo não arquetípico #278 em suínos. Este é o primeiro relato de toxoplasmose sistêmica em suínos com lesões musculares e, adicionalmente, demonstra a diversidade de genótipos de *T. gondii* causadores de doenças circulantes em animais no Brasil.

Palavras-chave: Doenças dos suínos, *Toxoplasma gondii*, miosite, genotipagem, PCR-RFLP, análise de microssatélites.

Introduction

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*, an obligate intracellular protozoan. Domestic and wild cats are the definitive hosts that excrete oocysts into the environment, with other species of mammals, including humans, acting as intermediate hosts (Tenter et al., 2000). Infections occur after the ingestion of food or water contaminated with oocysts or through the consumption of uncooked or undercooked meat containing tissue cysts, which usually results in subclinical disease in immunocompetent hosts (Dubey & Jones 2008; Dubey, 2008; Dubey & Lappin, 2011).

Central and South America are major hotspots for *T. gondii* genetic diversity, with almost 200 genotypes having been identified in the region. In contrast, North America, Europe, and Asia have fewer circulating genotypes, with a predominance of classical types II and III (Shwab et al., 2014). This genetic diversity can be associated with more severe forms of human toxoplasmosis; however, this association is unclear in animal hosts (Carme et al., 2009). Pork consumption may be an important source of *T. gondii* infection in humans (Feitosa et al., 2014). Acute toxoplasmosis in pigs is rare, and cases have been reported only in neonates (Dubey et al., 1990; Thiptara et al., 2006) and weaners (Liao et

al., 2006; Klein et al., 2010). This study aimed to present the pathological and molecular aspects of an outbreak of fatal toxoplasmosis with muscular lesions in a herd of growing-finishing pigs caused by an atypical *T. gondii* genotype infection.

Material and methods

Farm description

The farm had approximately 280 sows and 1500 growing-finishing pigs. The grower-finishers were housed in shared pens (1 m² per pig) in pig-sheds with sidewall curtains. The pens were separated by compact walls with a compact floor, pacifier-type drinking fountains that formed the water depth, and automatic feeders. There was no perimeter fence surrounding pig-sheds to prevent close contact between the housed pigs and other animals (domestic and wild). Domestic cats were raised by the producers and had free access to the swine sheds. All pigs were fed commercial diets, and the water provided to them was obtained from an artisanal well. The pigs were vaccinated against *Mycoplasma hyopneumoniae*, *Glaesserella parasuis* (autogenous vaccine), and porcine circovirus type 2 (two doses at 15 and 35 days of age).

Sampling and histopathology analysis

The outbreak of pig mortality occurred on a farm located in the municipality of Nova Veneza in Santa Catarina state (SC), in the southern region of Brazil (S-28.636908359871796, W-49.50109523771801). During the outbreak, one pig that died and six euthanized because of a poor prognosis were subjected to necropsy. Fragments of the main organs of the thoracic and abdominal cavities and samples of the skeletal muscles, brain, and spinal cord were collected. These fragments were fixed in 10% buffered formalin solution, routinely processed for histopathology, and stained with hematoxylin and eosin (HE). Additionally, fresh samples of several tissues were collected and stored at -20°C for subsequent molecular analysis.

Immunohistochemistry

Immunohistochemistry (IHC) for *T. gondii* was performed on selected sections of the lymph nodes, skeletal muscle, brain, and lungs from all pigs (7/7). For antigen retrieval, samples were incubated for 10 min with a polyclonal antibody (VRMD, Pullman, WA,

USA) at a 1:1000 dilution with 0.1% trypsin. A modified avidin-biotin-peroxidase complex method (LSAB Universal kit, Dako Cytomation, Glostrup, Denmark) was employed using 3-amino-9-tilcarbazoln (AEC, K3469, Dako Cytomation, Glostrup, Denmark) as the chromogen. Brain sections from a case of *T. gondii* encephalitis in a dog were used as positive controls as previously described by do Nascimento et al. (2017). Sections of the skeletal muscle and lymph nodes from all cases were subjected to IHC anti- porcine circovirus type 2 (PCV2) with a polyclonal antibody, as previously described (Corrêa et al., 2007). Sections of lymph nodes from a pig with circoviruses were used as positive controls. Primary antibodies were replaced with a universal negative control serum (BioCare Medical, CA, USA) in selected sections as negative controls for both tests (*Toxoplasma gondii* and PCV-2).

Molecular analyzes

DNA extraction from the tissues (lymph nodes, liver, lungs, spleen, muscle, or blood) of six pigs was performed using a Dneasy® Blood & Tissue commercial kit (Qiagen® Inc., USA) according to the manufacturer's protocols. Polymerase chain reaction (PCR) amplification of *T. gondii* was performed as described by Homan et al. (2000), using a 529 base pair (bp) repeat element (REP529) fragment as a target; DNA from the RH *T. gondii* reference strain was used as a positive control. The amplified DNA was visualized by electrophoresis on 2% agarose gels stained with SYBR® Safe DNA gel stain (Invitrogen, USA). Negative controls (ultrapure water) were included in all PCR reactions.

Three of the four *T. gondii* positive PCR samples were then genotyped by multilocus PCR Restriction Fragment Length Polymorphism (PCR-RFLP), compared and classified according to other previously characterized *T. gondii* Brazilian strains available in the ToxoDB database (http://toxodb.org/toxo/) and recent publications.

PCR-RFLP was performed as described by Su et al. (2010), using the genetic markers SAG1, SAG2 (3'5'SAG2 and alt. SAG2), SAG3, BTUB, GRA6, C22-8, C29-2, L358, PK1, Apico (Su et al., 2006), and CS3 (Pena et al., 2008). Reference archetypal strains RH (Type I), PTG (Type II), and CTG (Type III) and *T. gondii* non-archetypal strains (TgCgCa1, MAS, and TgCatBr5) were included as positive controls and ultrapure water was used as negative control in all reactions. To refine the genotyping results, microsatellite analysis (MS) using 15 markers (TUB2, W35, TgMA, B18, B17, M33, IV.1, X1.1, N60, N82, AA, N61, N83, M48, and M102) was performed according to the protocol

described by Ajzenberg et al., (2010), and the results were analyzed using Genemapper 4.1® (Applied Biosystems, Waltham, MA, USA). The classical Type II reference strain ME-49 was used as positive control and ultrapure water was used as negative control in all reactions.

This study was registered in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen; idenfication number: A3C5173).

Results

A pig farm owner contacted the Setor de Patologia Veterinária at the Universidade Federal do Rio Grande do Sul, after observing a significant increase in pig mortality in February 2020. A disease with a sudden onset was observed, affecting previously healthy grower-finishers. The affected pigs had anorexia, respiratory distress, and locomotion deficits characterized by muscle tremors, the inability to stand, or lateral recumbency, sometimes accompanied by a fever of up to 41.5ºC. The clinical course from the onset of clinical signs to death ranged from one to three days. Two disease peaks were also observed. In the first case peak (February 2020), 30 pigs died. One of these pigs was subjected to a postmortem examination by a field veterinarian, and tissue samples were submitted for histopathology. The microscopic findings in this case included interstitial pneumonia, interstitial nephritis, histiocytic and necrotizing hepatitis, and lymphadenitis. Based on these findings, a presumptive diagnosis of porcine circovirus-associated disease was established. The second peak occurred 30 days later (March 2020), in which 27 pigs died within three days. A total of 57 grower-finishers (57/1500) died in the first and second peaks of the disease, resulting in a mortality rate of 3.8 %. During the second peak, an on-site visit to the pig farm was conducted to investigate the outbreak and collect samples.

During the on-site visit, six grower-finishers pigs aged 102-135 days were euthanized and submitted to postmortem examinations. Gross findings included marked enophthalmos (dehydration) (4/6) and skin abrasions on the lateral aspect of the pelvic limbs (2/6). All pigs had markedly enlarged lymph nodes (generalized lymphadenomegaly), which were more prominent in the mesenteric and internal iliac lymph nodes (6/6). On the cut surface, the nodal parenchyma was nearly effaced by white tissue with a loss of corticomedullary differentiation. Mild to marked splenomegaly and mild ascites were observed in 5/6 and 4/6 cases, respectively. Liver enlargement was noted,

accompanied by random multifocal white spots < 2 mm on the capsule surface and on the cut section (4/6). Mild kidney enlargement with multifocal to coalescing white areas < 2 mm on the surface and the cut sections were also seen (3/6). In four cases, mild to severe and focally extensive to diffuse areas of pale discoloration were observed in the skeletal musculature. These areas were more evident in the muscles of the pelvic limbs, *psoas major,* and *psoas minor* (4/6).

Histopathological findings were compiled for all cases; tissue slides from the first case submitted prior to the on-site visit were revised and assessed in conjunction with the six cases examined during the field visit. Microscopic findings included multifocal to coalescing areas of marked coagulative necrosis in the lymph nodes with intense fibrin deposition, moderate infiltration of neutrophils and macrophages, and variable numbers of multinucleated giant cells (7/7). Muscle lesions were characterized by multifocal areas with multiple hypereosinophilic swollen muscle fibers with a loss of cytoplasmic striations (hyaline necrosis) and cytoplasmic fragmentation (flocculate necrosis). Affected muscle fibers were surrounded by, and sometimes contained, a cytoplasmic influx of satellite cells and mild inflammatory infiltrate of lymphocytes, plasma cells, and macrophages (6/7), and fewer multinucleated giant cells (1/7). Less commonly, necrotic myocytes had cytoplasmic mineralization, and the affected areas had regenerated muscle fibers (1/7).

Lung lesions were characterized by mild to moderate diffuse interstitial pneumonia with an inflammatory infiltrate composed predominantly of lymphocytes and plasma cells (7/7), accompanied by intense proliferation of type II pneumocytes (7/7) and syncytial cells (3/7). Other lesions in the lungs included multifocal areas of mild necrosis, with a central area containing neutrophils and fibrin deposition, often accompanied by macrophages and multinucleated giant cells (5/7) and multifocal thrombosis (2/7). Multifocal, mild to moderate histiocytic splenitis was observed in 6/7 cases, sometimes accompanied by multifocal areas of mild necrosis (3/7) and multinucleated giant cells (1/7).

Random foci of coagulative necrosis forming small nodules were observed in the hepatic parenchyma in all cases (7/7). These areas were accompanied by fibrin deposition and inflammatory infiltration of neutrophils, lymphocytes, and macrophages, fewer eosinophils, and multinucleated giant cells. Similar necrotic foci were also seen in the adrenal glands (1/7) and pancreas, accompanied by moderate peripancreatic fat necrosis (3/7). Multifocal to coalescing moderate lymphoplasmacytic interstitial nephritis was observed in 4/7 cases. Central nervous system lesions were present in three cases and were

characterized by mild multifocal perivascular lymphoplasmacytic infiltrates in the leptomeninges and parenchyma (3/7), and mild multifocal areas of microgliosis, especially in the white matter of the telencephalon (2/7).

In 4/7 cases, rare small, and rounded structures measuring 15-30 µm and filled with basophilic granules (encysted bradyzoites) were observed in areas of necrosis, mainly within the cytoplasm of type II pneumocytes and macrophages in the lung, and in macrophages in the liver. In these cases, oval or round structures measuring–3-5 µm, consistent with free tachyzoites, were observed in areas of inflammation and necrosis. *T. gondii* structures were not observed in histological sections of the remaining tissues.

Positive immunolabeling for *T. gondii* was observed in the tissue sections from all pigs (7/7). Positive immunolabeling was detected in sections of the lymph nodes (7/7), lungs (7/7), and brain (3/7). Granular immunolabeling of parasitic structures compatible with free tachyzoites was observed and was less frequent with encysted bradyzoites amid areas of necrosis and inflammation in sections of lymph nodes, lungs, and amid foci of gliosis in the brain. Encysted bradyzoites were more readily detected in the lung sections. No positive immunolabeling was detected in skeletal muscle tissue sections. All the cases were negative for IHC anti-PCV-2.

PCR for *T. gondii* was positive in four of the six cases examined (case 1: spleen; case 4: mesenteric lymph node; case 5: lungs; case 6: mesenteric lymph node). Genotyping was performed on three samples (spleen was not included in the study because DNA showed a very weak band in agarose gel using 529REP target). The strains were designated PS-TgPigBrSC1, PS-TgPigBrSC2, and PS-TgPigBrSC3 (PS, primary sample). The three strains were genotypically identical and corresponded to the atypical genotype PCR-RFLP #278 previously described by Pena et al. (2018) in a chicken isolate (TgCkBrSC4) from SC in Brazil (Table 1). Using MS, the three strains showed the same 15-allele profile, corresponding to a non-archetypal genotype and identical to the MS genotype from TgCkBrSC4 isolate (Table 2).

Discussion

The diagnosis of systemic toxoplasmosis in this study was based on clinical, gross, and histopathological findings, with confirmation by immunohistochemical and molecular results. The use of histopathology alone to diagnose similar cases may prove difficult because parasites are not always readily detected in tissue sections. Therefore,

immunohistochemistry and molecular assays are valuable tools for confirming toxoplasmosis diagnoses (Jones et al., 2012; Casagrande et al., 2015).

Generally, pigs infected with *T. gondii* are asymptomatic (Dubey, 2010). Several factors may be associated with clinical manifestations and death in pigs due to systemic toxoplasmosis, including the virulence of the strain, dose, and individual and environmental factors (Dubey, 2008). The origin of the toxoplasmosis was not investigated in this study. All infections occurred in grower-finishers, and cats had unrestricted access to the farm. Thus, food or water contaminated with *T. gondii* oocysts may have been the source of infection in pigs.

A non-archetypal genotype (PCR-RFLP #278) was identified by PCR-RFLP in this outbreak. This genotype is characterized by a combination of typical alleles I and III and was previously detected in an isolate from a free-range chicken (TgCkBrSC4), also from SC in Brazil (Pena et al., 2018); this isolate caused 100% of mortality in mice, but there is no direct association between virulence of strains in mice and other hosts, particularly because high virulence in mice seems to be a marked biological trait from strains isolated in Brazil (Pena et al., 2008). MS is a high-resolution tool useful to identify a common source of infection in an outbreak (Ajzenberg et al., 2010). Herein, although the exact source could not be established, it was confirmed that the animals were infected not only by a close strain, but also by the same non-archetypal lineage. This was the same lineage detected in the isolate TgCkBrSC4, suggesting a high circulation of this possible clone in the region, which is a risk factor for public health.

Although there are approximately 700 strains genotyped by PCR-RFLP from different domestic and wild animals and humans in Brazil (H.F.J.P, personal communication), information on *T. gondii* diversity is limited in SC. There are currently 14 genotyped strains in this state, which are classical clonal types I and II, and non-archetypal genotypes, including samples from cattle (Macedo et al., 2012), chickens (Pena et al., 2018; Trevisani et al., 2017), and cats (Pena et al., 2017). Therefore, this study adds to what is currently known about *T. gondii* diversity in this Brazilian state by reporting *T. gondii* infection in a different host.

Most descriptions of *T. gondii* genotypes identified in swine samples in Brazil are from the northeastern region of Brazil (Olinda et al., 2016; Melo et al., 2020). Olinda et al. (2016) reported an outbreak of toxoplasmosis in swine associated with another non-archetypal genotype (RFLP #9 or Chinese 1). Additionally, Paraboni et al. (2019) described

a new *T. gondii* non-archetypal genotype detected in pork samples from the state of Rio Grande do Sul in the southern region of Brazil.

Toxoplasmosis outbreaks with muscular lesions are uncommon in veterinary medicine; to the best of our knowledge, similar lesions have never been reported in pigs. Interestingly, no parasitic structures were observed in histological sections of the affected skeletal muscles. In humans, more information is available on muscle lesions associated with toxoplasmosis. Hassene et al. (2008) discussed cases of muscle damage evidenced by biochemical tests and biopsies in human patients who were seropositive for *T. gondii*. In that study, *T. gondii* was not identified through histopathology or PCR of muscle samples, similarly to that observed in this study. In this case, muscle necrosis may have been induced by an immunologic complication of toxoplasmosis associated with an immune complex-mediated systemic disease (Quilis et al., 1982). As this mechanism of injury is not well understood in animals, we were unable to establish the pathogenesis of the lesion.

An association between *T. gondii* seropositivity and increased muscle enzyme activity has been described in pigs, suggesting muscle injury, especially in sows with potentially compromised or suppressed immunity (Athanasiou et al., 2021). Although clinical toxoplasmosis in animals and humans is classically associated with immunosuppression, no signs of co-infection with PCV-2, an important immunosuppressive virus in pigs, have been found. Other diseases that cause systemic immunosuppression are classical swine fever and swine reproductive respiratory syndrome; however, the Brazilian herd was negative for these diseases.

Muscular lesions represented grossly by areas of pale discoloration, similar to those seen in our cases, have been previously reported in cases of granulomatous necrotizing myositis caused by PCV-2 infections (Konradt et al., 2018). Macroscopically, pigs affected by PCV-2-associated granulomatous myositis also had lymphadenopathy and interstitial nephritis, similar to the lesions found in this outbreak of toxoplasmosis in pigs. The similarities shared between these conditions led to a misdiagnosis in the first histopathological analysis. Although grossly indistinguishable, the muscular lesions associated with PCV-2 infections (Konradt et al., 2018) are richer in inflammatory cells and vasculitis, unlike the lesions displayed by pigs with toxoplasmosis *T. gondii* myositis. Lesions in these cases are predominantly necrotic, there is little association with inflammation, and there is no vasculitis, which is a special feature of circovirosis.

Toxoplasmosis in finishing pigs can occur as a feverish and fatal systemic disease and is noticeable owing to serious skeletal muscle injuries and clinical manifestations of locomotor deficits. To the best of our knowledge, similar necrotizing muscle lesions have not been previously associated with toxoplasmosis in pigs. Pathological findings characterized by systemic granulomatous and necrotizing lesions share similarities with PCV-2 infections, which appears to be the main differential diagnosis in similar cases. This study also contributes to the expanding knowledge on the diversity of *T. gondii* genotypes which circulate and cause clinical diseases in animal hosts in Brazil.

Acknowledgements

The authors thank the owners and field veterinarians for the availability of data. Financial support was supplied by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Finance Code 001, Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS), Pró-reitoria de Pesquisa da Universidade Federal do Rio Grande do Sul (Propesq/UFRGS), and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

Ethics declaration

The project that gave rise to the present data was approved to the Research Committee (COMPESQ) of the Universidade Federal do Rio Grande do Sul (UFRGS) (Project number 40376).

Conflit of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship or publication of this article.

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Tables

Table 1. Multilocus genotyping of *Toxoplasma gondii* by PCR - Restriction Fragment Length Polymorphism (RFLP) from pig samples during a toxoplasmosis outbreak.

Strains	Municipality	State	RFLP markers												PCR-RFLP genotype
			SAG1	5´3´SAG2	alt. SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico	CS3	
PS-TgPigBrSC1,2,3	Nova Veneza	Santa Catarina	I	I	I	III	III	III	III	III	I	I	I	I	#278*

*The same genotype was identified in an isolate from chicken (TgCkBrSC4) from Florianópolis, Santa Catarina state by Pena et al. (2018).

Table 2. Multilocus genotyping of *Toxoplasma gondii* by microsatellite analysis (MS) from pig samples during a toxoplasmosis outbreak.

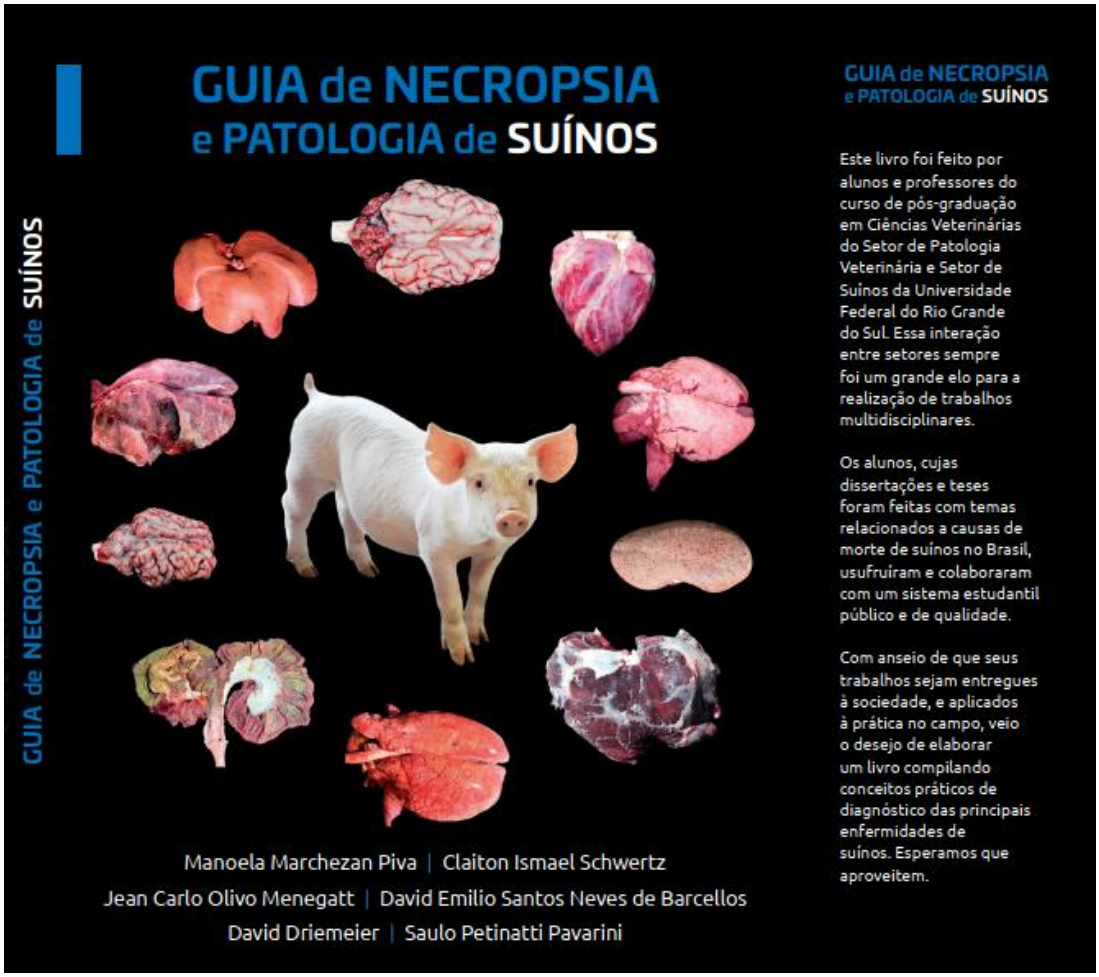
Strains	Municipality	State	Microsatellite markers														MS genotype
			TUB2	W35	TgM-A	B18	B17	M33	MIV.1	MXI.1	M48	M102	N60	N82	AA	N61	N83	
PS-TgPigBrSC1, 2,3	Nova Veneza	Santa Catarina	289	248	205	160	336	165	278	356	213	166	147	109	265	87	306	Non-archetypal*

*This genotype is a combination of alleles I/III, II/III, and III.

The same genotype was identified in an isolate from chicken (TgCkBrSC4) from Florianópolis, Santa Catarina state by Pena et al. (2018).

LIVRO

“Guia de necropsia e patologia de suínos”



Título

GUIA DE NECROPSIA E PATOLOGIA DE SUÍNOS

ISBN: 978-65-00-65826-2

Autores

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Patrocínio

Boehringer Ingelheim

Layout e editoração

Ting Comunicações

G943 Guia de necropsia e patologia de suínos / Manoela Marchezan Piva ... [et al.]. – Porto Alegre: SPV-UFRGS, 2023. 114 p.: il. color; 21 cm. ISBN: 9786500658262

1. Diagnóstico. 2. Suinocultura. 3. Doenças. I. Piva, Manoela Marchezan

CDD 636.089607

Catalogação na publicação: Maurício de Vargas Corrêa – CRB-10/2370

Apresentação do livro

A necropsia detalhada é a melhor oportunidade que temos para realizar um diagnóstico prático e fidedigno das doenças que estão presentes no rebanho. As lesões encontradas podem elucidar questões relacionadas ao manejo, ambiente, alimentação, genética, circulação de agentes infecciosos, intoxicações ou desequilíbrios nutricionais.

Este livro foi compilado por alunos de pós-graduação e professores do Setor de Patologia Veterinária (SPV) e do Setor de Suínos (SetSui), que realizaram trabalhos de mestrado e doutorado enfatizando causas de mortalidade em suínos de diferentes fases de criação.

Os alunos e professores são vinculados ao Programa de Pós-Graduação em Ciências Veterinárias da Universidade Federal do Rio Grande do Sul (PPGCV-UFRGS), da Faculdade de Veterinária, em Porto Alegre, Rio Grande do Sul.

O livro consiste em apresentar, de maneira ilustrativa, a metodologia aplicada durante o exame de necropsia de suínos, bem como as principais doenças que causam mortalidade nas diferentes fases de criação, desde a maternidade, passando pela creche, crescimento e terminação, além de matrizes. Essas doenças são abordadas de forma resumida, com enfoque nas lesões macroscópicas, principais características clínicas e epidemiológicas e particularidades referentes ao estabelecimento de um diagnóstico assertivo.

Este livro não visa substituir manuais ou atlas já existentes, e sim complementar o conhecimento com a experiência da rotina de diagnóstico dos autores. Ainda, são apresentados diversos achados de pouco ou nenhum significado clínico que podem ser encontrados em necropsias de suínos.

O material fotográfico deste livro é oriundo de projetos de pesquisa dos editores, arquivos pessoais, bem como da rotina do SPV-UFRGS e SetSui-UFRGS, que realizam visitas técnicas a propriedades para investigação de surtos de doenças ou de altas mortalidades, além de necropsias em laboratório, como forma de trabalho de extensão, pesquisa e ensino.

O objetivo deste livro é auxiliar médicos-veterinários a campo na investigação dos problemas mais frequentes que cursam com perdas produtivas e mortalidade de suínos nas diferentes fases de produção. Também, disponibilizar à sociedade e multiplicar o conhecimento produzido na Universidade Federal.

3 CONSIDERAÇÕES FINAIS

Nesse estudo, foram diagnosticadas várias condições que tornam os suínos caídos em granjas de crescimento e terminação. Geralmente ocorrem no final da terminação e estão relacionados a processos patológicos crônicos. As entidades diagnosticadas incluíram patologias do aparelho locomotor (artrite supurativa, epifisiólise e fraturas), SNC (meningoencefalite supurativa e encefalomielite não supurativa, mielite bacteriana ascendente, embolia fibrocartilaginosa, abscesso cerebral, polioencefalite de origem não determinada e meningoencefalite eosinofílica), e também patologias multissistêmicas, como doenças associadas ao PCV-2.

Lesões por mordedura de cauda foram um importante fator predisponente e atuaram como provável local primário de infecção para casos de artrite supurativa, espondilite e mielite bacteriana ascendente. As informações obtidas em investigações semelhantes são fundamentais para melhorar os esforços de diagnóstico de campo, mitigar as perdas económicas e melhorar o bem-estar animal na indústria suína.

Nessa tese, podemos demonstrar clinicamente, em rebanho comerciais, mortalidade associada a cepas de *Pasteurella multocida* de alta patogenicidade, atreladas a polisserosite em suínos de crescimento e terminação no Brasil. Esse trabalho corrobora com achados de frigoríficos e estudos experimentais no Brasil. *Pasteurella multocida* com gene pfhA positivo devem ser consideradas como diagnostico diferencial em quadros de polisserosite em suínos de crescimento e terminação no Brasil.

Casos clínicos e fatais de toxoplasmose em suínos são raros, e geralmente a infecção é assintomática. Quando ocorrem, apresenta-se como uma doença aguda, sistêmica e febril, que cursou com lesões musculares acentuadas. Até onde sabemos, lesões musculares necrosantes semelhantes não foram previamente associadas à toxoplasmose em suínos. Os achados patológicos caracterizados por lesões sistêmicas granulomatosas e necrosantes compartilham semelhanças com infecções por PCV-2, o que parece ser o principal diagnóstico diferencial em casos semelhantes. Este estudo também contribui para ampliar o conhecimento sobre a diversidade de genótipos de *T. gondii* que circulam e causam doenças clínicas em hospedeiros animais no Brasil.

O projeto realizado nessa tese proporcionou formação pessoal e profissional de aluno de doutorado, desenvolvendo principalmente aspectos relacionados a patologia suína, doenças infecciosas e não infecciosas de suínos, sanidade e produção de suínos e bem-estar animal. O desenvolvimento de material técnico é de extrema importância na divulgação científica, e nesse projeto foi realizado em forma de apresentações em congressos, palestras e entrega do livro intitulado “Guia de necropsia e patologia de suínos”.

O exame de necropsia é uma ferramenta essencial para monitorar as causas de mortalidade, no que tangem o aparecimento de novas enfermidades e monitoramento de rebanho, gerando informações essenciais sobre doenças infecciosas e não infecciosas que afetam os rebanhos comerciais.

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