

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

LESÕES DE PELE EM SUÍNOS

Paula Reis Pereira

Porto Alegre

2021

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Autor: Paula Reis Pereira

Tese apresentada como requisito parcial para obtenção do grau de Doutor em Ciências Veterinárias da Universidade Federal do Rio Grande do Sul na área de concentração em Medicina Veterinária Preventiva e Patologia: Patologia Animal e Patologia Clínica

Orientador: Prof. Dr. David Driemeier

Porto Alegre

2021

"O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001".

CIP - Catalogação na Publicação

Pereira, Paula Reis Pereira
Lesões de pele em suínos / Paula Reis Pereira
Pereira. -- 2021.
41 f.
Orientador: David Driemeier.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Veterinária, Programa de Pós-Graduação em Ciências Veterinárias, Porto Alegre, BR-RS, 2021.

1. Lesões de pele. 2. Suínos. 3. Patologia. 4. Imuno-histoquímica. 5. Erisipela suína. I. Driemeier, David, orient. II. Título.

Paula Reis Pereira

LESÕES DE PELE EM SUÍNOS

Aprovada em

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AGRADECIMENTOS

Agradeço à minha família, minha base, por terem me preparado para encarar as dificuldades e saber apreciar as alegrias da vida, o apoio de vocês foi fundamental para realizar meus sonhos, aos meus pais Paulo César Reis Pereira e Elaine Maria Pereira, ao meu irmão Juliano Reis Pereira e à minha cunhada Franciele da Silveira muito obrigada pela educação e amor. Às minhas sobrinhas, Maria Eduarda e Maria Clara, agradeço o amor e força na busca de sempre dar-lhes o melhor exemplo. Agradeço ao meu marido Marco Antônio Albé Bach pelo amor, companheirismo e paciência nos momentos de dificuldade.

Agradeço em especial as minhas avós, Dorilda e Derviria, que não estão mais presentes fisicamente, mas vivem em minhas lembranças e ensinamentos. Vocês ajudaram a moldar o que sou hoje, sou grata por cada segundo ao lado de vocês.

Ao professores David Driemeier, Saulo Petinatti Pavarini, a professora Luciana Sonne e a técnica veterinária Marcele Bandinelli agradeço a confiança, por toda ajuda e principalmente, por seus ensinamentos. O que aprendi durante esta jornada ao lado de vocês, vou levar para a minha vida pessoal e profissional. Foi um privilégio conviver e aprender com vocês.

Agradeço aos amigos do Setor de Patologia Veterinária, pelo companheirismo e ajuda de sempre, em especial a Andréia Vielmo, Cíntia de Lorenzo, Márcia Hammerschmitt, Ronaldo Bianchi e Welden Panziera. Aos demais, agradeço o auxílio ao longo destes anos, Anderson Gris, Claiton Schwertz, Claudio Laisse, Emanoelly Machado, Fernando Argenta, Felipe Auatt, Joanna Echenique, Lauren Mello, Luiza Ehlers, Manoela Piva, Marina Lorenzett, Matheus Bianchi, Mônica Slaviero, Paula Ribeiro, Rafael Biondo, Raquel Sales e Veronica Rolim.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela concessão da bolsa de doutorado.

RESUMO

As doenças de pele em suínos podem causar importantes prejuízos econômicos, com perdas relacionadas à morte dos acometidos, custo com tratamentos, atraso no crescimento e condenações nos frigoríficos. Para reduzir estas perdas, o diagnóstico correto e rápido é de suma importância, porém são escassos na literatura trabalhos que reúnam as lesões de pele em suínos e descrevam seus aspectos macroscópicos, histológicos e imuno-histoquímicos. Nesta tese estão incluídos dois artigos científicos relacionados ao tema. No primeiro artigo realizou-se um estudo retrospectivo de 2006 a 2018, para determinar a frequência e descrever os achados histopatológicos das doenças de pele em suínos nas diferentes faixas etárias. Foram analisados 154 casos conclusivos, incluindo as doenças restritas a pele e as secundárias a doenças sistêmicas. As lesões de pele bacterianas representaram 46,1%, as virais 26,6%, as alérgicas 12,3%, as neoplásicas 1,3 e outras corresponderam a 13,6%. A erisipela suína foi a enfermidade mais diagnosticada (47/154), seguida por síndrome dermatite nefropatia suína (23/154), dermatite alérgica (19/154) e epidermite exsudativa (15/154). Observamos ainda dermatite vesicular (9/154), pitiríase rósea (9/154), septicemia bacteriana com manifestações cutâneas (9/154), varíola suína (9/154) e hemorragias múltiplas de causa não determinada (7/154). Em menor número, cisto folicular (3/154), hiperqueratose sem causa definida (2/154), papiloma (1/154) e hemangioma escrotal (1/154). A idade foi informada em 138 casos, e a maior parte das lesões de pele foram observadas na linha de inspeção, durante o abate (56/138). Para o segundo artigo realizou-se um estudo retrospectivo de 2006 a 2019, com o objetivo de descrever os aspectos anatomopatológicos e avaliar o uso da imuno-histoquímica nos casos de erisipela em suínos de abate. A erisipela suína, é uma doença de distribuição mundial, que causa prejuízos econômicos e caracteriza-se como doença zoonótica de caráter ocupacional. Estima-se que 30% a 50% dos suínos sejam portadores da bactéria e fatores estressantes podem predispor o aparecimento da doença clínica. O diagnóstico de erisipela em lesões cutâneas de suínos de abate torna-se um desafio aos patologistas, uma vez que os processos de escaldagem e depila, rotineiros em abatedouros frigoríficos, geram artefatos histológicos que muitas vezes dificultam ou impossibilitam o diagnóstico final. Foram analisados 43 casos de erisipela oriundos de suínos de abate. Macroscopicamente, eram lesões multifocais romboides, retangulares ou quadradas, de coloração rosa, vermelho ou roxo característicos. Histologicamente, na derme e subcutâneo, havia vasculite supurativa associada a degeneração e necrose da parede de vasos, hidradenite e foliculite supurativas, bem como trombose, hiperemia de capilares e necrose multifocais. A técnica imuno-histoquímica se mostrou uma

eficaz ferramenta diagnóstica, com imunomarcção positiva em 93% dos casos. A marcação foi discreta em 23/40, moderada em 9/40 e acentuada em 8/40.

Palavras-chave: lesões de pele, suínos, dermatites bacterianas, patologia, imuno-histoquímica, erisipela, abate.

ABSTRACT

Skin diseases in pigs can cause important economic losses, with losses related to the death of the affected, cost of treatments, growth retardation and condemnations in the slaughterhouses. To reduce losses, the correct and quick diagnosis is of the utmost importance, however there are few studies in the literature that group skin lesions in pigs and describe their macroscopic, histological and immunohistochemical aspects. This thesis includes two scientific articles related to the topic. In the first article, a retrospective study was carried out from 2006 to 2018, to determine the frequency and describe the histopathological findings of skin diseases in pigs in different age groups. One hundred fifty-four cases were analyzed, including skin restricted diseases or skin lesions secondary to systemic diseases. Bacterial skin lesions accounted 46.1%, viral 26.6%, allergic 12.3%, neoplastic 1.3% and others 13.6%. Swine erysipelas was the most frequent diagnosis (47/154), followed by porcine dermatitis and nephropathy syndrome (23/154), allergic dermatitis (19/154) and exudative epidermitis (15/154). Vesicular dermatitis (9/154), pityriasis rosea (9/154), septicemia with cutaneous manifestations (9/154), swinepox (9/154) and multiple hemorrhages without definite cause (7/154) were also observed. Follicular cyst (3/154), hyperkeratosis without definite cause (2/154), papilloma (1/154), and scrotal hemangioma (1/154) were less frequently described. Age was reported in 138 cases, and most skin lesions were observed at the inspection process during slaughter (56/138). For the second article, a retrospective study was carried out from 2006 to 2019, with the objective of describe the anatomopathological aspects and evaluate the use of immunohistochemistry in cases of erysipelas in slaughter pigs. Swine erysipelas is a disease of worldwide distribution, that causes economic losses and considered an occupational zoonotic disease. It is estimated that 30% to 50% of pigs are carry the bacterium and stressors can predispose the appearance of clinical disease. The diagnosis of erysipelas in cutaneous lesions of slaughter pigs becomes a challenge for pathologists, since the scalding and dehairing processes, routine in slaughterhouses, generate histological artifacts that often make the final diagnosis difficult or impossible. Forty-three cases of erysipelas from slaughter pigs were analyzed. Grossly, were multifocal lesions characteristic rhomboid, rectangular or square pink, red or purple. Histologically, in the dermis and subcutaneous tissue, there were suppurative vasculitis associated with degeneration and necrosis of the vessel wall, suppurative hidradenitis and folliculitis, as well as thrombosis, capillary hyperemia and multifocal necrosis. The immunohistochemical technique proved to be an effective diagnostic tool, with positive

immunostaining in 93% of cases. The immunostaining was mild in 23/40, moderate in 9/40 and accentuated in 8/40.

Keywords: *skin lesions, swine, bacterial dermatitis, pathology, immunohistochemistry, erysipelas, slaughter.*

SUMÁRIO

1.INTRODUÇÃO	10
2. ARTIGO 1	15
3. ARTIGO 2	28
4.CONSIDERAÇÕES FINAIS.....	36
5.REFERÊNCIAS BIBLIOGRÁFICAS	37

1.INTRODUÇÃO

A suinocultura industrial brasileira tem destaque no cenário internacional e ocupa a quarta posição em produção e exportação mundial de carne suína (Associação Brasileira de Proteína Animal - ABPA, 2020). Na produção, o país fica atrás da China, União Europeia (EU) e Estados Unidos da América (EUA) e na exportação, da UE, EUA e Canadá (ABPA, 2020). De acordo com o Departamento de Agricultura dos Estados Unidos (USDA, 2019) em 2018, no Brasil foram produzidas cerca de 3% do total mundial de carne suína.

Segundo dados do Instituto Brasileiro de Geografia e Estatística (IBGE, 2021) no quarto trimestre de 2020 foram abatidas no Brasil 12,50 milhões de cabeças de suínos, a região Sul representou 65,3% desta produção, seguida pelas regiões Sudeste (18,8%), Centro-Oeste (14,8%), Nordeste (1,0%) e Norte (0,2%). No quarto trimestre de 2020, observou-se um crescimento de 21,4% na exportação de carne suína, em comparação com o mesmo período do ano anterior. A China foi o principal destino destas exportações, para compensar os efeitos da peste suína africana, que afetou o país ocasionando o colapso na sua produção (IBGE, 2021).

No ano de 2019, estimou-se que o consumo de carne suína foi em média 15,3 kg/habitante (ABPA, 2020), essa cada vez mais tem feito parte da dieta dos brasileiros. Os consumidores, tornaram-se exigentes e preocupados com o bem-estar animal, e em obter um alimento dentro dos padrões higiênico-sanitários (RODRIGUES *et al.*, 2009; LUNING *et al.*, 2015).

O Brasil apresenta bons índices produtivos e se destaca pelo seu *status* sanitário, há mais de 40 anos não registramos casos de peste suína africana (TOKARNIA *et al.*, 2004). E ainda não foram diagnosticadas, doenças como triquinelose, encefalomielite pelo vírus Nipah, síndrome reprodutiva e respiratória dos Suínos (PRRS) e diarreia epidêmica dos suínos (PEDV) (BRENTANO *et al.*, 2002; CIACCI-ZANELLA *et al.*, 2004; BRASIL, 2020; PEREIRA *et al.*, 2021).

Para atender a demanda da produção, os suínos são criados em sistemas intensivos, com aumento da densidade de animais nas instalações e uma maior concentração de granjas em determinadas áreas geográficas. Dessa forma, aumenta o risco do surgimento de doenças infecciosas (BARCELLOS *et al.*, 2008), e, portanto, a adoção de instalações e medidas de manejo adequadas devem ser priorizadas, para prevenir as doenças e sua disseminação (LUNING *et al.*, 2015). A biosseguridade e a vigilância sanitária são muito importantes, especialmente quanto às importações de animais vivos, seus produtos e subprodutos, material

de multiplicação animal (sêmen, embriões e óvulos), e produtos biológicos e patológicos que podem ser possíveis veiculadores dos agentes (AGUILAR *et al.*, 2015).

Dentre as doenças que podem acometer os suínos, as doenças de pele impactam negativamente a produção, pois causam uma diminuição significativa na taxa de crescimento, geram custos com tratamentos e aumento das taxas de mortalidade (TURTON, 2001; KESSLER *et al.*, 2003; BENDER *et al.*, 2011). E quando observadas em abatedouros frigoríficos, as lesões de pele podem causar condenações totais (BENDER *et al.*, 2011) ou impossibilitar o aproveitamento total das carcaças (DOSTER, 1995).

A pele suína é um importante subproduto das indústrias frigoríficas, é aproveitada na fabricação de gelatina, torresmo e pururuca, e melhora a qualidade de certos produtos industrializados, como os embutidos, pela sua quantidade de colágeno (SCHILLING *et al.*, 2003; SALMON, 2004). Além de ser utilizada para confecção de sapatos, luvas, casacos, bolsas, carteiras e agendas, bem como os pelos (cerdas) são matéria-prima para produção de pincéis (MURAD, 2017).

Além dos prejuízos econômicos, a preocupação com saúde pública deve ser considerada, pois algumas destas doenças são zoonóticas, como é o caso da erisipela suína, causada por *Erysipelothrix rhusiopathiae*. As infecções humanas, ocorrem via contato direto com animais infectados e são, portanto, doenças ocupacionais para veterinários, trabalhadores de frigoríficos e processadores de carnes, aves e peixes (COLAVITA; VERGARA; IANIERI, 2006). Em um estudo desenvolvido no distrito de Kamuli, na Uganda Oriental, que envolveu 302 pessoas que manipulavam carne suína crua, incluindo açougueiros, trabalhadores de abatedouros e cozinheiros, os autores observaram 9,9% de prevalência de infecção por *E. rhusiopathiae*. Porém, quando considerados apenas açougueiros e trabalhadores de abatedouros, a prevalência subia para 15% e 37%, respectivamente (MUSEWA *et al.*, 2021).

A pele é o maior órgão do corpo e forma uma barreira anatômica e fisiológica completa entre o animal e seu ambiente, é responsável pela termorregulação, síndrome vitamínica, imunorregulação, balanço eletrolítico, ações antimicrobianas e percepções sensoriais (DOSTER, 1995; TORRISON; CAMERON, 2019). A pele pode constituir 12-24% do peso corporal. No suíno, este órgão representa entre 10% e 12% do peso corporal no nascimento e cerca de 7% em animais adultos (TORRISON; CAMERON, 2019).

Histologicamente a pele dos suínos, é semelhante à de outros animais domésticos e, em comparação com outras espécies, tem muitas semelhanças com a pele humana. É composta pela epiderme, derme e hipoderme. A epiderme é relativamente espessa, composta por epitélio estratificado queratinizado, organizado em quatro camadas (estrato basal, estrato espinhoso,

estrato granuloso e estrato córneo), o estrato lúcido está ausente, exceto no focinho. A derme é subdividida em duas zonas, a derme papilar, que está em contato com a epiderme através da membrana basal e da derme reticular, que está em contato com a hipoderme subjacente. A derme é constituída por tecido conjuntivo contendo vasos sanguíneos, nervos, vasos linfáticos e apêndices epidérmicos associados. A hipoderme é constituída principalmente por adipócitos, e nela são originados os folículos pilosos e as glândulas sudoríparas. Os suínos contêm glândulas sudoríparas écrinas, no carpo e nas regiões nasolabiais. No entanto, a grande maioria das glândulas sudoríparas são apócrinas, presentes em todas as áreas, mas são poucas (cerca de 25/cm²) em comparação com outras espécies. As glândulas sebáceas, raramente foram encontradas em estudos que comparavam a pele de suínos e suas contrapartes em humanos (DEBEER *et al.*, 2013; TORRISON; CAMERON, 2019).

Uma variedade de doenças afeta a pele dos suínos, essas podem ser de natureza infecciosa (bacteriana, viral, fúngica e parasitária), hereditária, nutricional, neoplásica ou ambiental (JACKSON; COCKCROFT, 2007; TORRISON; CAMERON, 2019). O sistema de criação, sazonalidade, fatores nutricionais e de manejo interferem na expressão dessas doenças. Nos sistemas de criação extensivos ou livres, os suínos ficam sujeitos aos riscos ambientais, como queimaduras solares (TORRISON; CAMERON, 2019). Enquanto úlceras de contato, necrose de orelha e certas doenças bacterianas, geralmente são observadas em sistemas intensivos. Assim como a movimentação dos suínos, mistura de lotes e introdução de novos animais, podem resultar em brigas, além de introduzir novos ou diferentes agentes infecciosos (TORRISON; CAMERON, 2019).

As lesões de pele ocasionadas por picadas de insetos são exemplos de alterações cutâneas que podem ser influenciadas pela sazonalidade (TORRISON; CAMERON, 2019), a frequência varia conforme a região geográfica, devido às diferenças climáticas (BREWER; GREVE, 2019). A nutrição e a dieta também interferem, uma vez que deficiências de biotina, de zinco ou de ácidos graxos essenciais podem resultar em lesões cutâneas (SOBESTIANSKY; MORES; MORI, 1989; ALTROCK; HÖLTIG, 2013; TORRISON; CAMERON, 2019). As dietas com excesso de selênio podem dar origem a alopecia e separação da banda coronária dos cascos (JACKSON; COCKCROFT, 2007; GOMES *et al.*, 2014).

As doenças cutâneas podem ser restritas a pele (primárias) ou serem manifestações cutâneas de uma doença sistêmica (secundárias). Alguns exemplos de doenças que são restritas à pele são a pitiríase rósea e varíola, enquanto erisipela, peste suína clássica e a síndrome de dermatite e nefropatia são lesões cutâneas relacionadas a uma condição sistêmica (ALTROCK; HÖLTIG, 2013; TORRISON; CAMERON, 2019).

As dermatites bacterianas em suínos podem ser representadas pela erisipela suína, epidermite exsudativa, abscessos e septicemias bacterianas com manifestações cutâneas (DOSTER, 1995; ALTROCK; HÖLTIG, 2013; TORRISON; CAMERON, 2019). Dermatites fúngicas por *Microsporum nanum*, *M. canis*, *M. gypseum*, *Trichophyton mentagrophytes*, *T. rubrum*, *T. tonsurans*, *T. verrucosum*, e *Candida albicans* podem ser observadas em suínos (TORRISON; CAMERON, 2019). No Brasil, casos de zoonoses por *M. nanum* já foram relatados (CAMARGO *et al.*, 1992). Porém, as doenças bacterianas que afetam a pele são mais comuns, em comparação com as fúngicas, que são relativamente raras (JACKSON; COCKCROFT, 2007).

Como exemplos de dermatites virais podemos citar casos de síndrome dermatite e nefropatia suína por circovírus suíno tipo 2, varíola suína, peste suína clássica e dermatites vesiculares (ALTROCK; HÖLTIG, 2013; TORRISON; CAMERON, 2019). Doenças em suínos que cursam com dermatite vesicular, devem incluir como diagnósticos diferenciais, febre aftosa, doença vesicular suína, estomatite vesicular, exantema vesicular (TORRISON; CAMERON, 2019), doença vesicular associada ao Senecavirus A (SVA) (SEGALÉS *et al.*, 2017) e parvovírus suíno (KRESSE *et al.*, 1985).

Dermatites parasitárias incluem picada de insetos, pulgas, piolhos e ácaros (ALTROCK; HÖLTIG, 2013; TORRISON; CAMERON, 2019). Em suínos o mais importante ectoparasito é a *Sarcoptes scabiei* var *suis* (KESSLER *et al.*, 2003), podem ainda ser parasitados por *Haematopinus suis*, e menos frequentemente pela sarna demodécica (BREWER; GREVE, 2019). A ocorrência de demodicose por *Demodex phylloides* já foi relatada em suínos criados extensivamente em São Paulo (BERSANO *et al.*, 2016).

As doenças de pele congênitas ou hereditárias, como pitiríase rósea e epiteliogênese imperfeita também podem ser observadas. Em suínos, os tumores cutâneos são relativamente raros, já foram citados casos de linfangioma, rabdomioma, papiloma, adenoma de glândula sudorípara, fibroma e hemangioma (TORRISON; CAMERON, 2019). Melanocitomas e a melanomas podem ter uma alta frequência em certas raças de suínos, como Sinclair, Hormel, Duroc e minipigs *Melanoma-Bearing Libechov*, nas quais tem origem congênita e podem frequentemente ser observados no abate (GOLDSCHMIDT; GOLDSCHMIDT, 2017).

Como em outras doenças, uma história clínica completa é essencial para o diagnóstico. Essa deve incluir idade, distribuição de lesões, aparência e progressão de lesões e outros sinais clínicos que existem concomitantemente (DOSTER, 1995). De acordo com Torrison e Cameron (2019) o exame clínico geral pode fornecer informações importantes para determinar se a lesão de pele é oriunda de doença primária ou está relacionada a doença sistêmica.

Para o diagnóstico definitivo é necessário a coleta de amostras. As biópsias de pele são importantes e devem incluir todas as camadas da pele (epiderme, derme e subcutâneo) para realização de exame histopatológico. Da mesma forma, exames diretos para identificação de bactérias e fungos e cultura para isolamento e identificação de bactérias e vírus podem ser realizados (DOSTER, 1995; TORRISON; CAMERON, 2019).

O diagnóstico rápido e eficiente, é fundamental para a manutenção do *status* sanitário nacional adequado, assim como auxilia no serviço de defesa sanitária animal (CIACCIZANELLA; MORÉS; BARCELLOS, 2016). Devido à grande importância do diagnóstico eficaz das lesões de pele em suínos, esse estudo tem como objetivos: (1) determinar a frequência e descrever os achados histopatológicos das doenças de pele em suínos nas diferentes faixas etárias; e (2) descrever os aspectos anatomopatológicos e avaliar o uso da imuno-histoquímica nos casos de erisipela em suínos de abate. Desse estudo resultaram dois trabalhos científicos, que estão incluídos na íntegra a seguir.

2. ARTIGO 1

Nesse item é apresentado o artigo intitulado:

Primary skin diseases and cutaneous manifestations of systemic diseases in swine

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Artigo publicado no periódico **Pesquisa Veterinária Brasileira**. 2020, 40(8):579-588.

Pesq. Vet. Bras. 40(8):579-588, August 2020

Primary skin diseases and cutaneous manifestations of systemic diseases in swine¹

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ABSTRACT. - Pereira P.R., Bianchi R.M., Hammerschmitt M.E., Cruz R.A.S., Hesse K.L., Sonne L., Pavarini S.P. & Driemeier D. 2020. **Primary skin diseases and cutaneous manifestations of systemic diseases in swine.** *Pesquisa Veterinária Brasileira* 40(8):579-588. Setor de Patologia Veterinária, Departamento de Patologia Clínica Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, Prédio 42505, Porto Alegre, RS 91540-000, Brazil. E-mail: paula.rpereira@hotmail.com

Skin diseases in pigs can negatively impact the production. They cause losses related to the death of the affected pigs, to the cost with the treatment, growth retardation and condemnations in the slaughterhouses. This study was developed to determine the frequency and describe the histopathological findings of skin diseases in pigs in different age groups through a retrospective study from 2006 to 2018. A total of 154 conclusive cases were analyzed, including skin restricted diseases (allergic dermatitis, exudative epidermitis, vesicular dermatitis, pityriasis rosea, swinepox, follicular cyst, papilloma and scrotal hemangioma) or skin lesions secondary to systemic diseases (erysipelas, porcine dermatitis and nephropathy syndrome [PDNS], bacterial septicemia and multiple hemorrhages without definite cause). The skin lesions were classified as bacterial (46.1%), viral (26.6%), allergic (12.3%), neoplastic (1.3%) and others (13.6%). Swine erysipelas was the most frequent diagnosis (47/154), followed by PDNS (23/154), allergic dermatitis (19/154) and exudative epidermitis (15/154). Vesicular dermatitis (9/154), pityriasis rosea (9/154), septicemia with cutaneous manifestations (9/154), swinepox (9/154) and multiple hemorrhages without definite cause (7/154) were also observed. Follicular cyst (3/154), hyperkeratosis without definite cause (2/154), papilloma (1/154), and scrotal hemangioma (1/154) were less frequently described. Of the conclusive diagnosis, age was reported in 138 cases, with the highest frequency of skin lesions observed at the inspection process during slaughter (56/138).

INDEX TERMS: Swine, dermatitis, pathology, skin lesions, pigs, Brazil.

RESUMO. - [Doenças primárias da pele e manifestações cutâneas de doenças sistêmicas em suínos.]

As doenças de pele em suínos podem impactar negativamente a produção. Estas causam perdas relacionadas à morte dos acometidos, a custo com tratamentos, atraso no crescimento e condenações nos frigoríficos. Este trabalho foi desenvolvido para determinar a frequência e descrever os achados histopatológicos das doenças de pele em suínos nas diferentes faixas etárias, através de um estudo retrospectivo no período de 2006 a 2018. Foram analisados 154 casos conclusivos, incluindo as doenças restritas a pele (dermatites alérgicas, epidermite exsudativa, dermatite vesicular, pitiríase rósea, varíola suína, cisto folicular, papiloma e hemangioma escrotal) e as secundárias a doenças sistêmicas (erisipela, síndrome dermatite nefropatia suína [SDNS], septicemia bacteriana e hemorragias múltiplas de causa não determinada). Estas foram classificadas em bacterianas (46,1%), virais (26,6%), alérgicas (12,3%), neoplásicas (1,3%) e outras (13,6%). A erisipela suína foi a enfermidade mais diagnosticada (47/154), seguida por SDNS (23/154), dermatite alérgica (19/154) e epidermite exsudativa (15/154). Observamos ainda dermatite vesicular (9/154), pitiríase rósea (9/154), septicemia bacteriana com manifestações cutâneas (9/154), varíola suína (9/154) e hemorragias múltiplas de causa não determinada (7/154). Em menor número, cisto folicular (3/154), hiperqueratose sem causa definida (2/154), papiloma (1/154) e hemangioma escrotal (1/154). Dos casos conclusivos, a idade foi informada em 138 casos, sendo a maior frequência das lesões de pele observadas na linha de inspeção, durante o abate (56/138).

TERMOS DE INDEXAÇÃO: Dermatite, patologia, lesões de pele, suínos, Brasil.

INTRODUCTION

Swine production in Brazil ranks fourth in the world for pork production and export (USDA 2019). It is considered that the Brazilian pig herd has a good sanitary condition, proven by high productivity, with rates similar to those of other countries where pig farming is also developed. Even so, the rapid and efficient diagnosis of the most varied pathologies must be prioritized (Ciacci-Zanella et al. 2016). Skin diseases in pigs can negatively impact production, because they generate treatment costs, decrease the growth rate and, sometimes, can cause the death of the animal (Turton 2001, Bender et al. 2011). Swine skin is an important by-product of refrigeration industries, as it contributes to improving the quality of some products, as well as the amount of collagen (Schilling et al. 2003). Therefore, in addition to the losses mentioned in the production, the condemning and/or impossibility of utilization of carcasses of pigs with skin lesions can also cause important economic losses (Doster 1995, Bender et al. 2011).

A variety of diseases affect the skin of pigs and can be infectious (bacterial, viral, mycotic and parasitic), or non-infectious (environmental, nutritional, hereditary and neoplastic) (Torrison & Cameron 2019). These diseases may involve only the skin or be cutaneous manifestations of a systemic disease (Torrison & Cameron 2019). However, studies characterizing histologically the skin lesions in swine, whether primary or secondary, as well as establishing the age affected are scarce in the literature. Thus, the objective of this study was to determine the frequency and describe the histopathological findings of skin diseases in pigs in different age groups, diagnosed from January 2006 to December 2018 in southern Brazil.

MATERIALS AND METHODS

From January 2006 to December 2018, the files of the “Setor de Patologia Veterinária” of the “Universidade Federal do Rio Grande do Sul” (SPV-UFRGS) were reviewed, and cases of pigs with skin lesions submitted for histological analysis were select. Samples of a scientific experiment or submitted to other tests that did not include histological analysis were excluded from the research. The selected cases were from pig farms and slaughterhouses. The protocols were reviewed, and the information regarding age, history, anatomopathological descriptions and final diagnosis were analyzed and compiled. Pigs were classified into five stages of production, according to Machado (2014), in maternity (1-21 days), nursery (22-61 days), growth (62-120 days), finishing (121-160 days) and adults (over 161 days). Samples from the slaughterhouses were categorized as slaughter. Gross lesions of the main diseases were standardized according to the data provided in the histories and, to represent them, figures from the SPV-UFRGS database were used. The archived paraffin-embedded blocks were searched for making histological slides that were stained by hematoxylin and eosin technique, for later histological description. In all cases, the diagnoses were based only on the histological lesions observed, without the use of complementary exams. The study included cases in which the lesion was restricted to the skin (primary) or a cutaneous manifestation to systemic diseases (secondary). In the latter case, other organs were also analyzed when subjected together the skin. As an inclusion criterion for cases of septicemia, it was considered the anatomopathological description of erythema or cutaneous cyanosis, especially in the extremities (Torrison & Cameron 2019), and foci of necrosis in multiple organs associated with bacterial thromboembolism (Yaeger 1995). The cutaneous diseases were classified as bacterial, viral, allergic, neoplastic and “other” (when it was not possible to insert in one of the previous categories).

RESULTS

During the period of this study, 7.782 pig tissue samples were analyzed by the laboratory, of which 167 (2.1%) corresponded to skin lesions, which included both diseases restricted to the skin and systemic diseases with cutaneous manifestations. Of the 167 cases, seven (4.2%) were inconclusive and six (3.5%) unsuitable for histopathological evaluation, due to scalding and dehairing resulting from the slaughter process.

Allergic dermatitis, exudative epidermitis, vesicular dermatitis, pityriasis rosea, swinepox, follicular cyst, papilloma, and scrotal hemangioma were the primary skin lesions diagnosed. While swine erysipelas, porcine dermatitis and nephropathy syndrome (PDNS), bacterial septicemia and multiple hemorrhages without definite cause were the main skin lesions secondary to systemic diseases.

When considering conclusive diagnoses, bacterial diseases accounted for 46.1% (71/154) of cases, and included swine erysipelas, exudative epidermitis and bacterial septicemia with cutaneous manifestations. Viral infections were also frequent (26.6%, 41/154), and represented by cases of PDNS, swinepox and vesicular dermatitis. Allergic diseases accounted for 12.3% (19/154) and corresponded to cases in which the final diagnosis was suggestive of dermatitis allergic to scabies or insect bites. Neoplastic

category was the less frequent (1.3%, 2/154) and represented by papilloma and scrotal hemangioma. In the category “others”, 13.6% (21/154) of the cases were included, with diagnoses of pityriasis rosea, multiple hemorrhages without definite cause, follicular cyst and hyperkeratosis without definite cause. The frequencies of the main skin diseases diagnosed in this study are shown in Figure 1. Less frequent diagnoses were follicular cyst (1.9%), hyperkeratosis without a defined cause (1.3%), papilloma and scrotal hemangioma (0.6% each).

The age or production stage of the pigs was reported in 138 of the 154 conclusive diagnoses, with greater occurrence in the slaughter process (56/138). The occurrence of the main skin diseases by pig production stage is described in Table 1. Of the cases of follicular cysts, 2/3 and 1/3 were of the slaughter and adult categories, respectively. The cases of hyperkeratosis without a definite cause had no age information. Papilloma was observed in a slaughter pig and scrotal hemangioma in an adult pig.

Swine erysipelas caused by *Erysipelothrix* spp. was the skin disease most frequently diagnosed (47/154). Grossly, the disease was characterized by reddish multifocal lesions, with raised edges and a characteristic diamond shape (Fig.2A). Histologically, multifocal vasculitis was observed in the dermis and subcutaneous tissue, characterized by predominantly neutrophilic inflammatory infiltrate, surrounding and intermingling the blood vessels wall, associated with fibrinoid vascular degeneration and necrosis. There were also multifocal areas of necrosis in the dermis and subcutaneous tissue associated with fibrin deposition, as well as multifocal thrombosis and neutrophilic infiltrate in sweat gland ducts (Fig.2B and 2C).

The PDNS caused by porcine circovirus type 2 (PVC2) corresponded to 23/154 cases, of which 52.2% were diagnosed in the first two years of the study, 2006 and 2007. On the skin, dark red, multifocal macules and papules have been described, which sometimes coalesce to form erythematous plaques, predominantly in the pelvic limbs, ventral and perineal region (Fig.2D). Microscopically, the lesions were characterized by dermal vasculitis, associated with inflammatory infiltrate of neutrophils, lymphocytes, plasma cells and macrophages, as well as fibrinoid vascular degeneration, sometimes with multifocal hemorrhage (Fig.2E). There was also focal ulceration of the epidermis in two cases. In the kidneys, there was a marked accumulation of fibrin and neutrophils in glomeruli and in the urinary space (fibrinosuppurative glomerulitis), in addition to glomerulosclerosis and membranoproliferative glomerulonephritis. There were also marked tubular ectasia, hyaline cylinders and mononuclear interstitial nephritis (Fig.2F). In 21/23 cases, granulomatous lymphadenitis was also observed, characterized by inflammatory infiltrate of macrophages and occasionally multinucleated giant cells in the nodal parenchyma.

Allergic dermatitis corresponded to 19/154 cases, and were composed by small rounded, red and raised spots (Fig.3A). Histologically, they were characterized by a multifocal eosinophilic dermatitis. The inflammatory infiltrate of eosinophils, lymphocytes and plasma cells was often observed associated with weakly basophilic and myxomatous material (edema) and, sometimes, with perivascular distribution (Fig.3B). In three cases, multifocal ulceration of the epidermis was also observed, and in none of the 19 cases, mites were seen. The 15/154 cases of exudative epidermitis were characterized by marked greasy exudate and multifocal formation of crusts (Fig.3C). Microscopically, they consisted of focal areas of extensive ulceration of the epidermis, and crusted formations with marked deposition of fibrin, neutrophilic inflammatory infiltrate and coccoid bacterial aggregates. The inflammatory infiltrate also extended to the dermis and was associated with multifocal thrombosis (Fig.3D).

The cases of vesicular dermatitis (9/154) were observed only in 2015. The disease was characterized by vesicular and ulcerative lesions in the snout, hooves and coronary band (Fig.4A). It was noted that in eight cases the vesicles had ruptured and, histologically, they were composed by discrete multifocal areas of necrosis of keratinocytes, associated with infiltrate of intact and degenerate neutrophils, fibrin deposition and coccoid bacterial aggregates. In the adjacent epidermis hydropic degeneration and moderate multifocal hyperkeratosis were also observed in keratinocytes (Fig.4B). In the case where the vesicle remained intact, there was a cystic formation covered by keratinized epithelium.

Pityriasis accounted for 9/154 of the cases, initially observed as papules on the abdomen and medial face of the pelvic limbs, evolving into circular lesions outlined by a raised red border (Fig.4C). Histologically, the lesions were characterized by marked hyperplasia of keratinocytes, which formed digitiform projections towards the dermis, multifocal intracorneal pustules, in addition to marked multifocal inflammatory infiltrate of neutrophils, lymphocytes and macrophages in the dermis, sometimes, with perivascular distribution (Fig.4D).

In cases of bacterial septicemia (9/154), multifocal hemorrhages were observed in the dermis and subcutaneous tissue. In other organs there were multifocal thrombosis and necrosis, sometimes associated with fibrin deposition, neutrophilic infiltrate and bacterial aggregates. Swinepox, also diagnosed in 9/154 cases, was initially characterized by macules that evolved to vesicles and pustules, with subsequent formation of crusts (Fig.4E). Histologically, the lesions were composed by moderate multifocal acanthosis

of the epidermis with occasional vacuolization of spinous stratum cells. Sometimes intracytoplasmic eosinophilic inclusion bodies, of approximately 3µm, were also observed in keratinocytes (Fig.4F). Intracorneal pustules and necrosis of epidermis and dermis were also evidenced. Edema in the superficial dermis with infiltration of eosinophils and mast cells, compatible with allergic dermatitis was observed in eight cases. In the 7/154 cases of multiple hemorrhages without a defined cause, there was only hemorrhage and multifocal congestion in multiple organs, without associated inflammatory infiltrated or necrotic lesions.

DISCUSSION

Skin diseases can be restricted (primary) or be cutaneous manifestations of systemic (secondary) diseases. As primary, we can mention pityriasis rosea, vesicular diseases and swinepox, while secondary to erysipelas, classical swine fever (PSC) and PDNS (Torrison & Cameron 2019). In our study, as primary skin lesions, we observed cases of allergic dermatitis, exudative epidermitis, vesicular dermatitis, pityriasis rosea, swinepox, follicular cyst, papilloma and scrotal hemangioma. While erysipelas, PDNS, bacterial septicemia and multiple hemorrhages without a defined cause were secondary. These results confirm the wide variety of skin lesions that can affect pigs. Bacterial skin diseases, including primary and secondary diseases, accounted for 46.1% of the cases in this study. Similar to that described by other authors, bacterial dermatitis is considered common, unlike fungal diseases that are extremely rare in pigs (Jackson & Cockcroft 2007) and were not observed in this research.

Swine erysipelas was the main diagnosis observed, and can affect pigs of all ages, however, there is a greater susceptibility for pigs between two and 12 months of age (Wood 1999). In our study, most of the cases were from pigs subjected to slaughter and can be justified by the worsening of the disease due to transport stress (Schwartz 2002). In the present study, histopathological diagnoses were analyzed, of which 30.5% corresponded to swine erysipelas. Most studies related to erysipelas include evaluations of carcasses in refrigerators and bacteriological examinations, and present a wide variation, with frequencies of 0.41% (Piva Filho et al. 2011) and 84.3% (Bender et al. 2011). Brum et al. (2013), when studying swine infectious and parasitic diseases, diagnosed erysipelas in 1.4% of cases. Histological findings observed in this study were similar to those described by other authors (Barman et al. 2016, Mauldin & Peters-Kennedy 2016), which were mainly characterized by vasculitis, degeneration and fibrinoid vascular necrosis, as well as multifocal necrosis in the dermis and subcutaneous tissue associated with fibrin deposition, thrombosis and neutrophilic inflammatory infiltrate in sweat gland ducts.

Exudative epidermitis corresponded to 9.7% of the diagnoses in this study, similar to that observed by other authors, with 5.4% (Brum et al. 2013) and 6.25% (Coelho et al. 2017). This disease occurs worldwide, and the main etiological agent involved is *Staphylococcus hyicus* (Frana & Hau 2019), although some studies are related to a strain VA654 of *Staphylococcus chromogenes* (Andresen et al. 2005). In our study, the affected pigs were in the maternity and nursery, as described by Frana & Hau (2019) in which pigs aged three to 32 days are the most affected. We observed a case in an adult pig, but it is known that finishing animals and adults are rarely affected (Barcellos et al. 2012). As described by other authors (Andresen et al. 2005, Frana & Hau 2019) we observed ulcerative epidermitis with crust formation, marked fibrin deposition, neutrophilic inflammatory infiltrate and coccoid bacteria, as well as multifocal thrombosis in the dermis.

In the nine (5.8%) cases of septicemia, complementary exams were not performed to establish the etiological agent. However, many agents may be involved in cases of septicemia, such as *E. rhusiopathiae*, *Salmonella Choleraesuis*, *Actinobacillus suis*, *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Streptococcus suis*, *Pasteurella multocida* tipo A and *Escherichia coli* (Miniats et al. 1989, Sanford et al. 1990, Yaeger 1995, Del'Arco et al. 2008, Oliveira Filho et al. 2015, Torrison & Cameron 2019). As to the stage of production, five pigs were from the maternity, two from the nursery and two from the growth. Some of the agents that can cause septicemia mainly affect pigs in these stages, ranging from 21 days to four months of age in cases of salmonellosis by *Salmonella Choleraesuis* (Torrison & Cameron 2019), two to 28 days in cases of *A. suis* (Sanford et al. 1990) and *E. coli* in newborn pigs (Yaeger 1995).

Viral diseases represented here by PDNS, swinepox and vesicular dermatitis, accounted for 26.6% of cases. PDNS was the second most frequent diagnosis, with half of the cases in the years 2006 and 2007, the lowest number observed in the following years is explained by the commercialization of vaccines from 2008 (Ciacci-Zanella et al. 2016). We observed 14.9% cases of PDNS, rates higher than those observed by Segalés et al. (1998), which reports a low frequency, usually between 0.05 and 0.5%, and Corrêa et al. (2006), in Rio Grande do Sul, which described 4.1% of PDNS cases associated with suspected necropsies of circovirus. However, the frequency of our study is very similar to that observed in the United Kingdom and

other countries that had a frequency ranging from 0.25 to 20% (Gresham et al. 2000). In our study, most cases were observed in growth and finishing, agreeing, in part, with other authors, who report that this syndrome is more often described in nursery, growth and finishing pigs (Drolet et al. 1999, Gresham et al. 2000). PDNS is believed to be a type III hypersensitivity (Helie et al. 1995) and the microscopic findings observed in the kidneys, skin and lymph nodes in the cases were similar to that described in the literature, differing only in two cases, in which there was ulceration of the epidermis (Rosell et al. 2000, Wellenberg et al. 2004, Cadar 2009).

Vesicular dermatitis was associated with 5.8% of cases, a lesion that can be seen in cases of foot-and-mouth disease, swine vesicular disease, vesicular stomatitis, vesicular exanthema (Torrison & Cameron 2019), vesicular disease associated with Senecavirus A (SVA) (Segalés et al. 2017) and swine parvovirus (Kresse et al. 1985). We had information that they were negative for foot-and-mouth disease, and although there was no confirmatory examination for SVA, the involvement of this agent in our cases is suggested. These occurred in the year 2015, it was in this year and in 2014, that outbreaks occurred in Brazil of a vesicular disease of which the SVA was detected by Polymerase chain reaction (Vannucci et al. 2015, Leme et al. 2016). The affected pigs were all from the maternity, agreeing with data from the SVA outbreaks that occurred in Brazil, with the death of piglets in the first week of life (Vannucci et al. 2015, Leme et al. 2016). During the first half of 2015, of the 61 cases of swine diseases reported to the Official Veterinary Service of Rio Grande do Sul, 14.7% corresponded to idiopathic vesicular disease (Campos et al. 2016). In eight cases, the vesicles were ruptured and fibrinonecrotic dermatitis associated with bacterial colonies was observed in these areas. These histological lesions were similar to those described in the literature (Vannucci et al. 2015, Segalés et al. 2017). Segalés et al. (2017) report that ulcers begin to heal in seven days, and epithelial regeneration is usually complete within two weeks.

Swinepox corresponded to 5.8% of our diagnoses, in a study on swine diseases, it corresponded to 0.2% of the infectious and parasitic diseases diagnosed (Brum et al. 2013). This disease is caused by a poxvirus, has a wide distribution and outbreaks have already been described in São Paulo (Bersano et al. 2003, Medaglia et al. 2011), Tocantins (Bersano et al. 2003), in the Brazilian northeast (Olinda et al. 2016), Italy (Mariano et al. 2015) and India (Mech et al. 2018). The histological aspects were similar to those described by Olinda et al. (2016), of the nine cases, in eight, viral infection was associated with histological lesions suggestive of allergic dermatitis, suggesting the theory that *Haematopinus suis* lice and flies may be mechanical vectors for the transmission of the swinepox virus (Torrison & Cameron 2019).

Allergic dermatitis represented 12.3% of diagnoses, these generate economic losses due to reduced growth rate, reduced feed efficiency and loss of carcass value at slaughter (Kessler et al. 2003, Brewer & Greve 2019). Most of the cases corresponded to finishing and slaughter pigs, similarly to what reported by Cole (1990) in cases of hypersensitivity to insect bites in slaughter pigs and for Pedroso-de-Paiva et al. (2003) with lesions indicative of scabies in the slaughter line. Microscopy showed eosinophilic dermatitis, predominantly perivascular, in addition to edema and ulceration of the epidermis in three cases (Cole 1990, Mauldin & Peters-Kennedy 2016). In histological sections no mites were observed, however it does not mean that any of these cases cannot be sarcoptic mange, as the agent may not be detected in histology (Brewer & Greve 2019).

Neoplastic skin diseases represented a low number of diagnoses (1.3%), which was expected, since neoplasms in pigs are rare (Brum et al. 2015, Torrison & Cameron 2019). Papilloma occurred in a slaughter pig, with no information on the anatomical location, but it can affect growing pigs, especially around the neck, along the back and in the ears (Torrison & Cameron 2019). Hemangioma was observed in the scrotum of an adult pig, corroborating with Rech et al. (2013) who described that papillomas and hemangiomas can be seen in the scrotum of old necks and are considered lesions with no clinical significance. Papilloma and scrotal hemangioma represented 0.6% each of the total skin lesions diagnosed. Brum et al. (2015), in a study on swine neoplasms, diagnosed 2/37 (5.4%) cases of papilloma.

In the category "others", skin diseases were added that could not be inserted with bacterial, viral, allergic or neoplastic. Pityriasis rosea corresponded to 5.8% of diagnoses and affected animals in growth, as described by Kimura & Doi (2004), different from Altrock & Höltig (2013), who mention affecting nursery pigs. The cause of this disease is still unknown, but some authors believe that it is hereditary (Torrison & Cameron 2019), others cite *Scopulariopsis brevicaulis* as a possible agent (Purchio et al. 1980). Kimura & Doi (2004) describe three pigs and even using different methods of analysis (microbiological, histopathological, hematological and blood biochemistry) have not been able to elucidate the cause of this pathology. Histologically the lesions observed were similar to those described in the literature (Kimura & Doi 2004, Mauldin & Peters-Kennedy 2016).

In cases of multiple hemorrhages (4.5%) and hyperkeratosis (1.3%), it was not possible to confirm the diagnosis based on the information (history and anatomopathological findings) of the reports. Multiple hemorrhages in pigs can be seen in cases of coumarin poisoning (Amaral et al. 2015), purple piglet

thrombocytopenia (Forster 2007), prolonged treatment with sulfas, lead poisoning, zinc phosphate, ricin, aflatoxins and ophidian accident (Sobestiansky et al. 2012). In cases of multiple hemorrhages, histology showed only hemorrhage and severe congestion. Hyperkeratosis can be observed in metabolic disorders related to nutrition, such as deficiencies of vitamin A, zinc and fatty acids, or with the formation of local calluses due to trauma associated with pressure and friction (Sobestiansky et al. 1989, Altrock & Höltig 2013, Torrison & Cameron 2019). During the performance of this retrospective study, we observed that some skin diseases, which are known to occur in swine farms, such as injuries from fights, sunburn and imperfect epitheliogenesis, were not diagnosed. We believe that the recognition of these and other injuries by employees and veterinarians of swine producing units, prevents them from being sent to the laboratory for analysis, therefore interfering with the diagnoses of this study.

CONCLUSIONS

This retrospective study demonstrated that the diagnosed skin diseases were more frequent in slaughter pigs and most of the bacterial origin. The three main diagnoses were swine erysipelas, PDNS and allergic dermatitis. Followed by cases of exudative epidermitis, vesicular dermatitis, pityriasis rosea, bacterial septicemia with cutaneous manifestations, swinepox and multiple hemorrhages without a defined cause. Skin neoplasms in pigs were rare.

Acknowledgments. - The authors thank the “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES) for supporting this study.

Conflict of interest statement. - The authors declare having no conflicts of interest.

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Table 1. Occurrence of the main skin diseases in swine at different stages of production and slaughter histopathologically diagnosed by the “Setor de Patologia Veterinária” of UFRGS during the period from 2006 to 2018.

Disease	Stages of production						
	Maternity	Nursery	Growth	Finishing	Slaughter	Adults	NI*
Swine erysipelas	-	2	-	1	43	-	1
PDNS**	-	3	9	6	2	2	1
Allergic dermatitis	1	-	2	5	8	2	1
Exudative epidermitis	3	9	-	-	-	1	2
Vesicular dermatitis	9	-	-	-	-	-	-
Pityriasis rosea	-	-	9	-	-	-	-
Bacterial septicemia	5	2	2	-	-	-	-
Swinepox	-	-	-	-	-	-	9
Multiple hemorrhages	6	1	-	-	-	-	-
Total	24	17	22	12	53	5	14

*NI = not informed, **PDNS = porcine dermatitis and nephropathy syndrome.

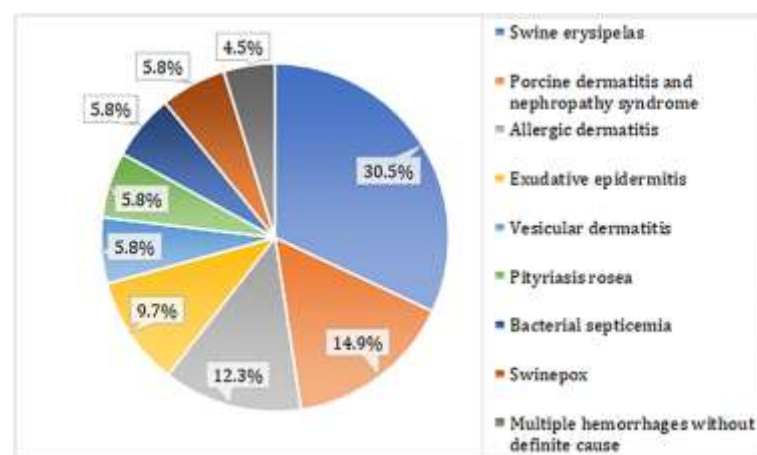


Fig.1. Frequency of the main skin diseases in swine diagnosed by the “Setor de Patologia Veterinária” of UFRGS from 2006 to 2018.

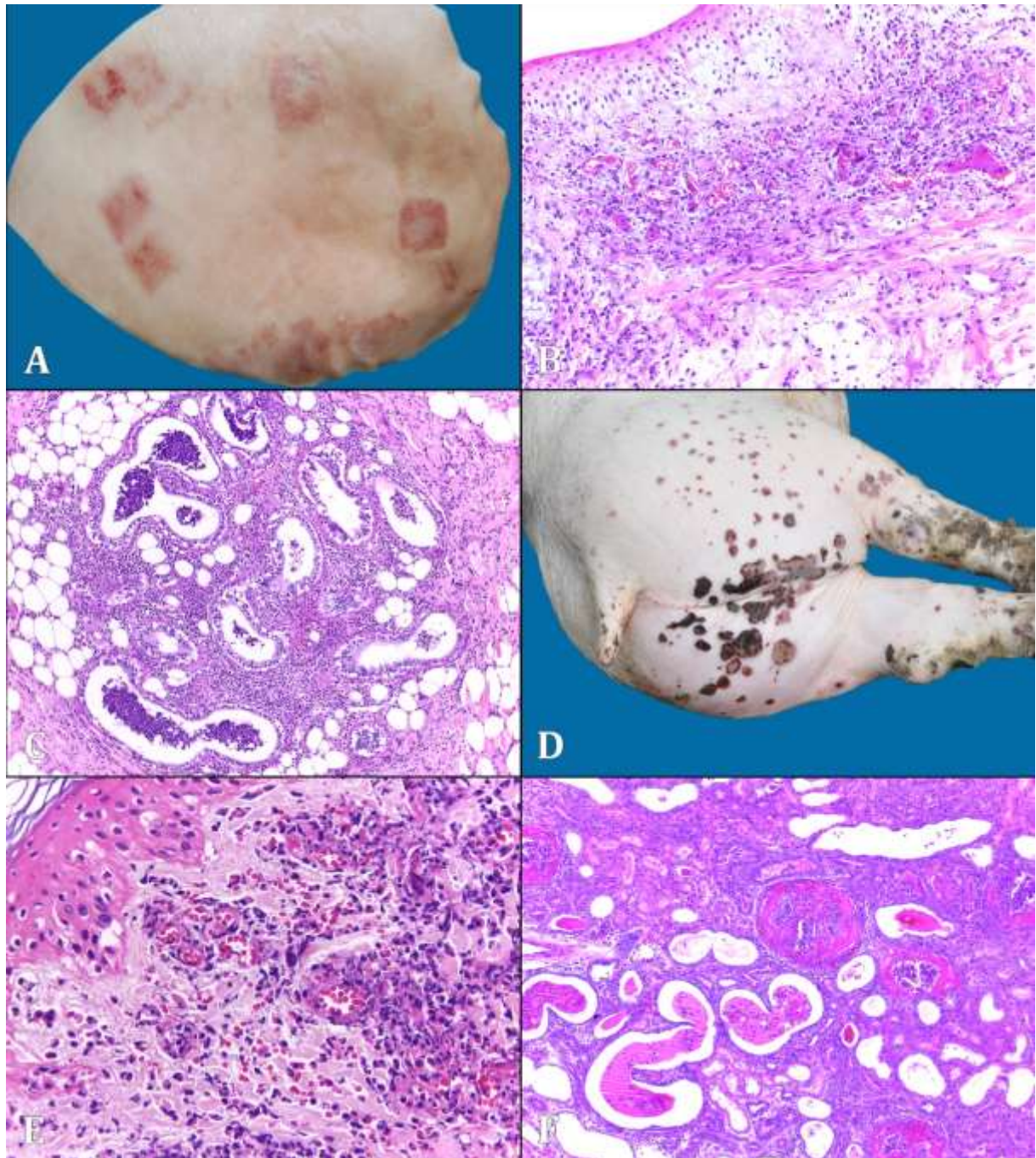


Fig.2. **(A)** Swine erysipelas grossly with reddish multifocal lesions, with raised edges and a characteristic diamond shape. **(B)** Swine erysipelas. Histologically, multifocal areas of necrosis were observed in the dermis and subcutaneous tissue, also multifocal vasculitis, fibrinoid vascular degeneration and thrombosis. HE, obj.20x. **(C)** Swine erysipelas. Marked inflammatory infiltrate surrounding and invading the sweat glands. HE, obj.10x. **(D)** Porcine dermatitis and nephropathy syndrome (PDNS). Gross appearance of the skin, dark red multifocal macules and papules in the pelvic limbs and perineal region. **(E)** PDNS. Microscopically, dermal vasculitis with mixed inflammatory infiltrate and fibrinoid vascular degeneration, also multifocal hemorrhage. HE, obj.40x. **(F)** PDNS. In the kidneys, there was a marked fibrinosuppurative glomerulitis. HE, obj.10x.

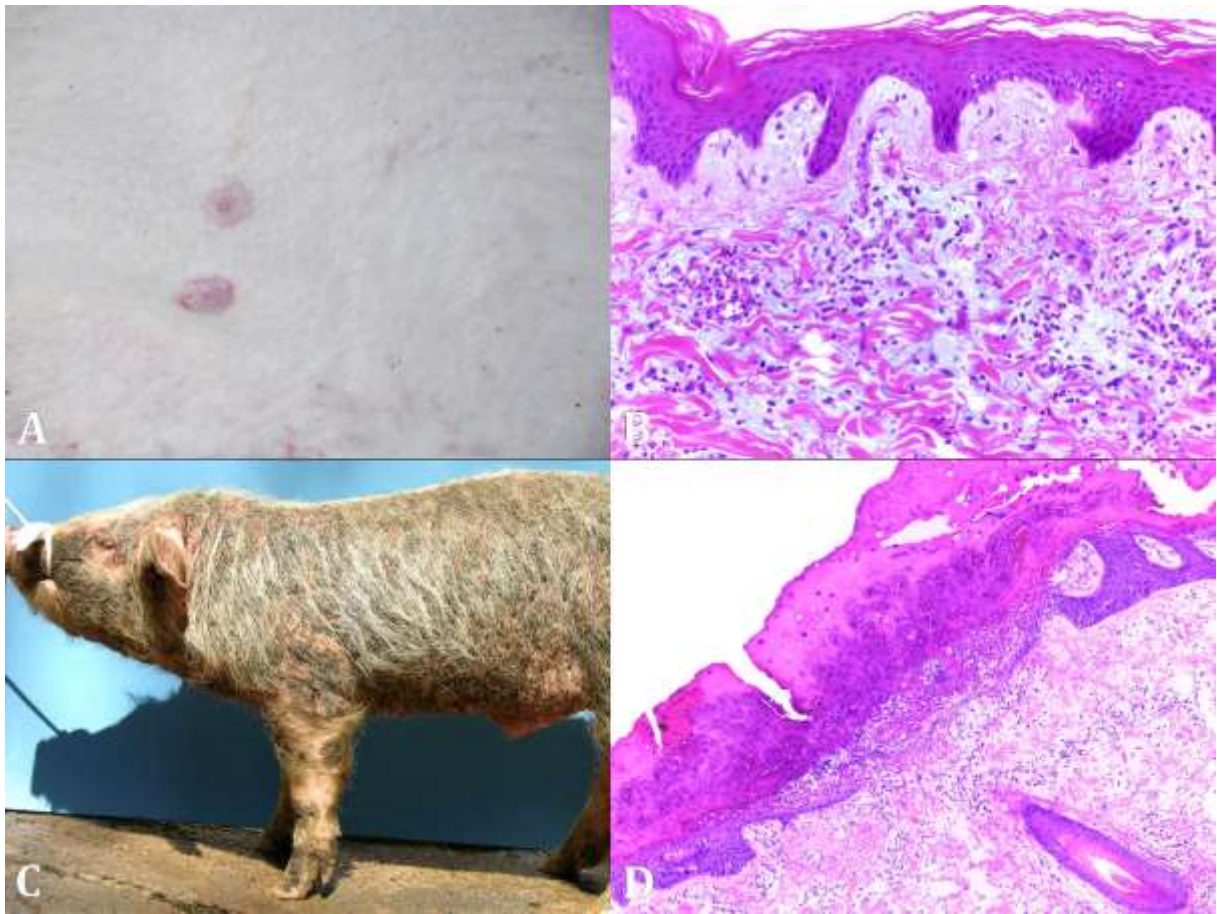


Fig.3. **(A)** Allergic dermatitis. Small round, red and raised spots grossly. **(B)** Allergic dermatitis. Multifocal eosinophilic dermatitis, sometimes perivascular, associated with edema. HE, obj.20x. **(C)** Exudative epidermitis. Marked greasy exudate and formation of crusts. **(D)** Exudative epidermitis. Microscopically observed ulceration of the epidermis, and crusted formations with marked deposition of fibrin, neutrophilic inflammatory infiltrate and coccoid bacterial aggregates. HE, obj.10x.

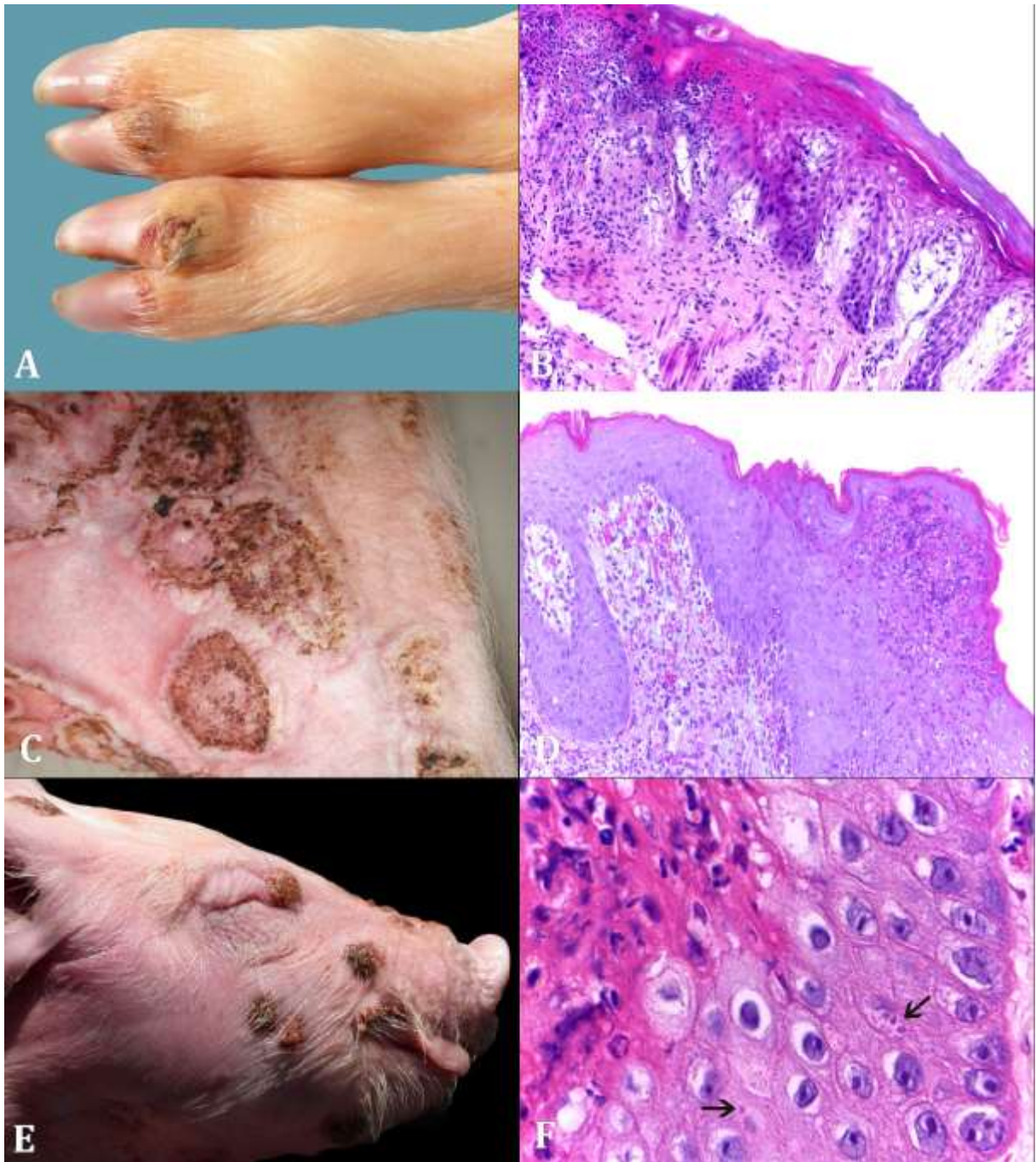


Fig.4. **(A)** Vesicular dermatitis. Ulcerative lesions in the coronary band in the hoof of a piglet. **(B)** Vesicular dermatitis. In the left corner of the figure, discrete focal necrosis of the epidermis with infiltrate neutrophils and fibrin. Adjacent epidermis hydropic degeneration in keratinocytes. HE, obj.20x. **(C)** Pityriasis rosea. Circular lesions outlined by a raised border on the abdomen and medial face of the pelvic limbs in pig. **(D)** Pityriasis rosea. Histologically, marked hyperplasia of keratinocytes and multifocal intracorneal pustules. In the dermis mixed multifocal inflammatory infiltrate. HE, obj.20x. **(E)** Swinepox. Vesicles, pustules and crusts on the face and ears. **(F)** Swinepox. Arrows show intracytoplasmic eosinophilic inclusion bodies of approximately $3\mu\text{m}$ in the cytoplasm of keratinocytes. HE, obj.100x.

3. ARTIGO 2

Nesse item é apresentado o artigo intitulado:

**Anatomopathological aspects and the use of immunohistochemistry in slaughter pigs
with cutaneous lesions of erysipelas**

Paula R. Pereira, Cíntia De Lorenzo, Bruna Correa Lopes, Luciana Sonne, Saulo P. Pavarini
and David Driemeier

Artigo a ser submetido ao periódico **Pesquisa Veterinária Brasileira**

Anatomopathological aspects and the use of immunohistochemistry in slaughter pigs with cutaneous lesions of erysipelas¹

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ABSTRACT.- Pereira P.R., De Lorenzo C., Lopes B.C., Sonne L., Pavarini S.P. & Driemeier D. 2021. **Anatomopathological aspects and the use of immunohistochemistry in slaughter pigs with cutaneous lesions of erysipelas.** *Pesquisa Veterinária Brasileira* 00(0):00-00. Setor de Patologia Veterinária, Departamento de Patologia Clínica Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, Prédio 42505, Porto Alegre, RS 91540-000, Brazil. E-mail: paula.rpereira@hotmail.com

Swine erysipelas is a disease of worldwide distribution, responsible for causing economic losses in swine and considered an occupational zoonotic disease. It is estimated that 30%-50% of pigs are carriers and stress can predispose the appearance of clinical disease. The diagnosis of erysipelas in slaughter pigs becomes a challenge for pathologists, since the scalding and dehairing processes, routine in slaughterhouses, generate histological artifacts that often make the final diagnosis impossible. This work describes the anatomopathological aspects and evaluate the use of immunohistochemistry as a diagnostic tool in these cases. Forty-three cases of erysipelas in slaughter pigs were analyzed. Grossly, the lesions were characteristic pink, red, or purple raised rhomboid, rectangular or square lesions ("diamond skin"). Histologically, in the dermis and subcutaneous tissue, there were suppurative vasculitis, hydradenitis and folliculitis, as well as degeneration and necrosis of the vessel wall, thrombosis and multifocal necrosis. Suppurative vasculitis and damage to the blood vessel wall were observed in all cases, with varying degrees of severity. The immunohistochemical technique proved to be an effective complementary method of diagnosis, with positive immunostaining in 93%. In most cases, we observed mild immunostaining (57.5%), moderate in 22.5% and marked in 20%.

INDEX TERMS: *Erysipelothrix*, skin lesions, histology, vasculitis, immunohistochemistry, swine, slaughter.

RESUMO.- [Aspectos anatomopatológicos e o uso da imuno-histoquímica em suínos abatidos com lesões cutâneas de erisipela.] A erisipela suína é uma doença de distribuição mundial, responsável por causar prejuízos econômicos na suinocultura, além de ser uma doença zoonótica com caráter ocupacional. Estima-se que 30%-50% dos suínos sejam portadores e fatores estressantes podem predispor o aparecimento da doença clínica. O diagnóstico de erisipela em suínos de abate torna-se um desafio aos patologistas, uma vez que os processos de escaldagem e depila, rotineiros em abatedouros frigoríficos, geram artefatos histológicos que muitas vezes impossibilitam o diagnóstico final. Este trabalho descreve os aspectos anatomopatológicos e avalia o uso da imuno-histoquímica como uma ferramenta diagnóstica nestes casos. Foram analisados fragmentos de pele de 43 casos de erisipela em suínos de abate. Macroscopicamente, eram múltiplas lesões romboides, retangulares ou quadradas rosa, vermelho ou roxo característicos ("pele de diamante"). Histologicamente, na derme e subcutâneo, havia vasculite, hidradenite e foliculite supurativas, bem como degeneração e necrose da parede de vasos, trombose e necrose multifocais. A vasculite supurativa e a lesão na parede de vasos sanguíneos foram observadas em todos os casos, em diferentes graus de severidade. A técnica imuno-histoquímica se mostrou um eficaz método complementar de diagnóstico, com imunomarcção positiva em 93%. Na maior parte dos casos observamos marcação discreta (57,5%), moderada em 22,5% e acentuada em 20%.

TERMOS DE INDEXAÇÃO: *Erysipelothrix*, lesões de pele, histologia, vasculite, imuno-histoquímica, suíno, abate.

INTRODUCTION

Swine erysipelas (SE) is a disease with worldwide distribution (Wang et al. 2010), responsible for causing economic losses in pig farming, related to the death of animals, reproductive failures, treatment costs, growth delay and condemnations in slaughterhouses (Wood 1984, Pescador et al. 2007, Bender et al. 2011). So far, the genus *Erysipelothrix* consists of eight species: *E. rhusiopathiae* (Skerman et al. 1980), *E. tonsillarum* (Takahashi et al. 1987), *Erysipelothrix* sp. strain 1, *Erysipelothrix* sp. strain 2, *Erysipelothrix* sp. strain 3 (Takahashi et al. 2008), *E. inopinata* (Verborg et al. 2004), *E. larvae* (Bang et al. 2015) and *E. piscisicarius* sp. nov. (Pomaranski et al. 2019). For pigs, the most relevant species is *E. rhusiopathiae*,

although studies have already isolated *E. tonsillarum* from carcasses in slaughterhouses in the United States (Bender et al. 2011, Opriessnig & Coutinho 2019).

E. rhusiopathiae, is characterized by being a small Gram-positive rod, facultative intracellular and anaerobic, non-motile and non-spore-forming (Brooke & Riley 1999, Opriessnig & Coutinho 2019). This bacterium has been isolated from many species of domestic and wild mammals, fish, birds, reptiles, as well as humans, in which the disease is known as erysipeloid (Eamens et al. 1988, Kitajima et al. 1998, Pomaranski et al. 2018). Human infections occur mainly through direct contact with infected animals and are, therefore, occupational diseases for veterinarians, abattoir workers and meat, poultry, and fish processors (Colavita et al. 2006). Musewa et al. (2021) report a 9.9% prevalence of *E. rhusiopathiae* infection in butcher, abattoir workers and cook who handling raw pork. However, when considering only butchers and abattoir workers, the prevalence was 15% and 37%, respectively.

About 30%-50% of pigs are believed to be asymptomatic carriers of *E. rhusiopathiae*. The bacteria remain in the tonsils and other lymphoid organs and when these carriers are exposed to stressful factors, such as transport, food or temperature changes, they can develop clinical disease (Haesebrouck et al. 2004). Three clinical forms of ES are recognized: 1) acute form is a septicemic disease with sudden onset that can present with acute death, abort, and characteristic "diamond skin" lesions; 2) subacute form, clinically less severe than the acute form, with little or no skin lesion; 3) chronic form, with development of chronic arthritis and vegetative valve endocarditis (Opriessnig & Coutinho 2019).

In Brazil, the disease has been described in outbreaks of arthritis, acute sepsis, abortions, and skin lesions during inspections carried out on carcasses (Reis et al. 1977, Pescador et al. 2007, Piva Filho et al. 2011). When skin lesions are observed at slaughter and submitted for analysis, the diagnosis is often compromised, as the scalding and dehairing processes generate artifacts that can make it impossible or difficult to observe the histological lesions. Therefore, the aim of this study was to describe the anatomopathological aspects and evaluate the use of immunohistochemistry as a diagnostic tool in erysipelas skin lesions in pigs slaughtered in southern Brazil.

MATERIAL AND METHODS

From January 2006 to December 2019, the files of pathological examinations of the "Setor de Patologia Veterinária" of the "Universidade Federal do Rio Grande do Sul" (SPV-UFRGS) were reviewed, selecting cases of pigs with skin lesions suspected of erysipelas. Only skins with lesions from the inspection lines of slaughterhouses were included in the study. The protocols were reviewed, and information, such as history, macroscopic description of the lesions and results of bacteriological examination were analyzed and compiled. Routine isolation was performed in blood agar (5% sheep blood; Mueller Hinton, Kasvi®, Brazil) and MacConkey (Kasvi®, Brazil) to aid in the detection of contaminants. The plates were incubated aerobically for 24–48 hours at 35°C, with increased growth in micro aerobiosis. Serial sections of the paraffin-embedded blocks were performed, and histological slides were prepared and stained using the hematoxylin and eosin technique for further microscopic description. Histological lesions were classified according to severity as mild, moderate, and marked. For the immunohistochemistry technique (IHC), Advanced Adhesive positive slides were used, for better adherence of the skin fragments. IHC was performed with a polyclonal antibody produced by inoculation of an ATCC strain of *E. rhusiopathiae* in rabbits, at a dilution of 1:1500, by the universal polymer method labeled with peroxidase (MACH 4, Universal HRP-Polymer, Biocare Medical). For antigen retrieval, protease XIV was used for 15 minutes, and the reaction was revealed with 3-amino-9-ethylcarbazole (AEC) and counterstained with Mayer's hematoxylin. As positive controls, routine cases with previous bacteriological culture of *E. rhusiopathiae* were used, and for negative control the primary antibody was replaced by phosphate-buffered saline (PBS). For IHC classification, immunostained *E. rhusiopathiae* were counted in 10 random fields under optical microscopy with 60x magnification. We classified it as mild, when there was one to four bacteria per field, moderate (five to 10 per field) and marked (more than 11 per field).

RESULTS

A total of 8,071 swine protocols were reviewed, of which 172 (2.13%) corresponded to skin lesions. Of these, 43 (25%) had macroscopic and histological lesions suggestive of SE and came from slaughterhouses. In the protocols, grossly pink, red, or purplish multifocal lesions were described, with rectangular to rhomboid shape, characteristics of "diamond skin".

There was information in the protocols, of six cases (14%) in which the skin was referred for bacteriological analysis and there was no growth of *E. rhusiopathiae*. In other cases, the skin fragments were fixed in 10% formalin, making it impossible to carry out a bacteriological examination. In the histological

analysis, we observed that in 33 cases (77%) the skin had artifacts resulting from the processes of scalding and dehairing, characterized by loss of the epidermis and dermis coagulation, it was noted that the skin structures became hypereosinophilic and sometimes with cells elongated epithelial and inflammatory lesions, making it difficult to observe and interpret the lesions (Fig.1A).

Inflammatory histological lesions were observed multifocally in the dermis and subcutaneous tissue, in cases where there was a high degree of artifacts, our analysis to describe the lesions below was restricted to the most preserved layers of the dermis and subcutaneous tissue, excluding the epidermis and superficial dermis. The infiltrate consisted mostly of neutrophils, and a smaller number of lymphocytes and macrophages. The most frequent histological lesion was vasculitis, observed in all cases, and characterized by an inflammatory infiltrate surrounding and intermingling the wall of blood vessels, associated with degeneration and necrosis of the wall of these vessels (Fig.1B). In 90.7% of cases, hidradenitis was observed, in which the infiltrate was around and in the ducts of sweat glands (Fig.1C). Hyperemia of the dermis capillaries was observed in 83.7%, while occlusion of blood vessels due to fibrin and cell debris deposition (thrombosis) was observed in 81.4% (Fig.1D). There were multifocal areas of coagulation necrosis in 74.4% of cases, mainly observed in the deep dermis and subcutaneous tissue, and in the 10 cases without artifacts it was also possible to observe in the superficial dermis. In 46.5% of the cases there was an inflammatory infiltrate surrounding the hair follicles and extending to the follicular wall (perifolliculitis and mural folliculitis). The classification according to the severity of the histological lesions is detailed in table 1.

When considering all histological lesions, we observed that most cases had mild severity, and few cases were marked. Selecting the 10 cases without artifacts, we noticed an increase in the severity of some lesions. Vasculitis was mild in 4/10, moderate in 5/10 and marked in 1/10, hidradenitis was mild in 2/10, moderate in 5/10 and marked in 2/10. Hyperemia in capillaries was observed in all cases, mild in 2/10, moderate in 4/10 and marked in 4/10. Thrombosis was moderate in 2/10 and marked in 6/10, while perifolliculitis and mural folliculitis were mild in 3/10, moderate in 3/10 and marked in 3/10.

The immunohistochemical test was performed in 43 cases, with immunostaining in 93% (40/43). Marking was mild in 57.5% (23/40), moderate in 22.5% (9/40) and marked in 20% (8/40). Multifocal immunostaining of antigens was observed, characterized by small rods, freely and occasionally visualized in the cytoplasm of macrophages, in the dermis and subcutaneous tissue, in areas of necrosis and surrounding blood vessels and accessory structures (sweat glands and hair follicles) (Fig.1E e 1F).

DISCUSSION

SE can present with skin, joint, cardiac, or septicemic lesions in pigs (Hoffmann & Bilkei 2002). We selected for the study only cases with skin lesions in slaughter pigs, through a retrospective study. The onset of lesions during slaughter can be explained by the worsening of the disease, triggered by transport stress, or by the mixture of animals from different origins in the pre-slaughter period (Schwartz 2002).

Considering that erysipelas is an occupational zoonotic disease, and that infection in humans occurs mainly through direct contact with infected animals, in the European Union, pigs with lesions in ante-mortem inspection must have their slaughter postponed for at least 15 days, and postmortem erysipelas carcasses should be condemned in order to ensure a good level of meat safety and reduce the risk of occupational disease (Colavita et al. 2006). In Brazil, according to Decree 10.468, carcasses with multiple skin lesions, arthritis aggravated by necrosis or signs of systemic effect must be condemned. When there is a discrete and localized skin lesion, without compromising the organ or the carcass, the conditional use of heat must occur, after removal of the affected area (Brasil 2020). Therefore, for the correct destination of the carcasses, accurate and quick diagnosis is very importance.

In slaughter pigs, skin lesions were described, as observed in the acute and subacute forms of the disease, with characteristic lesions of "diamond skin" (Opriessnig & Coutinho 2019, Mauldin & Peters-Kennedy 2016). It is believed that these injuries occur due to the invasion of bacteria in the vascular endothelium and triggering a hypersensitivity reaction (Shankar et al. 2009). The histological findings observed were suppurative vasculitis associated with degeneration and necrosis of the blood vessel wall, hidradenitis suppurativa, capillary hyperemia and thrombosis, in addition to necrosis of the dermis and subcutaneous tissue, corroborating data in the literature (Shankar et al. 2009, Mauldin & Peters-Kennedy 2016).

In the histological evaluation, we observed that in almost 80% of the cases, the skin presented artifacts generated by the processes of scalding and dehairing. The epidermis in these cases was not assessable, and in cases where there was a high degree of artifacts, our analysis was restricted to preserved layers (deep dermis and subcutaneous tissue). Therefore, only in the 10 intact cases we observed coagulation necrosis also in the superficial dermis. According to the literature (Shankar et al. 2009, Mauldin & Peters-Kennedy 2016) in cases of swine erysipelas, histological lesions are not observed in the epidermis,

however, in these cases, differential diagnosis of other lesions that may affect the skin of pigs is impaired (Pereira et al. 2020). During the study, few cases had marked severity of histological lesions, however when considering only the histologically preserved cases, we observed an increase in the severity of some lesions. As well, we observed that, with the exception of vasculitis, which was present in all cases, other lesions had different frequencies. This is possibly due to the difficulty in analyzing cases with artifacts, since a part of the sample is impracticable and sometimes the identification of inflammatory cells and histological lesions is impaired, interfering with the severity, frequency and description of the lesions.

For diagnostic confirmation, there are some alternatives such as bacterial isolation, IHC, polymerase chain reaction (PCR) and antibody detection by serological assays (Opriessnig & Coutinho 2019). The method of choice should be based on cost, required response time and availability in different geographic regions. In our study, most samples were received formalinized, without the possibility of bacterial isolation, and in the cases in which it was performed, there was no growth. Skin lesions and lesions associated with chronic forms can be difficult to isolate the agent (Markey et al. 2013). Isolation can be difficult because they are small colonies with a slow growth rate, in addition to having sensitivity affected by tissue conditions and by the antimicrobial treatment of the pigs (Bender et al. 2009).

The IHC technique proved to be an effective method of diagnosing SE, even in cases that presented histological artifacts of scalding and dehairing, with positivity in 93% of cases. Opriessnig and contributors (2010) demonstrated that IHC was quite sensitive and specific, especially in antibiotic-treated pigs, chronically infected, and reported that the technique was useful in skin lesions, which often present negative cultures. As noted, in cases where there was no bacterial isolation, and IHC positivity. Opriessnig et al. (2010) observed bacteria in the lumen and around superficial vessels in the dermis in experimentally inoculated pigs. We observed, in addition to immunostaining surrounding blood vessels, also in areas of necrosis and surrounding accessory structures (sweat glands and hair follicles). Thus, the IHC technique becomes an ally in the diagnosis, for samples paraffinized or sent in formalin, as well as for skin with negative cultures and for cases with artifacts arising from processes carried out in slaughterhouses.

CONCLUSIONS

In our retrospective study, we noted that scalding and dehairing processes make it difficult to analyze histological lesions of erysipelas skin lesions in slaughter pigs. In these cases, the immunohistochemical technique was essential for the definitive diagnosis, it proved to be an excellent diagnostic tool, and an efficient and easy-to-perform method. Histological findings of swine erysipelas were suppurative vasculitis associated with degeneration and necrosis of the blood vessel wall, observed in all cases. Other histological lesions observed were hidradenitis suppurativa, hyperemia in the dermis and thrombosis, in addition to necrosis of the dermis and subcutaneous tissue, perifolliculitis and mural suppurative folliculitis.

Acknowledgments. - The authors thank the “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES) for supporting this study (Code 001). The authors are grateful to PhD Tanja Opriessnig for having provided the first antibody, with which it was possible to establish the immunohistochemical protocol and to carry out pilot studies.

Conflict of interest statement. - The authors declare having no conflicts of interest.

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Table 1. Severity of histological lesions of swine cutaneous erysipelas.

Histological lesion	Mild	Moderate	Marked	Absence
Vasculitis and suppurative fibrinoid degeneration	20/43	18/43	5/43	0/43
Hidradenitis suppurativa	17/43	16/43	6/43	4/43
Hyperemia in the dermis	21/43	9/43	6/43	7/43
Thrombosis	14/43	12/43	9/43	8/43
Dermal and/or subcutaneous necrosis	17/43	10/43	5/43	11/43
Perifolliculitis and suppurative mural folliculitis	9/43	7/43	4/43	23/43

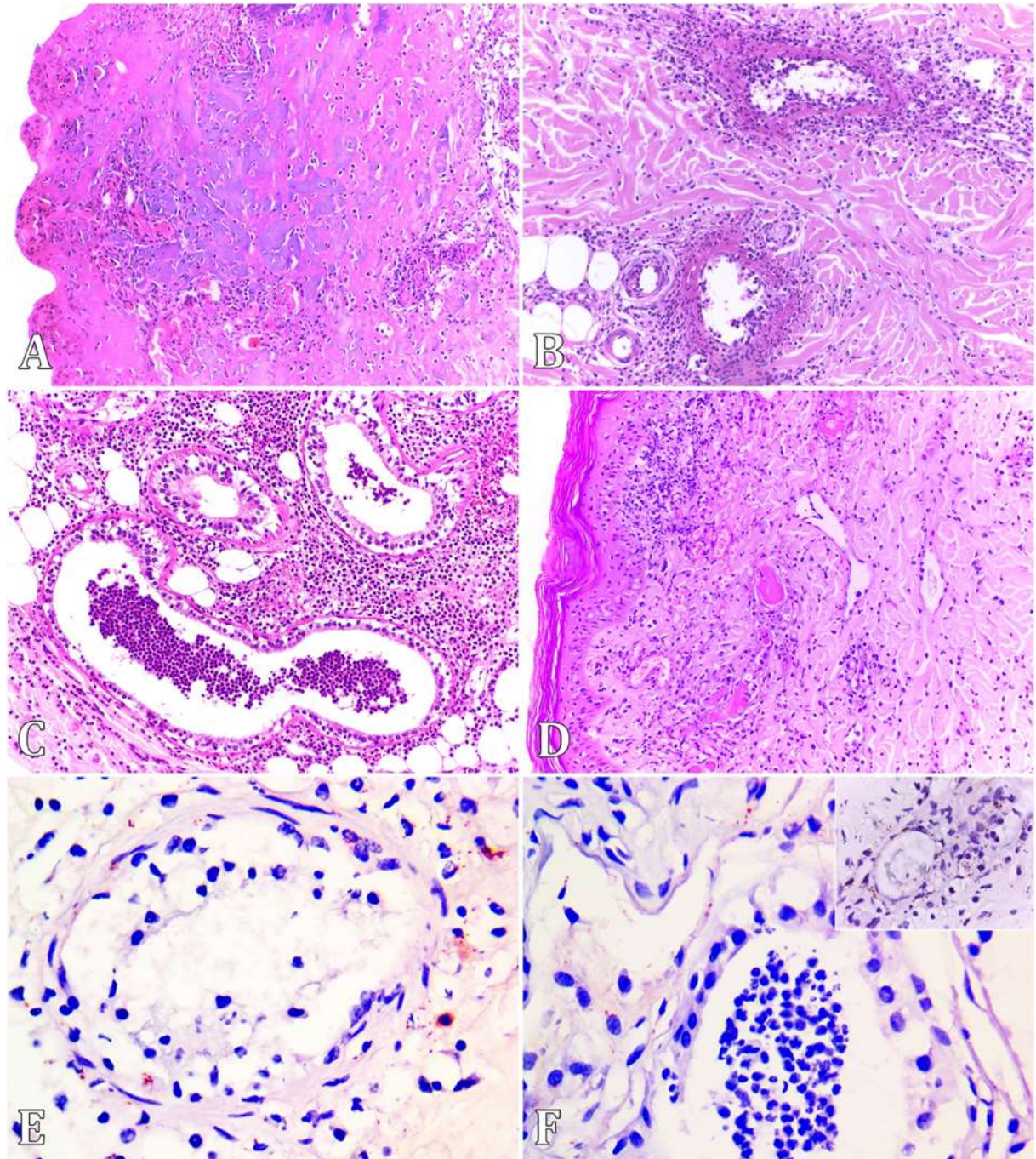


Fig.1. Swine erysipelas, skin (A) Artifacts resulting from scalding and dehairing, there is loss of the epidermis and dermis coagulation. HE, obj.20x. (B) Accentuated predominantly neutrophilic inflammatory infiltrate surrounding and intermingling the blood vessels wall, associated with fibrinoid vascular degeneration and necrosis of the wall of these vessels. HE, obj.20x. (C) Marked inflammatory infiltrate around and in the duct of sweat glands (hydradenitis). HE, obj.20x. (D) There is hyperemia of capillaries in the superficial dermis and occlusion of blood vessels by fibrin and cell debris (thrombosis). In addition to a multifocal inflammatory infiltrate in the dermis, predominantly perivascular. HE, obj.20x. (E and F) Immunolabelling anti-*E. rhusiopathiae*, multifocal, of small rods, free and in the cytoplasm of macrophages, surrounding blood vessels (E) and sweat glands (F). IHQ, 3-amino-9-ethylcarbazole (AEC), obj.60x. **Inset:** IHQ, AEC, obj.100x

4. CONSIDERAÇÕES FINAIS

- Através de estudo retrospectivo, de 2006 a 2018, analisamos 154 casos conclusivos, incluindo as doenças restritas a pele e as secundárias a doenças sistêmicas com manifestações cutâneas. Dessas, as dermatites bacterianas foram as mais frequentes em suínos, representaram 46,1% dos casos.
- Suínos de abate foram os mais diagnosticados.
- A erisipela suína foi a enfermidade mais diagnosticada, seguida por síndrome dermatite nefropatia suína, dermatite alérgica e epidermite exsudativa.
- Observamos ainda dermatite vesicular, pitiríase rósea, septicemia bacteriana com manifestações cutâneas, varíola suína e hemorragias múltiplas sem causa definida. Em menor número, cisto folicular, hiperqueratose sem causa definida, papiloma e hemangioma escrotal.
- Através de estudo retrospectivo, de 2006 a 2019, analisamos 43 casos de erisipela em suínos de abate. Observamos que os processos de escaldagem e depila, rotineiros em abatedouros frigoríficos, prejudicam a análise histológica. Nestes casos, a técnica imuno-histoquímica, mostrou-se uma ótima ferramenta diagnóstica, com imunomarcção positiva em 93% dos casos.
- Histologicamente, as lesões de erisipela suína foram vasculite supurativa associada a degeneração e necrose da parede de vasos sanguíneos, observadas em todos os casos. Observamos ainda hidradenite supurativa, hiperemia dos capilares e trombose, além de necrose da derme e tecido subcutâneo, perifoliculite e foliculite mural supurativas.

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