



## Anatomopathological aspects and the use of immunohistochemistry in slaughter pigs with cutaneous lesions of erysipelas<sup>1</sup>

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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% to 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 scalding and dehairing, routine procedures in slaughterhouses, generate histological artifacts that often make the final diagnosis impossible. This study 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 cutaneous 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, hidradenitis and folliculitis, as well as degeneration and necrosis of the vessel wall, thrombosis and multifocal areas of 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 cutâneas 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 áreas de 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.

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## 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. 2020). 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 butchers, abattoir workers and cooks, who handle raw pork. However, when considering only butchers and abattoir workers, the prevalence was 15% and 37%, respectively.

About 30% to 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 SE are recognized: 1) acute form is a septicemic disease with sudden onset that can present with acute death, abort, and the classical "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 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.

## MATERIALS 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 to 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 (HE) technique for further microscopic description. Histological lesions were classified according to severity as mild, moderate, and marked. For the immunohistochemistry (IHC) technique, 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 *Erysipelothrix 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 *Erysipelothrix 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.1).

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.2). In 90.7% of cases,

hidradenitis was observed, in which the infiltrate was around and in the ducts of sweat glands (Fig.3). Hyperemia of the dermal 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.4). 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.5 and 6).

## 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” (Mauldin & Peters-Kennedy 2016, Opriessnig & Coutinho 2019). 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, suppurative hidradenitis, 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, except for 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

**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   |

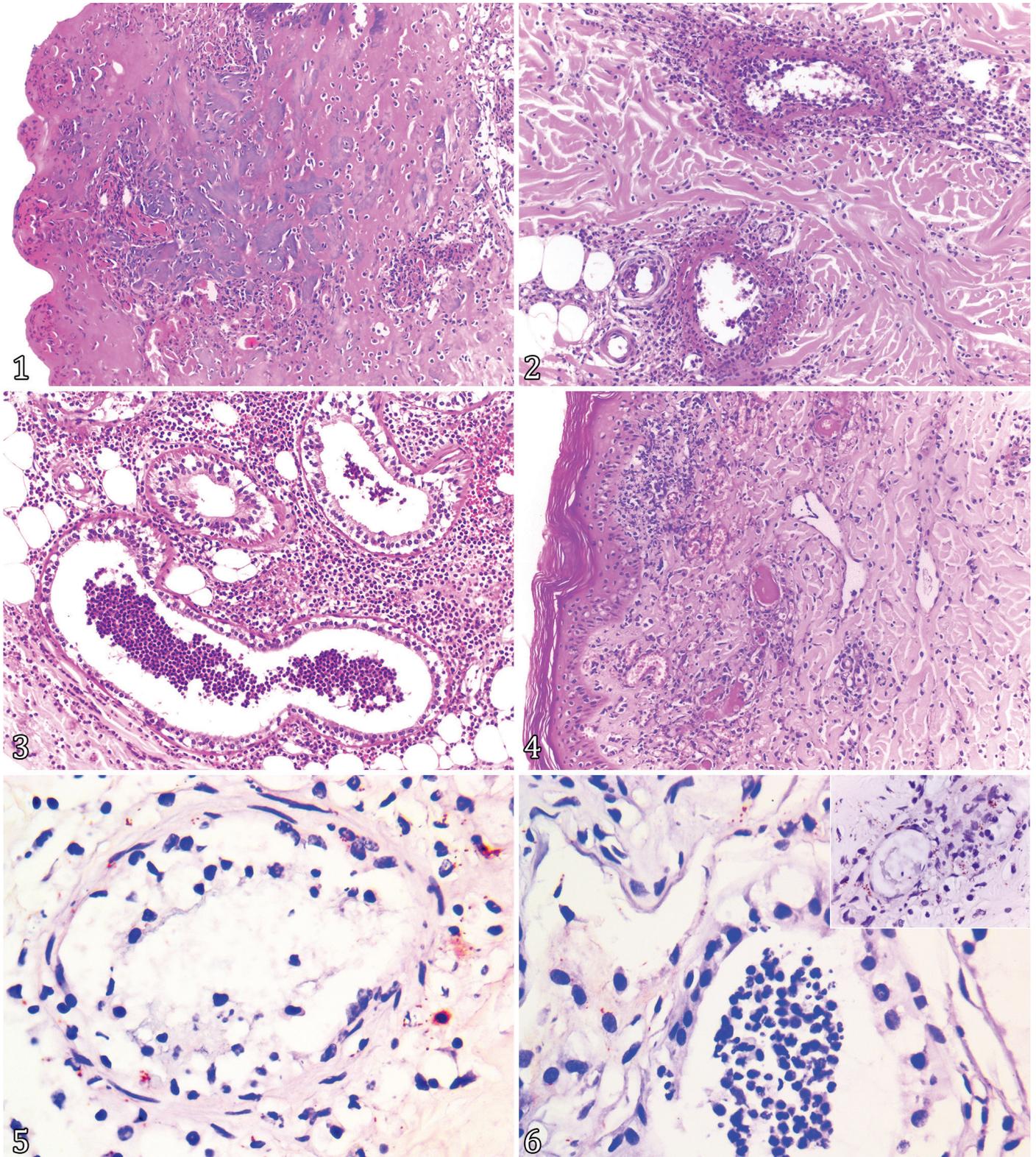


Fig.1-6. Swine erysipelas, skin (1) Artifacts resulting from scalding and dehairing, there is loss of the epidermis and dermis coagulation. HE, obj.20x. (2) Accentuated predominantly neutrophilic inflammatory infiltrate surrounding and intermingling the blood vessels wall, associated with fibrinoid vascular degeneration and necrosis of the wall of these vessels. HE, obj.20x. (3) Marked inflammatory infiltrate around and in the duct of sweat glands (hidradenitis). HE, obj.20x. (4) 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. (5 and 6) Immunolabelling anti-*Erysipelothrix rhusiopathiae*, multifocal, of small rods, free and in the cytoplasm of macrophages, surrounding (5) blood vessels and (6) sweat glands. IHC, 3-amino-9-ethylcarbazole (AEC), obj.60x. Inset: IHC, AEC, obj.100x.

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 et al. (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 impair the analysis 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.

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

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