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Economic impact of chronic pleural lesions and consequent disqualification of carcasses for export during inspection in swine slaughterhouses[1]

Nilson Rocha[2], Marcos A.Z. Mores[3]*, Diógenes Dezen[2], Nelson Mores[3], Arlei Coldebella[3], Raquel Rebelatto[3] and Jalusa D. Kich[3]

ABSTRACT.- Rocha N., Mores M.A.Z., Dezen D., Mores N., Coldebella A., Rebelatto R. & Kich J.D. 2022. **Economic impact of chronic pleural lesions and consequent disqualification of carcasses for export during inspection in swine slaughterhouses**. *Pesquisa Veterinária Brasileira 42:e07118, 2022.* Embrapa Suínos e Aves, BR-153 Km 110, Vila Tamanduá, Concórdia, SC 89715-899, Brazil. E-mail: marcos.mores@embrapa.br

Chronic pleuritis is the main reason for sending pig carcasses to the Department of Final Inspection (DIF), condemnation and led to economic losses to industries and producers. Most pleura lesions detected after slaughter are sequelae from bacterial infections by agents that do not pose risks to pork consumers. The objective of the present study was to generate science-based information for decision making in the evaluation and destination of chronic pleuritis by the Federal Inspection Service (SIF). Therefore, 200 carcasses, with and without pleurisy, from a swine slaughterhouse with SIF were assessed following the visual classification of the inspection agent. The study was carried out in two stages. In stage 1, 50 carcasses with pneumonic lesions adjacent to chronic pleuritis and 50 carcasses with only chronic pleuritis lesions were evaluated, through macroscopy, histopathology, and bacterial culture. In stage 2, 50 swine carcasses with chronic pleuritis and 50 without this lesion were sampled in the parietal pleura region to bacterial culture and PCR. The economic impact of not exporting these carcasses with chronic pleuritis was also assessed. Considering the stages of evolution of the lesions, the macroscopic examination showed high correlation with the histological examination. There was no bacterial isolation through pleural swabs, regardless of the presence or not of adjacent pulmonary lesions. Isolation was restricted to the adjacent pulmonary lesions of 70% samples, with *Pasteurella multocida* type A found in 48% of them, followed by *P. multocida* type D and *Streptococcus suis* in 12%, and *Actinobacillus pleuropneumoniae* in 3%. Only *Streptococcus suis* DNA was detected in 5/100 samples, with no correspondence to the isolation of viable bacteria. The reliability demonstrated in the macroscopic evaluation carried out during inspection, the absence of viable bacteria in the chronic pleural lesions, and the negative economic impact suggest that carcasses with chronic pleuritis can be submitted to pleura removal, with no need of sending to DIF.

INDEX TERMS: Slaughterhouse, condemnations, chronic pleurisy, swine.

RESUMO.- [**Impacto econômico de lesões pleurais crônicas e consequente desqualificação de carcaças para exportação durante inspeção em frigoríficos de suínos.**] Pleurite crônica é a principal causa do desvio de carcaças de suínos para o Departamento de Inspeção Final (DIF), podendo causar condenação e prejuízos econômicos às indústrias e produtores. A maioria das lesões de pleura detectadas após o abate são sequelas de infecções bacterianas por agentes que não oferecem riscos aos consumidores de carne suína. O objetivo do presente estudo foi gerar informações científicas para a tomada de decisão na avaliação e destino da pleurite crônica pelo Serviço de Inspeção Federal (SIF). Para tanto, 200 carcaças, com e sem pleurisia, provenientes de um frigorífico de suínos com SIF foram avaliadas seguindo a classificação visual do agente fiscalizador. O estudo foi realizado em duas etapas. No estágio 1, 50 carcaças com

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[2] Graduate Program in Animal Health, Instituto Federal Catarinense (IFC), Campus Concórdia, Rodovia SC-283 s/n, Fragosos, SC 89703-720, Brazil.
[3] Embrapa Suínos e Aves, BR-153 Km 110, Vila Tamanduá, Concórdia, SC 89715-899, Brazil. *Corresponding author: marcos.mores@embrapa.br

lesões pneumônicas adjacentes à pleurite crônica e 50 carcaças apresentando somente lesões de pleurite crônica foram avaliadas macroscopicamente, por histopatologia e cultura bacteriana. No estágio 2, 50 carcaças suínas com pleurite crônica e 50 sem esta lesão foram amostradas na região da pleura parietal para cultura bacteriana e PCR. O impacto econômico de não exportar essas carcaças com pleurite crônica também foi avaliado. Considerando os estágios de evolução das lesões, o exame macroscópico apresentou alta correlação com o exame histológico. Não houve isolamento bacteriano por meio de swabs pleurais, independentemente da presença ou não de lesões pulmonares adjacentes. O isolamento foi restrito às lesões pulmonares adjacentes de 70% das amostras, sendo *Pasteurella multocida* tipo A encontrado em 48% delas, seguido por *P. multocida* tipo D e *Streptococcus suis* em 12%, e *Actinobacillus pleuropneumoniae* em 3%. Apenas DNA de *Streptococcus suis* foi detectado em 5/100 amostras, sem correspondência com o isolamento de bactérias viáveis. A confiabilidade demonstrada na avaliação macroscópica realizada durante a inspeção, a ausência de bactérias viáveis nas lesões pleurais crônicas e o impacto econômico negativo sugerem que carcaças com pleurite crônica podem ser submetidas à remoção da pleura, sem necessidade de envio para DIF.

TERMOS DE INDEXAÇÃO: Frigorífico, condenações, pleurisia crônica, suínos.

INTRODUCTION

Pleurisy or chronic pleuritis is consequent of bacterial infection in the pleura, the lesion is characterized by proliferation of connective tissue, most often causing fibrous adhesion between the parietal and visceral pleura (Jäger et al. 2012).

Several lesions observed in swine slaughter, which are currently under the scope of the official inspection service, are known to not have impacts on public health. Moreover, many lack information to guarantee the absence of risk and support changes in regulations regarding final destination. Among these lesions, in terms of occurrence and reason for viscera and carcass condemnation, are chronic pleuritis, both in Brazil (Silva 2016, Coldebella et al. 2017) and in other countries (Bahnson et al. 1992, Christensen & Enoe 1999). Carcasses affected by pleurisy are deviated to the "Departamento de Inspeção Final" (Final Inspection Department - DIF) and, following legislation in place up to 2018, were disqualified for exportation, receiving a stamp marked "NE (no export)" (Brasil 1995). With the new legislation (Brasil 2018), the conditional use or not of these carcasses follows international requirements, and in some importing countries there are restrictions that state they must be rejected. In these cases, the disqualification for the export market reduces the aggregate value of the carcasses and their cuts (Morés et al. 2017), decreases the volume of exported pork cuts, in addition to other losses such as speed reductions in the slaughter line for the removal of these lesions (Jäger et al. 2012).

Pleuritis is often diagnosed in pigs as a result of bacterial infections, primary or secondary to pneumonia. The main bacteria involved in pleuritis are *Actinobacillus pleuropneumoniae*, *Glaesserella parasuis*, *Mycoplasma hyorhinis*, *Pasteurella multocida* A and D and *Streptococcus suis* (Jirawattanapong et al. 2010, Morés et al. 2016). However, in a qualitative risk evaluation, these agents are not listed as hazardous regarding the consumption of pork from industrial swine farming (Costa et al. 2017).

With changes to the zoonotic profile attributed to pork over time, inspection criteria must be modernized. However, the change in criteria must be supported by risk analyses that consider the occurrence of hazards, their amplification across the production chain, and consequences to the consumer. In this context, there is a lack of studies about the presence of viable pathogens in chronic pleuritis, characterized by parietal and visceral pleural adherence, and the implication of these agents to public health.

The present study aimed to identify the presence of viable bacteria in chronic pleuritis lesions causing carcass deviation to the DIF in pig slaughterhouses, generating science-based information for decision making regarding changes to criteria for the assessment and destination of carcasses with pleuritis by the Federal Inspection Service of Brazil.

MATERIALS AND METHODS

Study local and contextualization. This was a cross-sectional study carried out in a swine slaughterhouse, located in Santa Catarina State, Brazil, with a daily slaughter capacity of 4,000 animals. A total of 200 swine carcasses were sampled. The size of the sampling was determined through the formula of Cannon (2001). To obtain greater variability, a maximum limit of five samples per producer was established. To model the "p" proportion of isolations we used the Beta (s+1, n-s+1) distribution, in which "s" is the number of positive isolations and "n" the number of samples (Vose 2008). The study was conducted in two stages (Table 1). In stage 1, a total of 100 carcasses with chronic pleuritis were sampled, of which 50 presented adjacent pneumonic lesions and 50 only presented pleural lesions. In stage 2, another 100 carcasses were sampled in the parietal pleura region, considering that 50 of them had chronic pleuritis lesions and 50 did not.

Sample collection. Carcasses with pleural lesions were identified in the inspection lines and deviated to the DIF, always by the same veterinarian, following the usual inspection criteria. These were classified by one inspection agent as chronic, subacute, or acute. The lesions were classified as chronic when the pleura was thickened by an opaque and firm whitish material (fibrous tissue), there were fibrous adherences between the visceral and parietal pleura, without fibrinous or suppurative exudate; subacute cases also showed fibrous adherences between the pleurae, associated with the presence of a focal or multifocal wet yellowish exudate (fibrin); and acute lesions had loose adherences due to wet yellowish fibrinous exudate. Sampling was only conducted on carcasses with lesions classified as chronic in the visual examination. For the histopathological tests, samples were taken during stage 1, with the collection of the lesioned pleura and fragment of the adjacent lung. In cases where the lesions were diffuse, samples were collected from the dorsocaudal region of the lung. The samples were kept in a flask with 10% buffered formaldehyde, and transported to the laboratory. Likewise, sampling for the isolation analysis of viable bacteria from lung fragments was also carried out in stage 1 and only considering carcasses with pneumonic lesions. These samples were collected with sterilized tweezers and scissors and transported refrigerated. Swabs were collected from pleural lesions during stages 1 and 2, immediately after loosening the adhesion between the parietal and visceral pleura. The swabs were transported in sterile microtubes containing sterile saline solution and refrigerated. Swabs collected

in stage 1 were used for cultures to attempt bacterial isolation. In turn, the swabs from stage 2 were used for bacterial culture and PCR (Table 1). The attempts of bacterial isolation from lung fragments and swabs were conducted in up to eight hours after sampling.

Laboratory processing. The fragments for histopathology were kept in formaldehyde for at least 72 hours for complete fixation, then processed following a routine standardized methodology (Banks 1993). Slides were stained using hematoxylin and eosin (HE). To validate the classifications of chronic pleuritis made by the inspection agents, the macroscopic and microscopic assessment in 50 carcasses were compared. In the histopathologic analyses, pleuritis were characterized as subacute when they presented proliferation of connective tissue together with the presence of fibrinous or suppurative exudate, characterized by fibrin deposition with infiltration of degenerated neutrophils and few plasma cells and lymphocytes, in the pleural surface; chronic cases showed predominantly fibrous repair connective tissue, absence or scarce presence of plasma cells and lymphocytes, and absence of fibrin deposition. Routine bacteriological tests were carried out to detect viable bacteria and attempt to isolate the main bacterial agents involved in swine pulmonary diseases: *Actinobacillus pleuropneumoniae*, *Glaesserella parasuis*, *Pasteurella multocida* and *Streptococcus suis*. Therefore, lung fragments imprints and swabs, were streaked on blood agar (AS) culture medium, AS with a streak of *Staphylococcus aureus* supplying NAD (nicotinamide adenine dinucleotide), and MacConkey (MC) medium. The cultures in AS and MC media were incubated in aerophilic conditions and the cultures in AS media with the streak of *Staphylococcus aureus* were incubated in microaerophilic conditions. All were incubated at a temperature of 37°C and examined after 24 and 48 hours. The identification of the bacterial species was carried out through biochemical assays, according to Quinn et al. (1994). The A and D capsular types of *P. multocida* were identified through acriflavine and hyaluronidase tests (Carter 1984). Investigations on *Mycoplasma hyorhinis* were conducted with swab cultivation in Friis broth, incubated at 37°C for 21 days, with gentle agitation in a roller (Friis 1974). Bacterial DNA was assessed based on a swab pre-culture in a Brain Heart Infusion (BHI) medium supplemented with serum and NAD, incubated for 24 hours at 37°C. DNA was then extracted from this pre-culture using a Spin FastDNA® kit from MP Biomedicals. The DNA extracted was submitted to different PCR (Polymerase Chain Reaction) to detect *Actinobacillus pleuropneumoniae* targeting the gene *cpx* (Lo et al. 1998); *Glaesserella parasuis* using gene *tbp*A (De La Puente Redondo et al. 2003); *Mycoplasma hyorhinis* with detection of the conserved region in gene 16S rRNA (Stakenborg, et al. 2006); *P. multocida* with detection of gene *kmt*1 (species-specific portion), and genes *hya*D-*hya*C (capsular type A) and *dcb*F (capsular type D) (Townsend et al. 1998); and *Streptococcus suis* with detection of the conserved region 16S (Chatellier et al. 1998) and serotype 2, targeting gene *cps*2J (Marois et al. 2004). The isolates of *P. multocida* type A underwent PCR for detection of the virulence gene *pfh*A (Ewers et al. 2006).

Economic impact. an economic assessment was carried out based on a hypothetical situation of a slaughterhouse with capacity to slaughter 4,000 pigs per day and that exports 18.5% of its production (mean value for Brazil in 2018) to a country with restrictions for carcasses deviated to the DIF. Carcass weight was considered as 82.8 Kg, and the price for carcasses in the domestic market was set at 11.50 R$/Kg (Cepea 2020) and for the foreign market, 12.50 R$/Kg (Cepea 2020). The data on pleuritis and pericarditis are grouped in the "Sistema de Informações Gerenciais do Serviço de Inspeção Federal" (Managerial Information System of the Federal Inspection Service - SIGSIF) registries, so it was necessary to make a distinction between both based on the literature. Thus, we considered that 4.57% (Coldebella et al. 2017) of carcasses slaughtered are deviated to the DIF due to the presence of pleuritis and pericarditis, the historical mean slaughtered volume of condemnations due to pericarditis is approximately 2.6% (Schuh et al. 1998), and an estimated 12.38% of pericarditis is not correlated to pleuritis (Coelho et al. 2014). Therefore, 0.32% of the 4.57% of deviations to the DIF due to pleuritis and pericarditis cannot be considered as pleuritis. According to a study by Morés et al. (2017), 94.8% of carcasses deviated due to pleuritis and pericarditis received the NE stamp, which means they could not be exported, but were free to be marketed domestically with no restrictions, 3.72% were conditionally approved pending heat treatment and 1.45% were used for byproducts. Thus, for the hypothetical evaluation, an additional 161 carcasses (4.03% slaughter volume) were considered adequate for exportation if restrictions were lifted from the DIF.

RESULTS

Macroscopic evaluation and histopathological examination

The positive predictive value between the macroscopic evaluation carried out on the slaughter line (Fig.1 and 2) and the histopathological test (Fig.3) to characterize the evolution stage of the pleural lesion was 96%. In all lesions evaluated macroscopically as chronic pleuritis, the histopathological test showed predominant repair of fibrous connective tissue, with absence or discrete infiltration of lymphocytes, plasma cells and intact neutrophils. Two samples presented mild fibrin deposition above de pleura without any other associated injury, while in the other 48 samples there was no inflammatory

Table 1. Experimental design, contemplating the stages of the study, sampling, transport of samples, and assays carried out

Sampling	Stage	Lesion	Collection	Transport	Assay
Samples analyzed (200)	Stage 1 (100)	Chronic pleuritis with adjacent pneumonic lesions	Swab 1	Saline/4-8°C	Bacterial isolations
			Fragment 1	Sterile bag/4-8°C	
			Fragment 2	Formaldehyde/Room temperature	Histopathology
		Chronic pleuritis without adjacent pneumonic lesions	Swab 1	Saline/4-8°C	Bacterial isolation
			Fragment 1	Formaldehyde/Room temperature	Histopathology
	Stage 2 (100)	Chronic pleuritis	Swab 1	Saline/4-8°C	Bacterial isolation
			Swab 2		Isolation of *Mycoplasma hyorhinis*
			Swab 3		PCR
		No pleural lesions	Swab 1		Bacterial isolation
			Swab 2		Isolation of *Mycoplasma hyorhinis*
			Swab 3		PCR

exudate. Most samples that presented also lung lesions, the microscopy picture in the lung was characterized by areas of bronchopneumonia with infiltration of macrophages, plasma cells, lymphocytes and intact or degenerated neutrophils in the alveoli, infiltration of intact or degenerated neutrophils in the lumen of the bronchi and bronchioles, mild to moderate hyperplasia of the bronchi and bronchioles associated lymphoid tissue (BALT). Thickening of the interlobular septa by fibrous connective tissue was also observed in many samples.

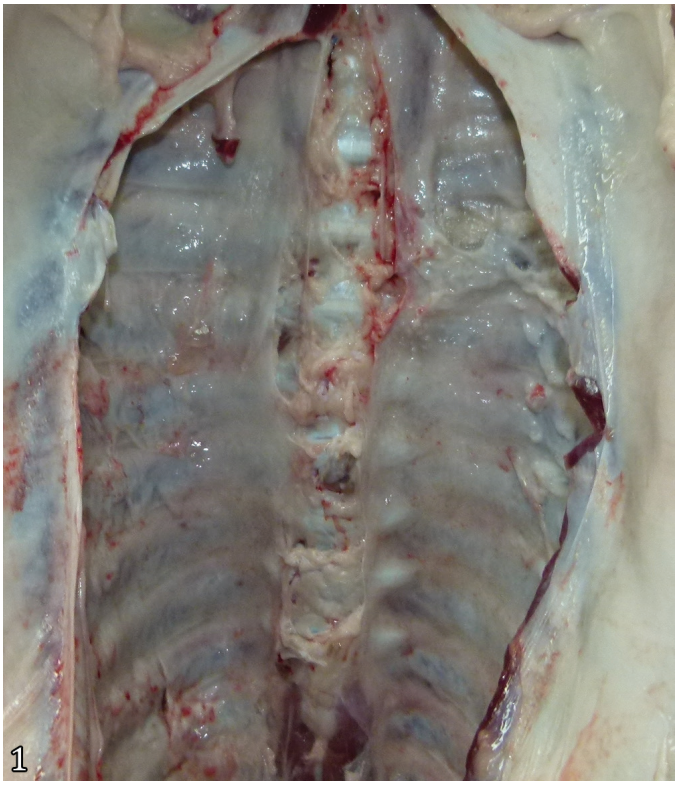

Fig.1. Pig carcass with pleuritis classified as chronic in the macroscopic evaluation conducted by the inspection agent: severe diffuse bilateral thickening of the pariteal pleura, which is whitish in color.

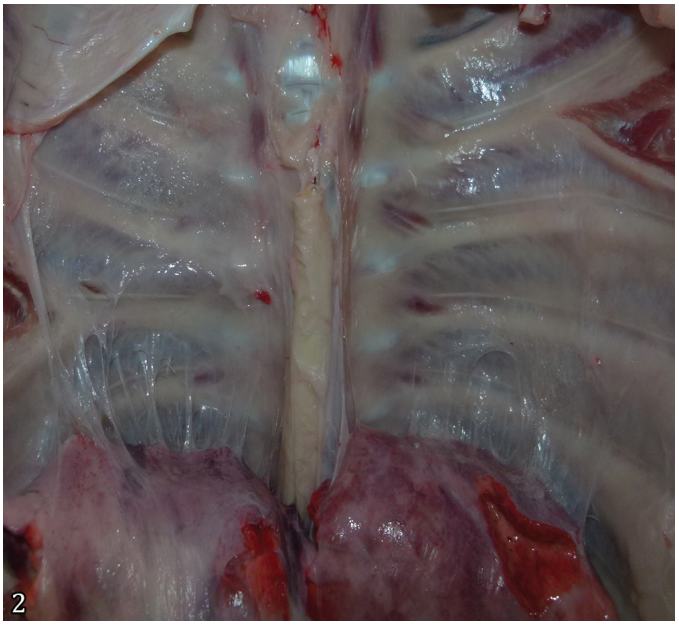

Fig.2. Pig carcass with pleuritis classified as chronic in the macroscopic evaluation conducted by the inspection agent: diffusely and bilaterally thickened pariteal and visceral pleura, with fibrous adherence to each other.

Isolation and PCR for bacterial agents

All 200 swabs from the chronic pleural lesions were negative for bacterial isolation, regardless the presence of adjacent pulmonary lesions. The *p* proportion of isolations can be represented by a Beta (1, 201) distribution, which had an expected value of 0.498%. Considering a confidence level of 95% and isolation sensitivity of 100%, the maximum percentage of isolations would be 1.48%. If isolation sensitivity were 90%, this maximum percentage would be 1.64%. And if we evaluated only carcasses that presented pleural adherences, a Beta distribution (1, 151) could be assumed to have an expected value of 0.66%. Again, considering a confidence level of 95%, sensitivity of 100% and 90%, the maximum percentage of isolations would be 1.96% and 2.18%, respectively.

Regarding the analysis of pneumonic lesion fragments, bacterial isolation reached 70% of samples (35/50), and the isolated agents were: *Pasteurella multocida* type A (21 samples); *P. multocida* type D (6 samples); *P. multocida* type A + *Streptococcus suis* (3 samples); *Actinobacillus pleuropneumoniae* (2 samples); *Streptococcus suis* (2 samples); and *Actinobacillus pleuropneumoniae* + *Streptococcus suis* (1 sample). The percentage of isolates of *P. multocida* type A that were positive in the PCR for the virulence gene *pfh*A was 12.5% (3/24).

The PCR analyses, carried out with samples collected in stage 2, five samples were positive for *S. suis*, four of which came from swine carcasses without pleural lesions and one from the carcass with pleural adherence. The rest of the samples were all negative.

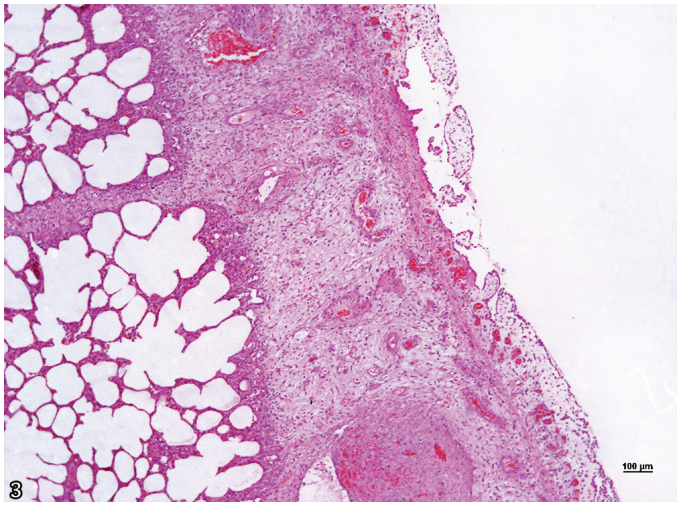

Fig.3. Microscopy of a pig lung fragment with severe thickening of the pleura and subpleural space by proliferation of fibrous connective tissue. There is also congestion, proliferation of blood vessels, and thrombosis. HE, obj.5x.

Economic impact

The negative economic impact, resulting from carcass not being eligible to export, was estimated at R$ 639,220.00 (Table 2), considering an exportation of 18.5% of processed carcasses. This scenario does not account for gains with offal (meat from the head, rectum, tripe, tongue, liver, among others) nor was the need for increments in labor measured, which vary according to the conditions of each plant.

DISCUSSION

An excellent association was found, regarding the developmental stage of the pleural lesions, between the macroscopic classification carried out in the inspection line and the results of the histopathological examination. The positive predictive value of this comparison was 96%, demonstrating security in the macroscopic evaluation of the inspector, since even in the two samples which had some divergence, the lesion was in an advanced stage and there was no bacterial growth.

The results from the pleuritis bacterial isolation must take into consideration the developmental stage of the lesion, observing if it is chronic or acute. In the present study the evaluation of chronic pleuritis demonstrated that there were no viable bacteria in these lesions, given that there was no bacterial growth in the pleura swab samples (0/150) even in samples with adjacent pulmonary lesions. Bacteria was isolated in 70% of pulmonary lesion samples (35/50). These results corroborate previous studies by Jirawattanapong et al. (2010), who also did not isolate bacteria from chronic pleural lesions, and by Morés et al. (2016), who isolated bacteria from lung fragments of chronic pulmonary lesions.

Type A *Pasteurella multocida* was the most frequently isolated bacteria from pulmonary lesions (found in 48% of samples), followed by *P. multocida* type D and *Streptococcus suis* both in 12% of samples, and *Actinobacillus pleuropneumoniae* in 3%. Morés et al. (2016) analyzed 150 lung fragments and isolated the same agents, except for *Streptococcus suis*. However, the percentages they found were different, with *P. multocida* type D present in 27.3% of samples, *P. multocida* type A in 24%, and *Actinobacillus pleuropneumoniae* in 14.6%. De Conti et al. (2021) isolated *P. multocida* type A from 54.2% of 150 samples of lung lesions in slaughter pigs, however, in this study, 68% of the samples had histopathological lesions suggestive of the involvement of more than one agent, with *Mycoplasma hyopneumoniae* and influenza virus being the most prevalent. Apart from *A. pleuropneumoniae* which is considered a primary agent of pleuropneumonia in pigs, the isolation of *P. multocida* and *Streptococcus suis* from pulmonary lesions in samples of chronic pleuritis is not a guarantee that the bacteria isolated were the cause of this pleural lesion since they are most times opportunistic agents and *S. suis* is part of the microbiota in the respiratory tract of pigs (Hansen et al. 2010).

The PCR assays only revealed DNA from *Streptococcus suis*, though the bacteria was not isolated from these samples. This can be explained due to the evolution of the disease, given that in chronic lesions a viable agent is not expected to be present though fragments of their DNA may remain. Another possibility, perhaps the most likely, is that the DNA detected originated from cross-contamination of samples of *S. suis* during slaughter procedures, since even samples with no lesions tested positive. In the study by Morés et al. (2016), DNA from *A. pleuropneumoniae* was found in pulmonary lesions without isolating the bacteria, which the authors correlated to the presence of other competitor agents. Another study that addressed pleuritis and pericarditis also failed to correlate the bacteria isolated from slaughter lesions with what was identified through the PCR. According to these authors, the diseases occurred in the initial phases of accommodation, and for this reason most infections were resolved in the termination phase and slaughter, with only fragments of DNA left behind (Coelho et al. 2014).

The reliability shown in the visual evaluation during inspection, the absence of viable bacteria in chronic pleural lesions, and the absence of respiratory bacteria listed as biological hazards in the swine production chain, since they are not considered as agents transmitted by consuming pork (Costa et al. 2017), secures the unrestricted use of carcasses with chronic pleural lesions.

The difference in carcass values for exportation in comparison to the domestic market was R$ 1.00 per kg, which represents R$ 0.615 per carcass slaughtered in a scenario where exportation makes use of the 18.5% of carcasses deviated due to chronic pleuritis. These values are slightly lower than those observed by Morés et al. (2017), who obtained an aggregated value of R$ 0.89 per carcass slaughtered, making use of 10% of carcasses marked as NE. This difference is mainly related to the lower difference in valuation of swine products between current internal and external markets. In this hypothetical case, the

Table 2. Estimated economic impact from not exporting swine carcasses due to chronic adhesions (pleuritis)

Variable	Unit	% from total	R$	U$*
Amount of pigs slaughtered/year	1,040,000.00			
Amount of pigs slaughtered/day	4,000.00			
Difference (carcasses per day) pleuritis	160.5	4.03		
Difference in exportable carcasses/year	41,730			
Carcass weight - kg	82.80			
Price per kg of carcass in the foreign market (FM)			12.50	2.20
Price per kg of carcass in the domestic market (DM)			11.50	2.02
Difference between the price of carcasses: FM-DM			82.80	14.9
Difference in annual revenue**			3,455,244.00	621,777.00
Difference in annual revenue exporting 18.5% of production (mean in Brazil in 2018)***			639,220.00	115,028.51

* Dollar quotation on 27 October, 2020 at 5.67R$/U$, ** In the hypothetical situation of there being a foreign market to export 100% of these pork products, *** In the hypothetical situation of exporting 18.5% of these additional carcasses (mean in Brazil).

difference in annual revenue would be R$ 3,455,244.00 for a company that exports 100% of its production.

CONCLUSIONS

In this study, it is possible to suggest that the macroscopic evaluation of chronic pleuritis was a good method to classify chronic lesions during the slaughter line.

These chronic lesions were free of viable bacterial agents and, thus, could be submitted to pleura removal without the need to forward the carcass to the DIF.

There is an important financial impact with the reduction in the number of carcasses that are disqualified for exportation due to chronic pleural lesions. This impact varies between pig lots and depends on the specific commercial requirements.

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Conflict of interest statement.- The authors declare that there are no conflicts of interest.

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