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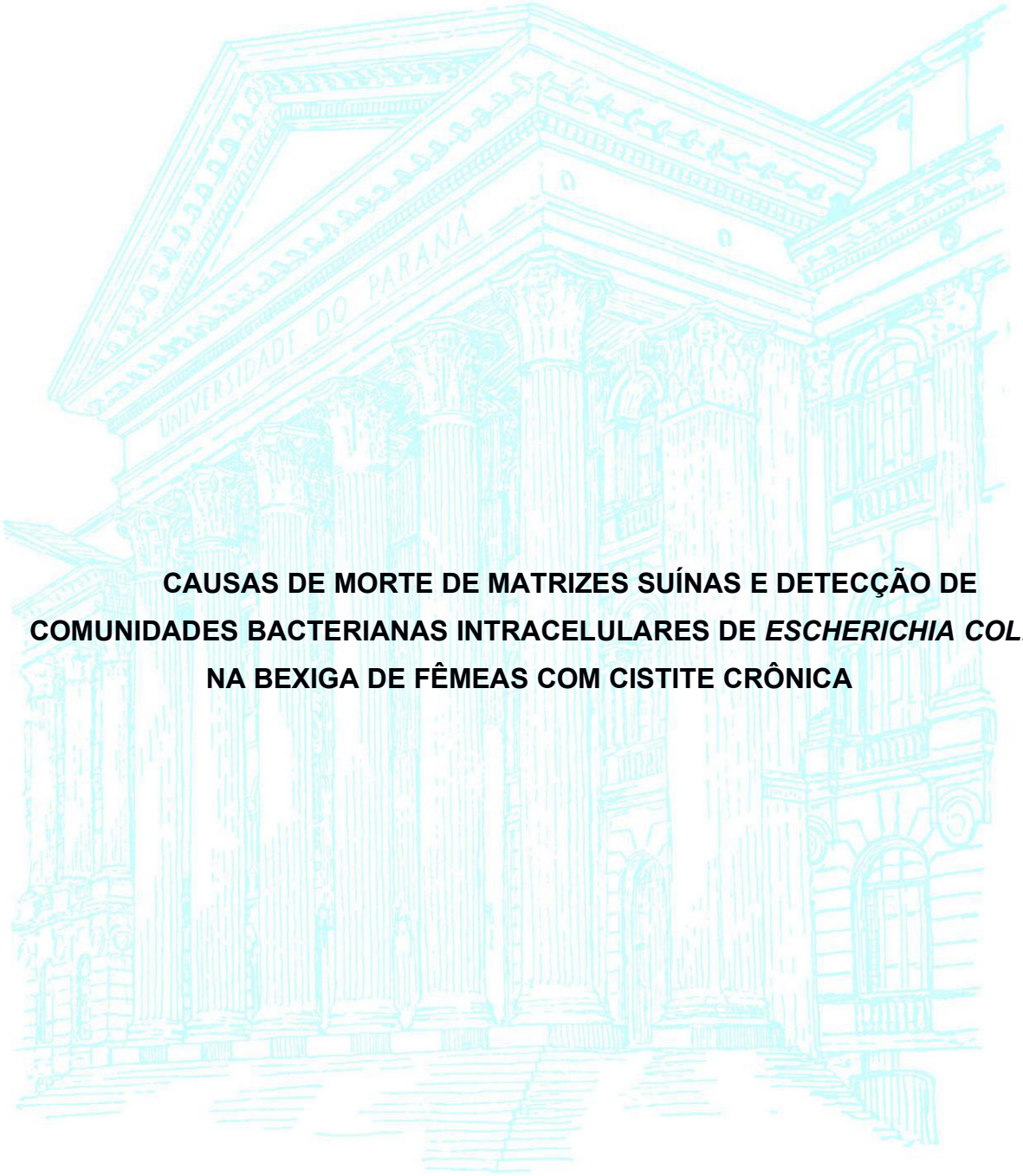

CAUSAS DE MORTE DE MATRIZES SUÍNAS E DETECÇÃO DE COMUNIDADES BACTERIANAS INTRACELULARES DE *ESCHERICHIA COLI* NA BEXIGA DE FÊMEAS COM CISTITE CRÔNICA

PALOTINA

2018

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RESUMO

As causas de morte de porcas e leitoas não são bem documentadas em todo o mundo e em particular no Brasil, sobretudo com relação a caracterização das lesões sob o ponto de vista anatomopatológico. Os trabalhos que se propõem a esclarecer as mortes de porcas são frequentemente focados em parâmetros produtivos, classificação de sinais clínicos como doenças, e categorização imprecisa de lesões observadas. Com base nisto, parte deste trabalho teve o objetivo de realizar diagnósticos anatomopatológicos precisos a partir da necropsia de porcas e leitoas que foram a óbito naturalmente ou por meio de eutanásia em duas unidades produtoras de leitões na região oeste do Paraná. No total, 74 animais foram necropsiados, sendo 17 leitoas e 57 porcas. As mortes naturais corresponderam a 66% (n=49) e as eutanásias a 34% (n=25) dos casos. Serosite fibrinosa foi a principal causa de morte espontânea em leitoas (n=8; 10,8%), enquanto em porcas a torção hepática foi a principal lesão (n=7; 9,5%). Das porcas eutanasiadas, 8,1% (n=6) apresentaram miosite supurativa, a lesão mais comum nesta categoria. Apenas quatro leitoas foram eutanasiadas (5,4%). Estas apresentaram: fratura de membro, mielomalacia, broncopneumonia supurativa e serosite fibrinosa. As lesões macro e microscópicas foram classificadas em principais e contributivas. As infecções urinárias são um problema historicamente importante em suínos, principalmente em matrízes. Os relatos de prevalência elevada são comuns e diversas estratégias de manejo, diagnóstico e tratamento vêm sendo empregadas ao longo dos anos. Sendo assim este trabalho investigou a ocorrência de colônias bacterianas intracelulares por *Escherichia coli* a partir de 13 amostras de vesícula urinária de porcas coletadas em abatedouros, com lesões macroscópicas de cistite. Foram realizados exames histopatológicos e de imunohistoquímica anti-*E. coli* para verificar a presença de colônias intracelulares por *Eschericha coli* no urotélio. Doze amostras (92,3%) foram classificadas como sendo de cistite crônica por meio da histopatologia e apresentaram marcação positiva intracitoplasmática das células uroteliais, comprovando a invasão celular por aquele agente. Trata-se do primeiro relato de colônias bacterianas intracelulares na bexiga de suínos, o que abre a possibilidade para novos estudos que esclareçam a importância epidemiológica destas colônias para a infecção urinária em suínos, além de formas de prevenção e tratamento efetivos contra agentes que provoquem este tipo de infecção.

Palavras-chave: Mortalidade. *Escherichia coli*. Porcas. Imunohistoquímica.

ABSTRACT

Mortality causes of sows and gilts are not well reported worldwide and particularly in Brazil, specially when it comes to lesions characterization under an anatomopathological standpoint. Researches with the objective of elucidate sow mortality usually focus on performance parameters, classify clinical signs as specific diseases and use imprecise characterization of observed lesions. Based on that, part of this dissertation had the objective of establishing precise pathological diagnostics from the necropsy of sows and gilts spontaneously dead or euthanasiated in two piglet producing farms located in western Paraná. A total of 74 animals were necropsied, comprising 17 gilts and 57 sows. Spontaneous deaths corresponded to 66% (n=49) and euthanasia to 34% (n=25). Fibrinous serositis was the main cause of spontaneous death among gilts (n=8; 10.8%), while on sows, hepatic torsion was the most common lesion (n=7; 9.5%). Among the euthanasiated sows, 8.1% (n=6) had suppurative myositis, the most common lesion in this category. Only four gilts (5.4%) were euthanasiated, comprising limb fracture, myelomalacia, suppurative bronchopneumonia and fibrinous serositis. Macroscopic and microscopic lesions were classified in major and contributory. Urinary tract infections are a historically relevant problem for sows. Reports of elevated prevalence are common and numerous management, diagnostic and treatment strategies have been employed through the years. Therefore, this study investigated the occurrence of intracellular bacterial colonies of *Escherichia coli* from 13 samples of urinary bladder of sows collected in slaughterhouses, showing macroscopic lesions compatible with cystitis. Samples were submitted to histopathological and anti-*E. coli* immunohistochemistry analysis with the objective of detecting urothelial *Escherichia coli* intracellular colonies. Twelve samples (92,3%) were histologically classified as chronic cystitis and had positive intracytoplasmic marking, therefore proving cellular invasion by such microorganism. It is the first report of intracellular bacterial colonies on the urinary bladder of swines, what opens the possibility of new studies aiming to elucidate the epidemiological importance of such colonies for urinary tract infection in swines, as well as forms of prevention and treatment effective against agents that cause this type of infection.

Keywords: Mortality. *Escherichia coli*. Sows. Immunohistochemistry.

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LISTA DE ABREVIATURAS E SÍMBOLOS

CBI – Colônia Bacteriana Intracelular

CKD - Chronic Kidney disease

ECUP - Escherichia coli Uropatogênica

HE - Hematoxylin and Eosin

IBC - Intracellular Bacterial Colonies

IHC - Immunohistochemistry

IHQ – Imunohistoquímica

ITU – Infecção do Trato Urinário

PPF - Piglet Producing Farm

SIMEPAR – Sistema Meteorológico do Paraná

UPEC – Uropathogenic Escherichia coli

UTI - Urinary Tract Infection

® - Marca registrada

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1 INTRODUÇÃO

Morte e descarte inesperado de matrizes suínas são fonte importante de prejuízo à suinocultura que são frequentemente negligenciados pelos administradores das granjas quando tais índices não extrapolam os valores esperados (MORÉS, 2007). O aumento do custo de produção é relacionado à mão de obra, leitegada perdida, consumo de ração e reposição da fêmea. Também é importante salientar que altas taxas de mortalidade de porcas podem indicar a existência de agravos ao bem-estar dos animais (JENSEN et al. 2012). A taxa de mortalidade de matrizes suínas em granjas comerciais apresenta grande variação entre diferentes países produtores, sendo reportadas taxas de 5,45% no Brasil, 6,49% no Canadá e 7,8% nos Estados Unidos (PALOMO, 2006); esta ampla faixa de variação é decorrente de diferenças de manejo, status sanitário, genética, nutrição e bem-estar. Entre granjas de uma mesma região pode haver diferenças importantes na taxa de mortalidade; Morés (2007), encontrou valores anuais entre 1,39% e 11,80% em granjas brasileiras entre os anos de 2004 e 2006. Da mesma forma, as perdas econômicas relacionadas sofrem variação semelhante. O autor considera taxas acima de 5% altas, porém Almond et al. (2006) consideram taxas entre 5% e 8% como metas.

A morte de matrizes pode ocorrer de forma natural (súbita ou esperada) ou por eutanásia. As causas naturais relatadas com maior frequência em granjas de alta produção são relacionadas a distúrbios do trato gastrointestinal, infecções do sistema urinário, doenças cardiovasculares e problemas no parto ou no puerpério. A eutanásia costuma ser realizada quando da ocorrência de afecções locomotoras, como fraturas, artrites, epifisiólise, apofisiólise, abscessos e osteocondrose (PALOMO, 2006; MORÉS, 2007; VEARICK et al. 2008).

As informações disponíveis sobre as causas de morte de matrizes suínas são escassas em todo o mundo e imprecisas quanto a qualificação de tais causas. Nota-se que muitos estudos que têm o objetivo de elencar as causas de morte de porcas apresentam categorização deficiente das lesões encontradas, frequentemente recorrendo a termos genéricos, zootécnicos ou mesmo a sinais clínicos como entidades nosológicas distintas, comprometendo a acurácia dos relatos e consequentemente a possibilidade de correção de fatores causadores ou predisponentes. Além disso, muitos destes trabalhos são realizados na Europa ou

na Ásia e não necessariamente refletem as condições ambientais, genéticas, sanitárias e de manejo do Brasil.

A prevalência de infecções urinárias em matrízes suínas é historicamente elevada. Tais infecções consistem na colonização ascendente da vesícula urinária isoladamente (cistite) ou da vesícula urinária, ureter e pelve renal (pielonefrite), por microrganismos patogênicos, sobretudo *Escherichia coli, Proteus mirabilis, Staphylococcus sp., Streptococcus sp., Aeromonas hydrophila, Trueperella pyogenes* e *Actinobaculum suis,* presentes normalmente na microbiota do trato urinário inferior, nas fezes e na pele periuretral. Destas, *E. coli* é a mais prevalente em casos graves de cistite ou pielonefrite em suínos, sendo responsável por ao menos 50% dos casos (FAIRBROTHER; GYLES, 2006; MAZUTTI et al. 2013), sendo que Meister, (2006), encontrou prevalência de até 70% em porcas com infecção urinária. As cepas de *E. coli* que causam ITU são comumente designadas uropatogências (ECUP). As infecções do trato urinário (ITU) constituem importante causa de mortalidade de suínos com mais de um ano de idade, além de provocarem falhas reprodutivas em matrizes suínas, como redução do tamanho da leitegada, aumento da taxa de retorno ao cio, abortos, anestro e de predispor ao desenvolvimento de síndrome da mastite, metrite e agalaxia (FAIRBROTHER; GYLES, 2006; DA SILVEIRA et al. 2007).

As infecções urinárias são favorecidas por características anatômicas das fêmeas de várias espécies (uretra curta e larga), associados à criação em gaiolas, manejo inadequado dos dejetos, que propiciam o contato de material fecal com a vulva e consequente colonização bacteriana, estresse, baixa ingestão de água e pouca movimentação (ALBERTON et al. 2000).

Geralmente as ITU são recorrentes, tantos nos suínos como nas outras espécies (GAROFALO et al. 2007), sendo que as cepas de *E. coli* uropatogências responsáveis por estas infecções são frequentemente semelhantes, do ponto de vista genético e fenotípico entre as manifestações clínicas, indicando que o agente permanece no trato urinário mesmo após resolução clínica da enfermidade (STAPLETON; STAMM, 1997). Trabalhos desenvolvidos em humanos e ratos revelaram que a persistência das ECUP na bexiga é possível graças à habilidade destas bactérias em se estabelecerem no interior das células epiteliais da mucosa vesical e constituírem comunidades duradouras nestes locais, permitindo que estes microrganismos evadam a resposta imune e a ação de antibióticos e posteriormente

reativem o quadro de cistite (ANDERSON et al. 2004a; ANDERSON et al. 2004b; ROSEN et al. 2007).

Diversos métodos de tratamento e prevenção já foram estudados, por isso este trabalho teve o objetivo de investigar um mecanismo de infecção ainda não estudado na medicina veterinária, que consiste na formação de colônias bacterianas inracelulares (CBI) por *E. coli* no urotélio da bexiga de porcas acometidas por cistite.

1.1 JUSTIFICATIVA

A morte de porcas tem importante impacto nas granjas, relacionado não apenas ao custo de cada e fêmea e a perda de produtividade, mas também ao ânimo dos funcionários (MORÉS, 2007). Os custos foram estimados por Palomo (2006) em US$275 a US$350 por porca. Deen e Xue (1999), afirmam que os custos podem chegar a US$ 400 ou US$ 500 por porca. No Brasil, trabalhos estimaram este custo em cerca de R$ 791 (MORES et al. 2007). Além disso, os trabalhos que se propõem a esclarecer as causas de mortes em porcas e leitoas são frequentemente imprecisos e pouco úteis para esclarecimento das doenças que afetam os rebanhos do ponto de vista anatomopatológico.

Dentre as principais causas de morte de matrizes suínas, vários autores indicam uma alta prevalência de infecções bacterianas do trato urinário, particularmente cistite. Dentre os agentes implicados nestas infecções, a *Escherichia coli* destaca-se (SIPOS et al. 2014). Além da ITU, *E. coli* também são relacionadas a outras afecções, como abortos, anestro, predisposição síndrome mastite, metrite, agalaxia, além de morte (FAIRBROTHER; GYLES, 2006; DA SILVEIRA et al. 2007).

Um melhor entendimento das causas de mortalidade e das lesões mais frequentes é essencial para amparar ações de redução da mortalidade, redução de custos de produção e melhorias de bem-estar animal.

Com a comprovação em humanos e ratos de que as ITU recorrentes podem decorrer da reativação do processo inflamatório a partir de CBI no epitélio da bexiga, surge a suspeita de que tal mecanismo também possa estar relacionado a alta prevalência da doença em porcas.

A comprovação da ocorrência de CBI de *E. coli* na bexiga de porcas abre espaço para novas pesquisas que visem comprovar a importância epidemiológica

deste mecanismo, reavaliar e criar técnicas de controle e tratamento para esta doença.

1.2 OBJETIVOS

1.2.1 Objetivo Geral

Estabelecer diagnósticos anatomopatológicos precisos a partir de necropsias de porcas e leitoas mortas naturalmente ou eutanasiadas em duas unidades produtoras de leitões;

Avaliar a ocorrência de CBI por *E. coli* em amostras de bexiga de porcas com sinais macroscópicos de cistite como forma de comprovar um mecanismo de infecção urinária ainda não estudado nestes animais.

1.2.2 Objetivos Específicos

a) Descrever a casuística das principais doenças que acometem porcas e leitoas e as lesões envolvidas nestes diagnósticos
b) Averiguar a ocorrência de CBI na mucosa de porcas com sinais macroscópicos de cistite.

2 CAPÍTULO 1 – CAUSES OF DEATH OF SOWS AND GILTS IN TWO PIGLET PRODUCING FARMS IN BRAZIL

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2.1 ABSTRACT

Background: Mortality causes of sows and gilts are not well documented worldwide and particularly in Brazil, specially when it comes to lesions characterization under an anatomopathological standpoint. Researches with the objective of elucidate sow mortality usually focus on performance parameters, classifying clinical signs as specific diseases and use imprecise characterization of observed lesions. Based on that, this paper aimed on establishing precise pathological diagnostics from the necropsy of sows and gilts spontaneously dead or euthanasiated in piglet producing farms located in western Paraná, Brazil.

Material, Methods and Results: A total of 74 animals proceeding from two piglet producing farms in western Parana, Brazil, were post mortem examined immediately after death, between January and May 2017. Temperature control in PPF A maternity consisted in curtain management and forced ventilation directed to the sows through individual pipes. In PPF B, ambiance of every sheds was controlled by exhaust fans and evaporator plates, keeping

temperature around 23ºC. Necropsies comprised 17 gilts (23%) and 57 sows (77%). Spontaneous deaths and euthanasias were considered for this study. Depending on the necropsy findings or clinical hypothesis, samples of various organs were collected in 10% buffered formalin for histopathological evaluation. Samples were processed by routine methods for hematoxylin and eosin stain and analyzed with optic microscope. Macroscopic and microscopic lesions were classified in major and contributive. Major lesions were the ones considered to be the main cause of death or euthanasia, while contributive ones were lesions that did not pose direct life threat but that could complicate a clinical condition. Data about daily maximum temperatures were provided by Sistema Meteorológico do Paraná and information about the females were supplied by farm administrators. Spontaneous deaths corresponded to 65% (n=48) and euthanasia to 35% (n=26). Fibrinous serositis was the main cause of spontaneous death among gilts (n=8; 10.8%), while on sows, hepatic torsion was the most common lesion (n=7; 9.5%). Of the euthanasiated sows, 8,1% (n=6) had suppurative myositis, the most common lesion in this category. Only four gilts were euthanasiated, comprising limb fracture, myelomalacia, suppurative bronchopneumonia and fibrinous serositis. The most common contributive lesion in gilts was lymphohistiocytic hepatitis (41.2%; n=7) while in sows it was gastric ulcers (59.3%; n=32).

Discussion: There is little information about the causes of death of sows and gilts because necropsies are still rarely performed during farms routine, thus the relevance of this study. Despite the great prevalence of gastric ulcers in sows, only 2.7% (n=2) of deaths were a direct consequence of such lesion, although 70.2% (n=56) of the 74 females had some degree of ulcer. Other authors reported mortalities due to gastric ulcers ranging from 3,6% to 10,4%. Such differences among studies are difficult to explain given the diversity of possible etiologies for gastric ulcers in swine. Literature cites gastrosplenic torsion as the most common cause of death related to torsion of abdominal organs with 13.1% of the deaths,

different from this report in which hepatic torsion was the most common in this category of lesions (9.5%; n=7). Gilts accounted for 71.4% (n=10) of cases with some type of fibrinous lesion as the main or contributive lesion. In gilts these lesions were always considered the cause of death and six of the nine gilts had lymphohistiocytic hepatitis as a contributive lesion associated, evidencing the septicemic character of the etiologic agents. The present research gathers evidence of a potential shift in death causes from the ones historically cited, such as heart failure, arthritis, osteochondrosis and urinary tract infections.

Keywords: Euthanasia; Natural death; Swine; Torsion

2.2INTRODUCTION

Mortality and culling of sows are an important source of economical losses in swine industry that are frequently neglected by farm administrators when such indexes do not exceed the expected values. Morés (2007) found annual values ranging from 1,39% to 11,80% in Brazilian farms during years of 2004 to 2006. Economical losses related to sow death show similar variation. High sow mortality ratios can also indicate welfare problems (Jensen et al., 2012).

Sows death increases production costs, which are related to waste of labor, litters, feed and replacement of the animal. Deen & Xue (1999) state that costs can reach US$ 400 or US$ 500 per sow. In Brazil some researches indicate these costs to be around R$791 (MORÉS et al., 2007).

Death of sows may occur by natural means or by euthanasia. Natural causes in high production farms are historically linked to gastrointestinal tract diseases, urinary tract infection, cardiovascular diseases, troubled parturition or puerperium. Euthanasia is usually performed when locomotor conditions occur, such as fractures, arthritis, epiphysiolysis, apophysiolysis, abscesses and osteochondrosis (MORÉS, 2007).

Therefore, this research has the objective of establishing precise anatomopathological diagnostics as a form of elucidating the types of lesions observed in sows and gilts during routine necropsies because a better understanding of death causes is essential for supporting actions oriented to reduce mortality, production costs and improve welfare.

2.3 MATERIALS AND METHODS

Between January and May 2017 necropsies were performed in sows and gilts of two piglet producing farms (PPF) located in western Paraná, Brazil, both separated by a distance of roughly 43 kilometers. The first PPF (A) was visited five days a week, over 32 days during January and February, while the second PPF (B) was visited over 58 days during March and May, with the same frequency. The farms had, 4926 and 4250 sows and mortality rate in the 12 months previous to the study of 11,26% and 10%, respectively. PPF A dams had DanBred® genetics, while PPF were Agroceres PIC®. In both farms, females were kept in individual cages during gestation, maternity and resting periods. The floor of the gestation, reposition, discarded and resting sows sheds were fully made of compact concrete. In the maternity cage the floor was entirely made of plastic grids. Replacement gilts were kept in concrete floor pens with 20 to 30 animals. Feeding was provided twice a day in gestation, maternity and resting. Gilts were fed *ad libitum* in garners type feeders. Water was supplied by nipple waterers. Temperature control in PPF A maternity consisted in curtain management and forced ventilation directed to the sows through individual pipes. In replacement and gestation thermal control was made, respectively, by fans and exhaust fans. In PPF B, ambiance of every sheds was controlled by exhaust fans and evaporator plates, keeping temperature around 23ºC. This PPF was populated one year ago, therefore most of the sows were still on the first parturition.

Necropsies were performed on sows and gilts that undergone spontaneous death or that were subject of euthanasia due to unfavorable clinical condition. Necropsies consisted on the evaluation of all internal organs, skin, hooves, brain, as well as elbow, shoulder, stifle and hip joints. When necessary, spinal cord was also evaluated. Macroscopic findings were photographed and registered on a written report. Depending on the necropsy findings or clinical hypothesis, samples of various organs were collected in 10% buffered formalin for histopathological evaluation. Samples were processed by routine methods for hematoxylin and eosin stain and analyzed with optic microscope. Special stains were used as needed. Information about productive indexes of the females and the farms were provided by its administrators.

The pathological findings were sorted as main lesions and contributive lesions. Main lesions are the ones which nature and/or intensity were sufficient for causing death or to explain a clinical impairment which justified euthanasia. Contributive lesions are the ones that did not impose enough prejudice to the animal's clinical condition to justify natural death or euthanasia, or yet that are secondary to a main morbid process.

Gastric ulcers were sorted into four grades depending on the type and intensity of lesions on pars oesophagea, according the method proposed by Sobestiansky et al. (2007). The maximum environmental temperature value was provided by Meteorological System of Paraná (SIMEPAR), from the meteorological stations closest to the farms.

2.4 RESULTS

A total of 74 females were submitted to necropsy, 25 of which from farm A and 49 from farm B, comprising 17 gilts (23%) and 57 sows (77%). From the total of 74, 48 (65%) of the females had spontaneous death and 26 (35%) suffered euthanasia. Of necropsies performed in

PPF A, 17 (68%) were spontaneous death and 8 (32%) were euthanasia. In PPF B, 31 (63%) of the animals died spontaneously and 18 (37%) had suffered euthanasia.

During the research it became evident that morphological diagnostics only would not be enough to make clear the causes of some deaths, therefore some cases were accounted in broader categories which include process comprising several lesions, but that better indicate the etiology of the death.

The average parity of euthanasiated females was 2.37 and that of the ones spontaneously dead was 1.68 making evident a higher mortality of gilts due to natural causes.

The commonest cause of spontaneous death in gilts was fibrinous serositis (n=8; 10.8%), characterized by presence of white to yellowish material over serous membranes in thoracic and/or abdominal cavities (Fig. 1A, 1B). Other causes included enteritis (n=2; 2.7%), intestinal volvulus (Fig. 1C), hyperthermia and fibrinosuppurative pericarditis (Fig. 1D), each of them with one occurrence (table 1). In sows, hepatic torsion (n=7; 9.5%) (Fig. 2A) and the undefined causes (n=6; 8.1%), were the main responsible for spontaneous deaths. The rest of the diseases found are described in table 2.

Table 1. Causes of death in 13 gilts spontaneously dead in two piglet producing farms from western Paraná, between January and May 2017.

Diagnostic	n	%
Fibrinous serositis	8	61,5
Enteritis	2	15,4
Intestinal volvulus	1	7,7
Hyperthermia	1	7,7
Fibrinosupurative pericarditis	1	7,7
Total	13	100

SOURCE: The author (2017)

SOURCE: The author (2017)

Figure 1. A. Fibrin deposits are observed covering the intestinal serosa. **B.** Area of adherence of visceral and parietal pleura (arrow). **C**. Focus of intestinal volvulus (arrow). **D.** Pericardium. There is great amount of pus and fibrin inside pericardial sac and covering the epicardium.

Table 2. Causes of death of 35 sows in two piglet producing farms in western Paraná between January and May 2017.

Diagnostic	n	%
Hepatic torsion	7	20
Undefined	6	17,1
Fibrinous serositis	3	8,6
Prolapse	3	8,6
Intestinal volvulus	2	5,7
Paresis	2	5,7
Uterine rupture	2	5,7
Splenic torsion	2	5,7
Gastric ulcer	2	5,7
Hyperthermia	1	2,9
Fibrinosupurative Pleurobronchopneumonia	1	2,9
Endometritis	1	2,9
Uterine torsion	1	2,9
Suppurative cystitis	1	2,9
Miscellaneous	1	2,9
Total	35	100

SOURCE: The author (2017)

The most important lesions to cause euthanasia of sows were suppurative myositis (n=6; 8.1%) (Fig. 2B), prolapse (Fig. 2C, 2D) and osteochondrosis (Fig. 3), each of them with four occurrences (5.4% each) (table 3).

SOURCE: The author (2017)

Figure 2. A. Liver, visceral surface, the left lobe is rotated, severely congested and enlarged. **B.** Pelvic limb. Big multifocal abscesses (arrows). **C.** Perineal region. Rectal and vaginal prolapse. **D.** Large intestine is accentuated and diffusely distended (arrows) and filled with feces. Serosa is heavily congested.

Only four gilts were submitted to euthanasia during the observed period. The causes were: limb fracture, myelomalacia, suppurative bronchopneumonia (Fig. 4A, 4B) and fibrinous serositis.

Contributive lesions were found in 71 animals. Such lesions were predominantly of mild intensity, except for gastric ulcers, which intensity was mostly moderate or accentuated (grades 3 and 4). Mild multifocal lymphohistiocytic and necrotizing hepatitis (Fig. 4C, 4D) were the most frequent finding in gilts (n=7; 9,9%), whereas gastric ulcers, suppurative bronchopneumonia and lymphohistiocytic pneumonia had two cases (2,8%) each. Occurrences of lymphohistiocytic hepatitis were almost always concomitant to fibrinous

serositis. Other lesions were suppurative arthritis and myositis, each with one occurrence and chronic kidney disease (table 4). There was a great prevalence of gastric ulcers in sows (n=32; 45.1%); there were also seven cases (9.9%) of chronic splenic torsion and/or rupture (Fig. 4E, 4F) and five of osteochondrosis (7%). Other contributive lesions in sows are listed in table 5.

Table 3. Causes of euthanasia in 22 sows from two piglet producing farms in western Paraná between January and May 2017.

Lesion	n	%
Suppurative myositis	6	27,3
Prolapse	4	18,2
Osteochondrosis	4	18,2
Paresis	2	9,1
Brain abscess	2	9,1
Miscellaneous	1	4,5
Splenic torsion	1	4,5
Vertebral abscess	1	4,5
Vertebral fracture	1	4,5
Total	22	100

SOURCE: The author (2017)

Table 4. Contributive lesions in 17 gilts necropsied in two piglet producing farms in western Paraná between January and May 2017.

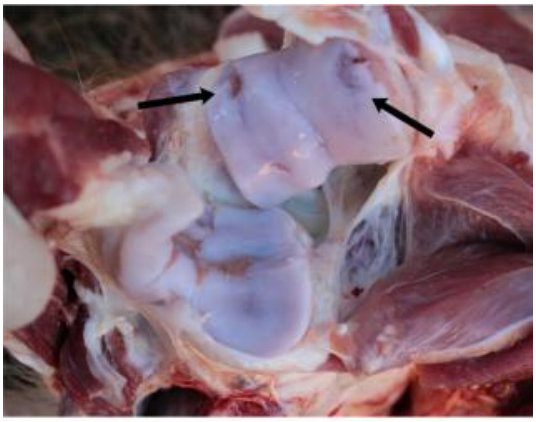
Contributive lesion	n	%
Lymphohistiocytic hepatitis	7	41,2
Gastric ulcer	2	11,8
Suppurative bronchopneumonia	2	11,8
Lymphohistiocytic pneumonia	2	11,8
Chronic Kidney Disease	2	11,8
Suppurative arthritis	1	5,9
Suppurative myositis	1	5,9
Total	17	100

SOURCE: The author (2017)

Table 5. Contributive lesions in 54 sows necropsied in two piglet producing farms in western Paraná between January and May 2017.

Contributive lesion	n	%
Gastric ulcer	32	59,3
Chronic splenic torsion/rupture	7	13
Osteochondrosis	5	9,3
Arthrosis/ proliferative synovitis	3	5,6
Lymphohistiocytic hepatitis	2	3,7
Suppurative arthritis	1	1,9
Suppurative bronchopneumonia	1	1,9
Suppurative myositis	1	1,9
Lymphohistiocytic cystitis	1	1,9
Fibrinous pleuritis	1	1,9
Total	54	100

SOURCE: The author (2017)



SOURCE: The author (2017)

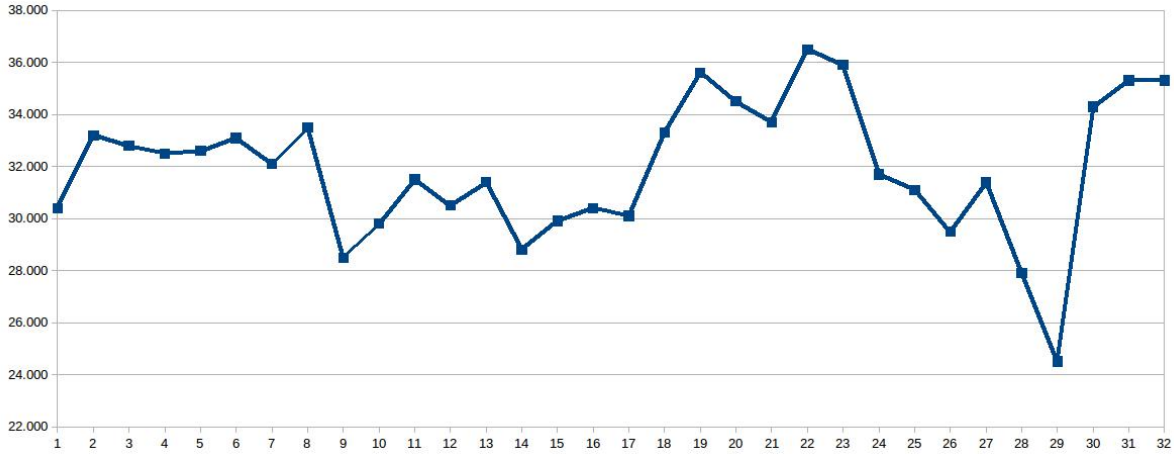
Figure 3. Elbow joint, there are multifocal ulcers and erosions on the articular cartilage (osteochondrosis) of the humerus (arrows).

Cases sorted as fibrinous serositis include lesions affecting abdominal, thoracic or both cavities concomitantly. These were predominant in gilts destined to herd replacement, with average age of 103 days, although sows were also affected. Of the nine cases of necrotizing lymphohistiocytic hepatitis, six were associated to fibrinous serositis, suggesting sepsis.

Two cases of enteritis were observed affecting gilts. The first comprised jejunitis and ileitis, mucosa was moderately reddish and the content was liquid and red. Histopathological diagnosis was of acute, moderate, necrohemorrhagic and neutrophilic enteritis with bacterial

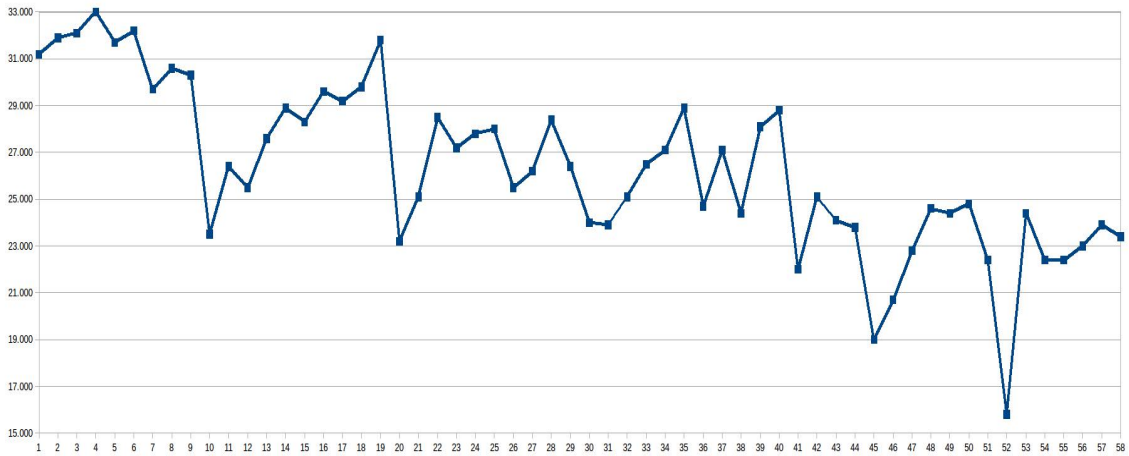
colonies associated. The second one was of moderate hemorrhagic jejunitis, typhlitis and colitis. Etiology of such cases was not established.

Hyperthermia was considered a cause of death when there was edema and pulmonary congestion without other pathological findings. Both cases happened in maternity, in days which maximum temperature was above 30ºC. We do not discard the possibility of heat being a contributing factor to other deaths, however this study considers only cases in which hyperthermia alone was the cause of death. The maximum environmental temperature during the study period in each farm are described in graphs 1 and 2.



SOURCE: The author (2017)

Graph 1. Variation of maximum daily temperature during observed period in PPF A (ºC)



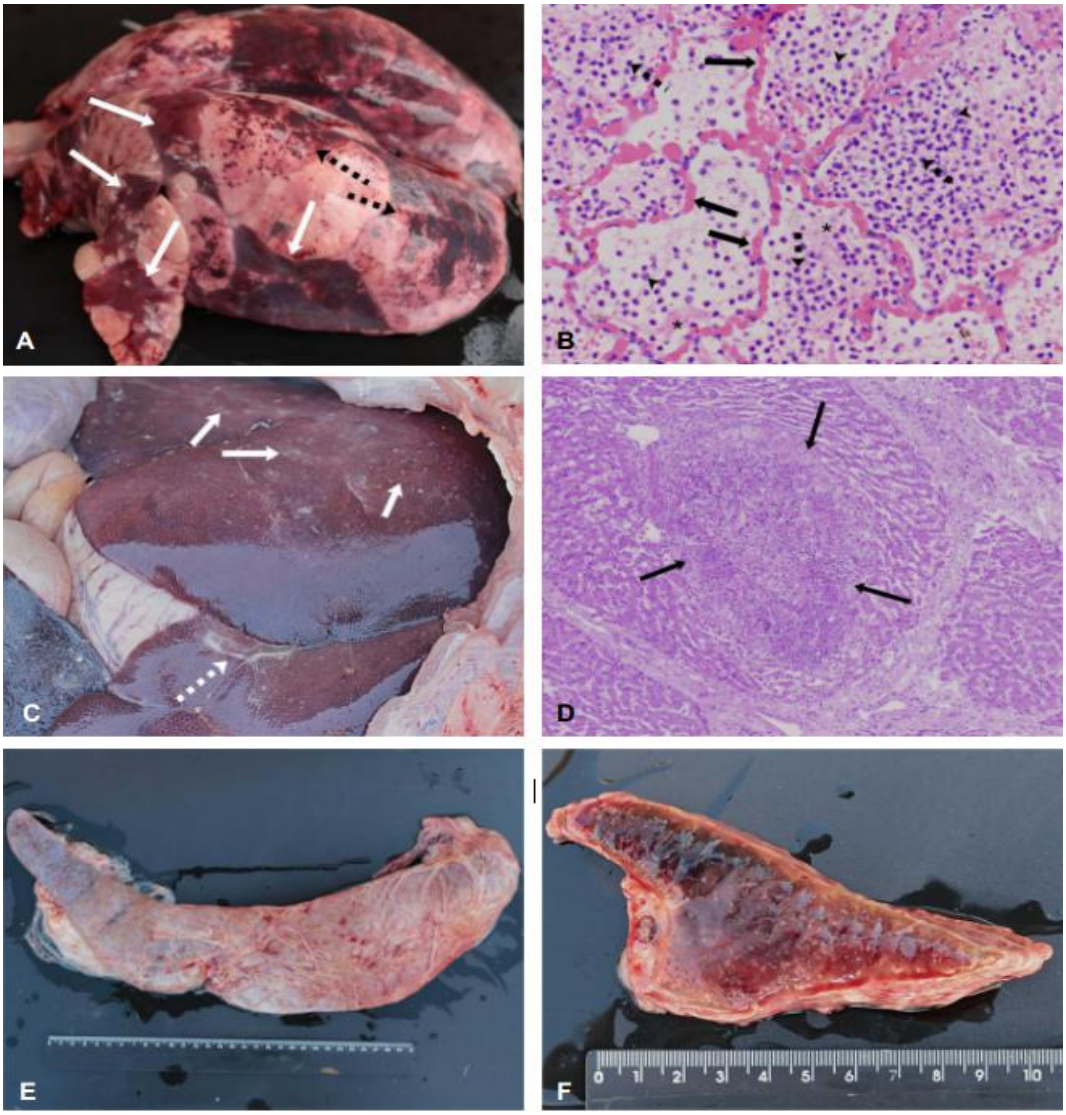
SOURCE: The author (2017)

Graph 2. Variation of maximum daily temperature during observed period in PPF B (ºC)

There was a single occurrence of myelomalacia (Fig. 5A, 5B), which deductible etiology was trauma for there was a focal area of hemorrhage in lumbar musculature and mild

intramedullary hemorrhage.

All cases of hepatic torsion were followed by acute spontaneous death. On every cases, torsion of the left hepatic lobe was observed as well as intense hemoperitoneum and deposition of fibrin over liver's capsule. The affected portion of the liver was deeply red and the edges were round (congestion). Sows with at least two deliveries, during maternity were the most affected. Average age was 553 days.



SOURCE: The author (2017)

Figure 4. A. Lungs; in the caudal part of the left cranial lobe and cranial portion of the left caudal lobe there are multifocal to coalescent areas of consolidation (arrows) and multifocal areas of acute or sub-acute hemorrhage (dashed arrows). In both areas lung parenchyma in depressed (atelectasis). **B**. Lung. H&E. There is accentuated quantity of erythrocytes inside alveolar capillaries (hyperemia) (arrows), inflammatory infiltrate composed by neutrophils (dashed arrows) and macrophages (arrow head) inside alveoli. There is moderate fibrin and erythrocytes inside alveoli (hemorrhage). Similar exudate is also present in the bronchioles and bronchi. **C.** Liver, there are random, marked, multifocal white spots (arrows). **D.** Liver, H&E, focus of necrosis (arrows) and inflammatory infiltrate composed of lymphocytes and macropha ges. **E.** Spleen, the omentum is diffusely

adhered to the splenic capsule. **F.** Spleen, cut surface, capsule is moderately and diffusely thickened (fibrosis), the parenchyma is pale and friable (necrosis).

The seven cases of prolapse involved mostly dams from third delivery, during maternity phase. The most affected organ was rectum (six times) and were associated to prolapse of other organs such as vagina, urinary bladder and uterus in five cases. There was one occurrence of prolapse of urinary bladder alone. The average age of affected sows was 620 days and average parity was 3.2. One frequent finding in rectal prolapse cases was the large intestine distended by large amount of feces, with congested walls and sporadic intraluminal hemorrhage.

Deaths which could not be linked to any macroscopic or microscopic lesion, whether for the absence of lesion or because observed lesions were insufficient to cause death were sorted as undefined. Among such there are three cases of gastric ulcers, although no signs of hypovolemia nor sepsis to corroborate a death directly linked to the ulcer.

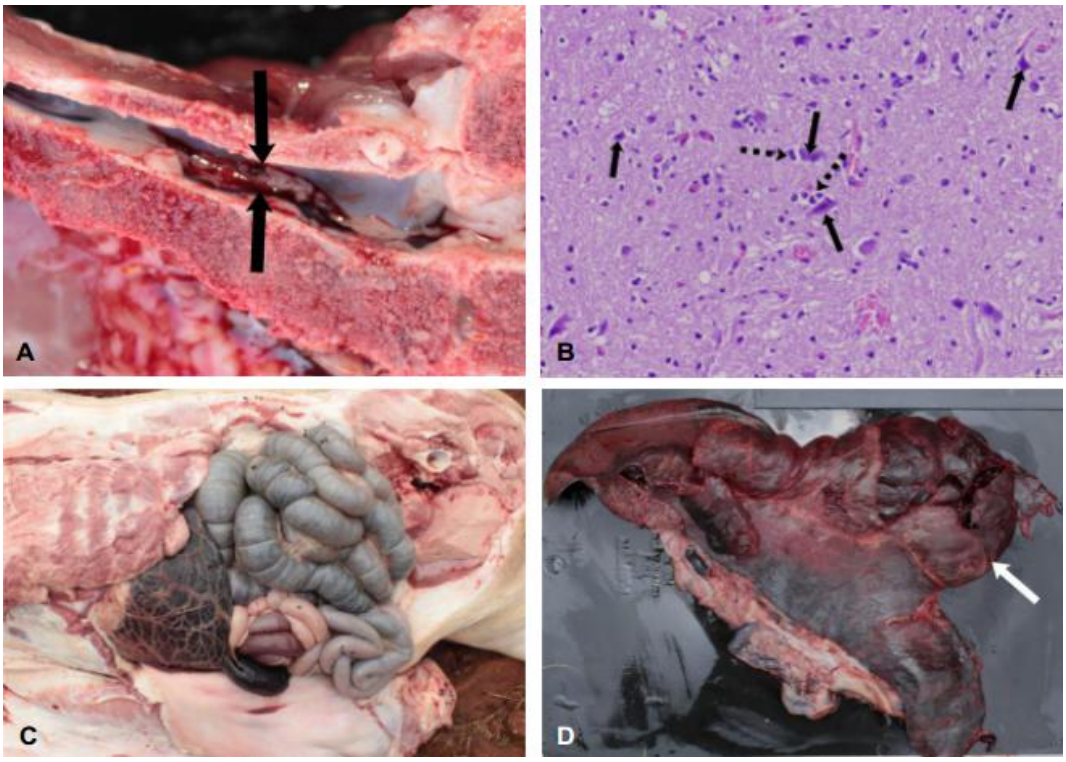
Suppurative myositis was characterized by the presence of abscesses or pus interspersed along limbs musculature. In all cases in which myositis was responsible for death (n=6; 8.1%), there was only one limb affected, four of which were one of the thoracic and all of them in sows. In five of six cases, there was also at least one articular lesion associated, mostly suppurative arthritis and one case of proliferative synovitis.

Osteochondrosis caused death of four sows (5.4%); three of them affected elbow joint and one the left hip and stifle joints. All sows were euthanasiated.

Paresis, a clinical sign, was considered a specific cause of death because of the occurrence of four cases in which sows remained in decubitus for a prolonged time, followed by emaciation and diminishing in milk production when nursing. These dams did not had any lesions or history that justified such clinical presentation. Three cases affected dams in maternity and one in gestation; two were from first parity females, one of parity two and another one did not have this data available. On two of the four cases, dams were

euthanasiated and two others resulted in spontaneous death.

Three sows were affected by acute splenic torsion (Fig. 5C, 5D). Two were associated to severe hemoperitoneum, resulting in death due to hypovolemia and one sow was euthanasiated.

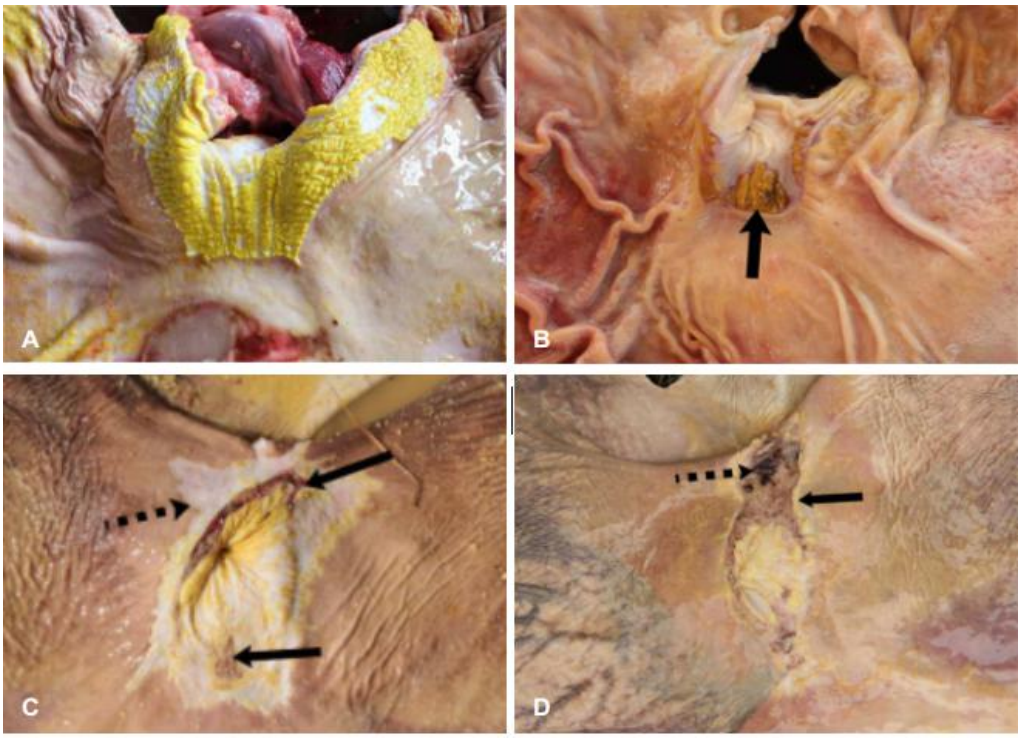


SOURCE: The author (2017)

Figure 5. A. Vertebral canal, sacral portion, there is focal hemorrhage (arrows). **B.** Spinal cord. On the gray matter there are mild astrocytes surrounding neuronal bodies (satellitosis) (dashed arrows). Neuronal bodies are retracted and lightly eosinophilic (malacia) (arrows). **C.** Abdominal cavity; spleen is rotated around its axis, shifting the ventral extremity caudally. **D.** Spleen is severely congested and there is a focal rupture, with a blood clot adhered (arrow).

There were 36 dams affected by ulcers on the on *pars oesophagea,* two of which caused death and the 34 others (47.9%) were sorted as contributive lesions. Both cases in which sow death was due to gastric ulcers had the *pars oesophagea* affected and were given grade 4. There was great volume of blood in stomach's lumen, digested blood in the intestines and the carcass was severely pale, characteristic of death due to hypovolemia. Ulcers were more frequent in first parity females (n=10; 27.8%) among which grade 3 was the most frequent (7 occurrences). Parity two sows were the second most affected category, with six cases of ulcers, yet these included grades 2, 3 and 4, each one with two occurrences. Grades 3 and 4

(severe ulcer) added up to 25 cases (69.4%). Figure 6 shows an example of the lesions that comprises the four grades of *pars oesophagea* ulcers used in this research.



SOURCE: The author (2017)

Figure 6. A. Pars oesophagea, diffuse hyperkeratosis. **B.** Hyperkeratosis with focal area of ulceration (arrow). **C.** Severe erosion (dashed arrow) with multifocal ulcers (arrows). **D.** Severe, diffuse, erosion (arrow) and severe focal ulcer (dashed arrow).

Three cases of grade 3 ulcers were associated to ulcers on the fundic or cardic glands portion of the stomach. In all this cases a concomitant infectious condition was found, either being suppurative endometritis, fibrinous serositis or muscle abscesses.

Gastric ulcers diagnosis were concentrated in maternity (50%, n=18), followed by gestation (25%, n=9). Maternity also concentrated the majority of the severe cases (grades 3 and 4) comprising 15 out of 25 (60%). In gestation grades 3 and 4 were diagnosed three times each, besides two occurrences of grade 2 and one of grade 1.

Cases of chronic splenic torsion and/or rupture (n=7; 9.9%) were more observed in parity four sows with an average age of 760 days. Despite the designation chosen for this paper, omentum torsion was concomitant in these cases. Macroscopically these were characterized by splenomegaly, adherence of the omentum to the spleen, thickening of the splenic capsule

and congestion of omentum blood vessels.

Miscellaneous lesions were considered the cause of death in two sows (2.7%), which included discrete fibrinous serositis, chronic focal torsion of the spleen, mild chronic focal torsion of the spleen, multifocal mild acute bronchopneumonia and mild focal necrotizing pancreatitis, among others.

Maternity was the phase with highest number of deaths (n=30; 40.5%), followed by replacement (n=15; 20.3%), gestation (n=13; 17.6%) and culling (n=11; 14.9%). Weaning to estrus period had five deaths (6.8%).

Considering parity alone (table 6), parity 0 (gilts) corresponded to 23% (n=17) of deaths, while sows (parity equal or superior to 1) corresponded to 77% (n=57) of deaths. There were 18 sows (24.3%) which parity data was not available in the farm's database.

Table 6. Distribution of spontaneous deaths and euthanasias in sows and gilts according to parity order in two piglet producing farms in western Paraná between January and May 2017

Parity	Spontaneous death (n)	%	Euthanasia (n)	%	Total	%
0	13	27,1	4	15,4	17	23,0
1	8	16,7	4	15,4	12	16,2
2	7	14,6	4	15,4	11	14,9
3	4	8,3	3	11,5	7	9,5
4	4	8,3	-	-	4	5,4
5	1	2,1	1	3,8	2	2,7
6	-	-	2	7,7	2	2,7
9	1	2,1	-	-	1	1,4
Unavailable	10	20,8	8	30,8	18	24,3
Total	48	100	26	100	74	100

SOURCE: The author (2017)

2.5 DISCUSSION

There is little information about the causes of death in sows because necropsies are still rarely performed during farms routine and the same applies to gilts deaths (D'ALLAIRE; DROLET, CHAGNON, 1991). The present study congregates conditions found in two farms with important differences in the premises quality and age of the herds. Farm A had and older

herd and poorer sanitary conditions while farm B had being recently filled and ambiance control was more effective. Despite evaluation of only two farms, for a limited period not necessarily demonstrate the situation of Brazilian pig industry or the seasonal effect, it is relevant a study that demonstrate the anatomopathological findings of swine dams in an objective manner, given the scarcity and imprecision of such data in the world and especially in Brazil.

Of 74 sows and gilts evaluated, 20 (27%) did not have complete productive data available, especially about age and/or parity, what limited the possibility of establishing correlations between pathological findings and the productive situation of the dams.

Differently from other studies, (ENGBLOM et al., 2008; KIRK et al., 2005; VEARICK et al., 2008) arthritis and synovitis were not relevant causes of spontaneous death or euthanasia in this research. The cases found were uniarticular, except for one, and considered to be contributive. Both cases of arthritis were suppurative and chronic, and both cases of synovitis were proliferative type. Such low occurrence may be due to better management, less abrasive floor or because the dams of this study were primarily young, since some authors cite higher prevalence of arthritis in sows above the third parturition (SANZ et al., 2007).

The present study found 63.5% (n=47) of all deaths in parity three or less, very close to the reported by Sanz et al. (2007) who evaluated a farm with 5200 sows and also found 63% of deaths in this category although, as stated by the authors cited, this result may be a consequence of the low average parity order of the herd, given that other authors state that higher mortality is observed in sows with more than three parturition and in gilts (KOKETSU, 2000; LOUIS, REMPEL, MICKELSON, 1993; SASAKI & KOKETSU, 2008). An agreement point among various authors is the biggest mortality during maternity, especially peripartum, what was corroborated by this study because death in maternity accounted for 40.5% (n=30) of the total. Concentration of mortality in this phase is linked primarily to the

increase of cardiovascular exigence and labor, which leads to prolapses and hearth failure (CHAGNON, D'ALLAIRE, DROLET, 1991; DEEN & XUE, 1999; ENGBLOM et al., 2007; SASAKI & KOKETSU, 2008).

In this study most of the dead sows with parities 1 to 4 suffered spontaneous death (n=23), while other authors found euthanasia as the main reason for death (SANZ et al., 2007). Such difference stems from the fact that on the present study main lesions in this group were torsion of abdominal organs (n=8; 35%), while on the cited study the most important causes were arthritis and osteochondrosis, which do not provoke death, but debility requiring euthanasia.

Swine are highly sensible to high temperatures, with most of deaths occurring during summer, when temperatures reach 28 ºC to 32 ºC or higher (LOUIS, REMPEL, MICKELSON, 1993; REAMS et al., 1994). In the present paper only two deaths were attributed directly to heart failure due to hyperthermia. The most important lesions in these cases were pulmonary edema and congestion, hydropericardium and hydroperitoneum. Absence of other relevant lesions was determinant for settlement of death cause. Both deaths, one sow and one gilt, were in PPF A, which has poor ambiance control. Maximum temperature on the day of the deaths was, respectively, 30.4 ºC and 32.5 ºC. Both deaths had acute characteristics, something consistent with other authors who found predominance of acute deaths due to hyperthermia (DROLET, D'ALLAIRE, CHAGNON, 1992). Cases like these are usually preceded by a stressful event such as change of barn, labor or fights, although this research did not manage to identify the occurrence of such event on the observed animals. At PPF B the better ambiance control apparently eliminated the effect of temperature on mortality given the fact that no death in PPF B was attributed to hyperthermia. Probably, the quantity of deaths by hyperthermia in this study were significantly lower than other reports due to the high efficiency of thermal control in farm B. The importance of heat

and heart failure is very variable among studies that addresses culling and mortality of sows, with some of them claiming it to be the most important cause and others stating that its importance is small (CHAGNON, D'ALLAIRE, DROLET, 1991; D'ALLAIRE, DROLET, BRODEUR, 1996). No death was attributable to porcine malignant hyperthermia because hallmark lesions such as pale, soft and edematous muscles were not observed (LOUIS, REMPEL, MICKELSON, 1993). Nevertheless diagnosis of this disease is also based on history of a stressful triggering event, which was not evaluated in this study.

There was significant prevalence of pars oesophagea ulcers ($p<0.05$) in sows when compared to gilts. Sanz et al. (2007) found 10.3% of deaths due to gastric ulcers, in a total of 16% of sows affected by any grade of ulcer, whilst in the present study only 2.7% (n=2) of deaths were a direct consequence of this lesion, although there was 36% (n=27) of severe gastric ulcers (grades 3 and 4) and a total of 70.2% of the 74 dams with some degree of ulcer. Other authors report mortality due to gastric ulcers of 3.6% (CHAGNON, D'ALLAIRE, DROLET, 1991) and 10.4% (ENGBLOM et al., 2008). An explanation for such different prevalence is controversial, since factors that influence gastric ulcers formation are not well understood. It is possible that lower mortality is a consequence of feed intake at the analyzed farms be realized twice a day or yet to the fact that most of the dams are still young, given that authors previously cited report higher mortality in sows with parity equal or superior to three and fed once a day. Sobestiansky & Kieckhofer (2007) state that sows recently transferred to maternity or under adaptation to cage are more commonly affected by *pars oesophagea* ulcers, what was corroborated by this study because 50% (n=18) of ulcers cases affected dams in maternity, being 44.4% (n=16) of first or second parity. Apart from stress, other causes linked to gastric ulcer development include feed granulometry below 500 micrometers, prolonged fasting for any reason, movement restriction and infectious causes such as porcine circovirus type 2, *Fusobacterium gastrosuis, Helicobacter pylori* and *H. suis* (BAELE, et al., 2008;

CORRÊA, et al., 2007, CORRÊA, et al., 2008, DE WITTE et al., 2016).

The three cases of ulcers in the fundic region of the stomach were related to presence of suppurative endometritis, severe fibrinous serositis and suppurative myositis, with signs of sepsis such as subcutaneous and lung edema, microabscesses in serosas and generalized hyperemia. Presence of ulcers in this region is a possible consequence of systemic diseases such as salmonellosis. The presence of other lesions compatible with sepsis and focuses of concomitant bacterial infection on the studied cases suggest that such ulcers are related to a systemic bacterial infection which is not necessarily due to *Salmonella enterica* (SKILLMAN et al., 1969; SOBESTIANSKY, 2007).

This study also found quantities of cystitis/pyelonephritis highly discordant from other authors. There were no pyelonephritis. Cystitis was present in only two cases, one of which was chronic and the other was acute. A research based on the necropsies of 78 sows in Brazil found 30.8% of deaths due to urinary infection, of which 54.2% had more than five labors (VEARICK et al., 2008). The fact that the examined population was primarily of low parity certainly had major influence for the low incidence of urinary infection found in this report. The tendency is that as the herd ages, a higher occurrence of such disease might arise (CHAGNON, D'ALLAIRE, DROLET, 1991). It must also be considered the precision of the diagnostics of cystitis among different studies and the importance attributed to this disease as a cause of death, because there could exist different interpretations among authors.

Torsion of abdominal organs, chronic or acute, affected 28.4% (n=21) of females, different than reported by previous studies which vary from 9.3%, 24% (ALMEIDA et al., 2007) to 47.4% (SANFORD, JOSEPHSON, REHMTULLA, 1994). Of sows affected by torsion or accidents of abdominal organs. Hepatic torsion was found to be the most common lesion in this category (n=7; 9.5%), which is different from the cited authors since they found 5.2% of liver torsion and gastrosplenic torsion as the most common, comprising 13.1% of

deaths. Causes for torsion of abdominal organs are related to movement of the dam either for excitement during feeding or walking to the maternity or empty sows barns. In this study, beyond the cited possibilities, hepatic torsions may also be a consequence of a practice of transferring some dams to other maternity room few days after parturition, what certainly may be a predisposing factor, although such hypothesis was not tested in this research. Genetic predisposition might also be possible. All three cases of intestinal torsion affected the jejunum and were characterized macroscopically by presence of large amount of blood in the intestinal lumen, fibrin deposits over the serosa, edema and congestion of the affected segment and hemoperitoneum. Death due to intestinal torsion may be superior to 4%, (BARCELLOS & DRIEMEIER, 2007) compatible with the present report index which was 4% (n=3). Etiology is not well understood, but there are risk factors such as highly fermentable feed, irregular intervals for feeding, low water intake and poor hygiene of premises, neither of which detected at the farms evaluated on this study, making evident the idiopathic nature of the disease.

This study also found seven cases of chronic torsions and/or ruptures of abdominal organs (9.45%), what is not reported by other authors. There were significant differences on the occurrence of this kind of lesion among the farms ($p<0.05$); 24% (n=6) of the animals from farm A had such lesions, all of them affecting the spleen, while only 4% (n=2) of the animals from farm B had it, one case affecting the spleen and other the uterus. Average age of the affected dams was 760 days. Five of the total seven affected were multiparas and two did not have this information available. Genetics of females may be a predisposing factor for occurrence of this type of lesion, in addition to management or premises factors, but a well established cause is not yet known and these lesions are rarely reported. Similarly to previous authors, the present study report cases of sows submitted to necropsy which spleen was involved by a thick fibrous capsule and the parenchyma was friable and degenerated

(GEIGER, IRWIN, PRETZER, 1999). As well as the authors cited, the present research could not determine an etiology for this process.

The suppurative lesions observed (myositis, arthritis, brain abscess and vertebral osteomyelitis) are a consequence of opportunists bacterial infections related to traumas, cannibalism or infection of cutaneous wounds. The agent most commonly isolated from such lesions is *Trueperella pyogenes*, a gram negative bacteria present in the normal microbiota of the superior respiratory and genital tracts of swines. Other possible agents include *Streptococcus zooepidemicus, S. equisimilis, Fusobacterium necrophorum, Bacterioides* sp.; *Staphylococcus hyicus, Staphylococcus aureus, S. epidermidis, E. coli, Pseudomonas aeruginosa, Actinobacillus* sp., among others. After initial infection of the traumatic lesion, microorganisms disseminate through blood circulation or lymphatics to internal organs, forming abscesses filled with greenish or yellowish pus. Localization and intensity of the lesions determine the clinical signs. Usually, young swines are more severely affected (OLIVEIRA & BARCELLOS, 2007). Both occurrences of brain abscess involved gilts with clinical signs of head tilt, walking on circles to the affected side and limp. The cross cutting of the cranium revealed the destruction of the structures of the internal ear and substitution by pus, as well as the presence of an unilateral abscess in the brainstem adjacent to the vestibulocochlear nerve, confirming it to be consequence of an internal otitis (ZACHARY, 2009). Internal otitis are an evolution of external otitis, which starts with accumulation of dirtiness in external ear. *Trueperella pyogenes, Pasteurella multocida* and *Streptococcus* spp. are the bacteria most frequently associated to otitis in swine. Otitis media can be caused by bacteria found in the nasopharynx of healthy animals or causing respiratory disease, such as *P. multocida, Streptococcus* spp., *Actinobacillus pleuropneumoniae, Fusobacterium necrophorum* and others. Absence of other relevant lesions on the evaluated sows allows us to reject the hypothesis of an otitis secondary to respiratory disease (ALBERTON et al., 2007;

SHIMADA et al., 1992).

Of the nine cases (12.1%) of osteochondrosis, four were the cause of euthanasia and five were contributive lesions in sows spontaneously dead due to other causes. There were no spontaneous deaths from osteochondrosis, something already expected (ENGBLOM et al., 2008). Elbow joint was the most affected (n=6) and stifle joint was affected in three cases, one of which associated to fracture of the femur neck, which joint was already showing arthrosis, indicating the lesion to be chronic. Occurrence of bone fractures as a consequence of osteochondrosis is a well know fact, so is the importance of recognizing signals of lesion chronicity, such as fibrosis of joint capsule and absence of hemorrhage and edema, as a way of avoiding an incorrect diagnosis of fracture, which in some cases may be simply secondary to a degenerative process. The predominance of these lesions on the femorotibial and humeroulnar joints is supported by previous studies (ALBERTON et al., 2000; YTREHUS et al, 2004). No gilt was affected by osteochondrosis, which is expected as the critical age for this disease is from 150 to 450 days. The fact that only one of the dead sows was from discard barn makes evident that osteochondrosis usually does not cause clinical signs. All other causes were from gestation or maternity, meaning they were in regular production cycle. Absence of zootechnical data hinders a more detailed evaluation of factors linked to occurrence of osteochondrosis in this study.

Gilts accounted for 71.4% (n=10) of 14 cases with some type of fibrinous lesion as the cause of death or contributive lesion. In gilts these lesions were always the cause of death and six of the nine gilts had lymphohistiocytic hepatitis as a contributive lesion, evidencing the septicemic character of the etiologic agents associated to fibrinous serositis, of which *Haemophilus parasuis, Escherichia coli, Streptococcus suis* and *Mycoplasma hyorhinis* are the most probable to be isolated from the lesions (JOHN, MILCOCK, KLERSTEAD, 1982; LIN et al., 2006; NIELSEN et al., 1975; REAMS et al., 1994; VAHLE, HAYNES,

ANDREWS, 1995; WILKIE, 1981). Detection of such lesion suggests a failure of the vaccine cover against these agents. There was a great predominance of mortality of gilts due to fibrinous serositis at PPF B ($p<0,05$).

The advanced age of the sows affected by prolapse (average of 620 days) is consistent with the reported in literature. Sporadic prolapses are more common in older animals though exclusively rectal prolapses are more common in 56 to 140 days gilts. In this study most of the prolapses involved more than one organ in animals older than 340 days, which suggests that age is the most important predisposing factor. Other risk factors for sporadic prolapses are edema or accumulation of fat in the pelvic region, increase of intra-abdominal pressure during defecation, cough or parturition or inclination of the floor where animals stand or lay down (PAPATSIROS et al., 2012; SOBESTIANSKY, 2007). Cases of death due to prolapse affected primarily dams from third labor during maternity. The organ most frequently involved was the rectum (n=6), associated to prolapse of another organs such as vagina, urinary bladder and uterus (n=5). There was a single case of prolapse of the urinary bladder alone.

Mortality due to pneumonia was 2.7% (n=2) and a total of 9.45% (n=7) of the dams had some type of pneumonia or bronchopneumonia. Such numbers are compatible with values found by other authors but there are big differences in prevalence and mortality due to pneumonia among different studies with values ranging from 63.6% in culled sows (VEARICK et al., 2008), 12.5% in sows found dead or submitted to euthanasia independently of the cause (ENGBLOM et al., 2008) and 9.7% in sows culled in the United States (KNAUER et al., 2007). A study found that pneumonia or bronchopneumonia were responsible for 3.7% of euthanasia and abrupt death of sows (SANZ et al., 2007).

2.6 CONCLUSIONS

Elucidate the causes of death of swine females requires additional exams, particularly necropsies, apart from the mere clinical evaluation, because diagnostics based only in clinical signs are frequently wrong. Detailed postmortem anatomopathological diagnostics are scarce through the world and particularly in Brazil. Despite this study demonstrate mainly the necropsy findings expected in farms of high sanitary status and low parity order, the approach oriented to anatomopathological aspects is important to practitioners dealing with routine diagnostics at the field.

The chronic splenic lesions reported in this paper are one of the findings that diverge the most in relation to other reports; the etiology and importance of this lesions must be better studied in subsequent researches.

2.7 ETHICAL APPROVAL

This study was approved by the ethics committee of Universidade Federal do Paraná, sector Palotina, number 52/2017.

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3 CAPÍTULO 2 – FIRST REPORT OF INTRACELLULAR BACTERIAL COMMUNITIES IN URINARY BLADDER OF SOWS

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First report of intracellular bacterial communities in urinary bladder of sows

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ABSTRACT.- Cestari F.K., Lorenzo C., Viott A.M., Da Costa D.B., Morés N. & Alberton G.C. 2017. **First report of intracellular bacterial communities in urinary bladder of sows.** Pesquisa Veterinária Brasileira 00(00):0000-0000. Laboratório de Patologia Animal, Faculdade de Medicina Veterinária, Universidade Federal do Paraná (UFPR), Setor Palotina, Rua Pioneiro 2153, Palotina, PR 85915-000, Brazil. E-mail: filipekraces@gmail.com

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3.1 ABSTRACT

Reports of elevated prevalence of urinary tract infections are frequent in swine industry and various management, diagnostics and treatment strategies have been employed through the years, with variable success. Therefore, this study investigated the occurrence of intracellular bacterial communities due to *Escherichia coli* in urinary bladders of sows chronically affected by cystitis. Studies of murine and human cases of chronic cystitis demonstrated that one of the major uropathogens, *E. coli*, have the capacity of invading epithelial cells of the urinary bladder and form intracellular bacterial colonies (IBCs). Urinary bladder samples from discarded sows were analyzed macroscopically and through histopathology. The ones that had evidence of chronic cystitis were selected for anti-*E. coli* immunohistochemistry. Of the 13 samples with signals of chronic cystitis, 12 were positive for *E. coli* either on the surface of the urothelium and inside cytoplasmatic vesicles on the urothelium. This indicates that such IBC mechanism does also occur in sows and its importance must be better studied in this species as well as effective treatments for chronic cystitis which should be driven to avoid IBC formation.
INDEX TERMS: uropathogen, *Escherichia coli*, sow, cystitis, swine.

3.2 RESUMO - (PRIMEIRO RELATO DE COMUNIDADES BACTERIANAS INTRACELULARES POR *ESCHERICHIA COLI* NA BEXIGA DE PORCAS COM CISTITE CRÔNICA.)

Relatos de prevalência elevada de infecções urinárias em suínos são frequentes na indústria a várias estratégias de manejo, diagnósitico e tratamento tê sido empregadas ao longo dos anos, com sucesso

variável. Sendo assim, este estudo investigou a ocorrência de comunidades bacterianas intracelulares por *Escherichia coli* na vesícula urinária de porcas acometidas por cistite crônica. Estudos com cistite crônica em ratos e humanos demonstraram que um dos principais uropatógenos, *Escherichia coli*, possui a capacidade de invadir células epiteliais da bexiga e formar colonias bacterianas intracelulares (IBC). Amostras de bexiga de porcas descartadas de granjas comerciais foram analisadas macroscopicamente e por meio de histopatologia. Aquelas que apresentavam evidências de cistite crônica foram selecionadas para exame de imunohistoquímica anti-*E. coli.* Das 13 amostras com sinais de cistite crônica, 12 foram positivas para *E. coli* tanto na superfície do urotélio quanto dentro de vesículas no citoplasma das células uroteliais. Isto indica que o mecanismo de IBC ocorre também em porcas, devendo sua importância ser melhor estudada nesta espécie, além de que tratamentos para cistite recorrente devem ser direcionados para evitar a formação de IBCs.
TERMOS DE INDEXAÇÃO: uropatógeno, *Escherichia coli,* porca, cistite, suíno.

3.3 INTRODUCTION

In the present swine industry, use of highly selected animals for zootechnical performance and the intensification of farming exigences led to an increase of the ambience, management, nutrition and health exigences. In this context, a large incidence of multifactorial etiology diseases is noted, including urinary tract infections (UTI), a long known disease in the swine industry, linked to reproductive failures in sows, such as litter reduction, return to estrus, abortions, anestrus, predisposal to mastitis, metritis, agalactia syndrome and sow death (Fairbrother and Gyles 2006, Da Silveira et al. 2007). Alberton et al. (2000), reported 30% prevalence of urinary infection in sows housed in commercial farms.

Normal urinary bladder and urethra are sterile thus bacterias causative of UTI in female swine are the ones present in the feces, perineum, and vagina; they access urinary tract through the urethra. Microorganisms related to the development of UTI are also the agents of endometritis in sows, such as *Escherichia coli*, *Streptococcus* spp, *Enterococcus* spp e *Candida* spp (Sipos et al. 2014).

UTI is favored by anatomical characteristics of the female such as short urethra, cage housing, prolonged contact of feces with the vulva, stress, low water intake and restrained movement (Alberton et al. 2000). These infections consist of ascending colonization of urethra and urinary bladder alone (cystitis) or bladder, ureter and renal pelvis (pyelonephritis), by pathogenic microorganisms, mainly *Escherichia coli, Proteus mirabilis, Staphylococcus sp., Streptococcus sp., Aeromonas hydrophila, Trueperella pyogenes* and *Actinobaculum suis,* normally present in the inferior urinary tract microbiota, feces and periuretral skin (Alberton et al. 2007, Menin et al. 2008). *Escherichia coli* is the most commonly isolated bacteria in mild or severe cases of cystitis and pyelonephritis in swines, being responsible for at least 50% of the cases (Fairbrother and Gyles 2006, Mazutti et al. 2013). Pôrto et al. (2003) found up to 57% of *E. coli* in sows with UTI. *E. coli* is also a cause of UTI in dogs, cats and humans, composing up to 40% of UTI in such domestic species and up to 85% in women (Ling et al. 2001, Johnson et al. 2003, Rosen et al. 2007, Litster et al. 2011).

UTI are usually recurrent in all host species and the uropathogenic *E. coli* strains (UPEC) are usually genetically and phenotypically similar among different clinical manifestations (Garofalo et al. 2007). Whether recurrence is due to reinfection by gastrointestinal bacteria or by agents previously present in the urinary tract even after clinical resolution and absence of viable organisms in urine samples was an issue to be explained (Russo et al. 1995, Stapleton and Stamm 1997, Scott et al. 2015). Subsequent studies demonstrated that persistence of *E. coli* in urinary tract and reactivation of infection are possible due to formation of intracellular bacterial communities (IBC) in superficial epithelial cells of urinary bladder transitional epithelium, a mechanism initially demonstrated in mice and latter in humans (Mulvey et al. 2001, Anderson et al. 2004a, Rosen et al. 2007). Human and swine UPEC have the same pathogenicity factors, such as type 1 pili, what allows to speculate that IBC mechanism and pathogenicity factors also occurs in swines (Wiles et al. 2008).

This research had the objective of investigating the occurrence of IBCs in the urinary bladder of sows presenting macroscopic signals of cystitis, thus contributing for understanding the mechanisms possibly involved on the elevated occurrence of cystitis in such animals and providing elements for further researches aiming to expand this topic.

3.4 MATERIALS AND METHODS

This study was approved by the ethics committee of Universidade Federal do Paraná, sector Palotina, number 42/2017.

Thirteen urinary bladder samples of sows eliminated from commercial farms from western Paraná and Santa Catarina states, for any reason, showing macroscopic lesions consistent with cystitis, such as hyperemia, hemorrhage, edema, and exudate were selected. The samples were photographed and collected fragments were conditioned in 10% buffered formalin, processed for hematoxylin and eosin (HE) stain according to standard procedures for histopathological evaluation. Samples were also submitted to immunohistochemistry (IHC) technique using polyclonal anti-*E. coli* antibodies according to the method of De Lorenzo et al. (2017).

Microscopically, samples were considered positive for cystitis when inflammatory cells in the lamina propria and submucosa were present. Intensity of the lesions observed were arbitrarily classified according to the proportion of affected tissue in 5 categories: discrete (up to 10%), mild (10% to 30%) moderate (30% to 60%), accentuated (60% to 90%) and severe (90% to 100%).

3.5 RESULTS

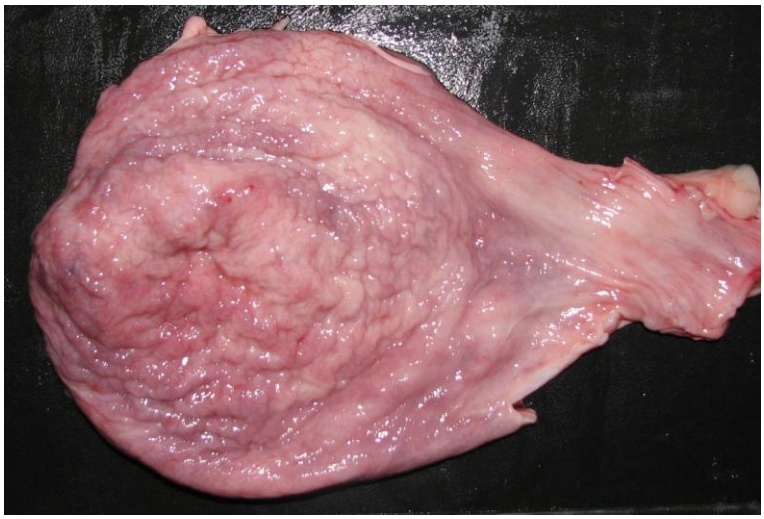
Urinary bladders evaluated had macroscopic characteristics of acute or subacute inflammation, with hyperemia, petechiae, ecchymosis and edema. Intensity varied from mild to accentuated (figures 1 and 2).

All 13 samples showed inflammatory infiltrate, 12 of which composed of lymphocytes, histiocytes, and plasma cells, multifocal or diffusely distributed along the lamina propria and submucosa. Intensity varied from mild to moderate (figure 3). One sample showed, an infiltrate composed of lymphocytes, histiocytes and neutrophils (table 1).

Table 1. Microscopic lesions on urinary bladder samples of 13 sows with chronic cystitis

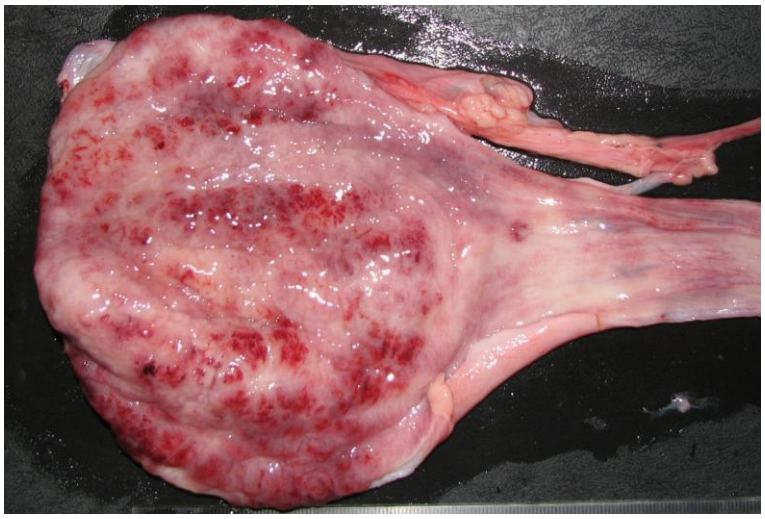
	Lymphohistiocytic and plasmacytic infiltrate	Lymphohistiocytic and neutrophilic infiltrate	Urothelial "pods" filled with basophilic bacterial colonies	Translucent urothelial "pods"	IHC positive marking of intermediate or basal layers
1	~~x~~		~~x~~		
2	~~x~~		~~x~~		x
3	~~x~~			x	x
4	~~x~~		~~x~~		x
5	~~x~~			x	
6	~~x~~			x	
7	~~x~~		~~x~~		
8	~~x~~			x	
9	~~x~~			x	
10	~~x~~			x	
11	x			x	x
12	~~x~~			x	x
13		~~x~~			

SOURCE: The author (2017)



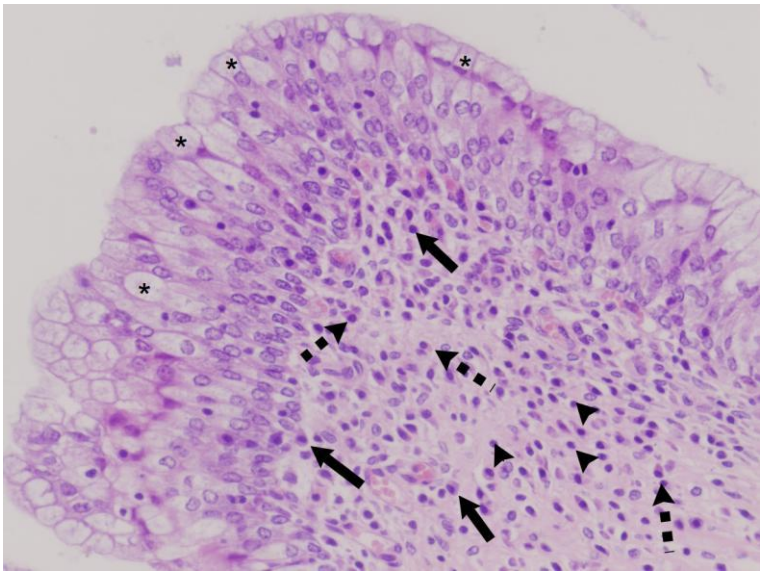
SOURCE: The author (2017)

Figure.1 - Swine. Urinary bladder. Mucosa. Mild to moderate diffuse hyperemia.



SOURCE: The author (2017)

Figure.2 – Swine. Urinary bladder. Mucosa. Multifocal moderate petechiae and hemorrhages.



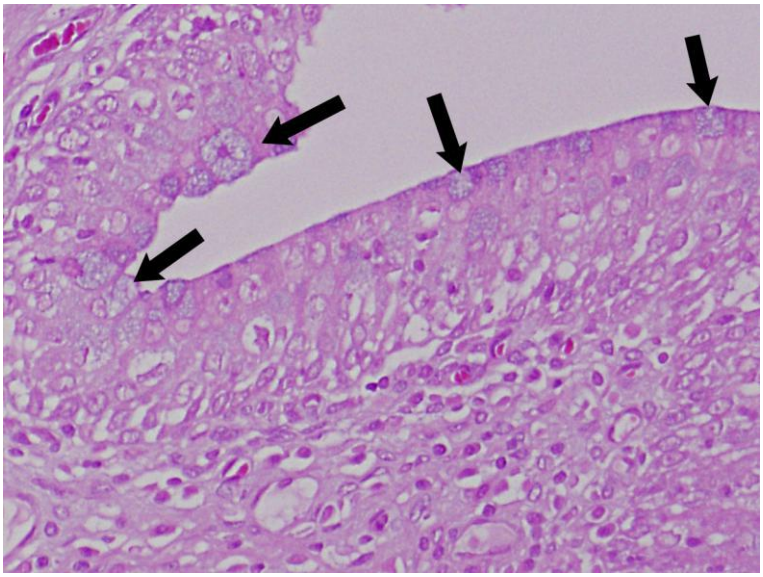
SOURCE: The author (2017)

Figure.3 - Swine. Urinary bladder. Hematoxylin and eosin. Through submucosa and lamina propria there is inflammatory infiltrate constituted by lymphocytes (arrowhead), histiocytes (arrow) and plasma cells (dashed arrow). The urothelium sows multifocal cytoplasmic dilations (asterisk).

Multifocal intracytoplasmatic dilations were observed in all samples, in different intensities. Most of the time such structures were translucent and found inside superficial or intermediate urothelial cells. In four samples (30.7%) such dilations were observed on the apex of superficial urothelium cells, therefore named pods (figure 4), filled with basophilic structures compatible with bacterial colonies (Anderson et al. 2003, Scott et al. 2015). Other lesions included hemorrhage, edema and hyperemia.

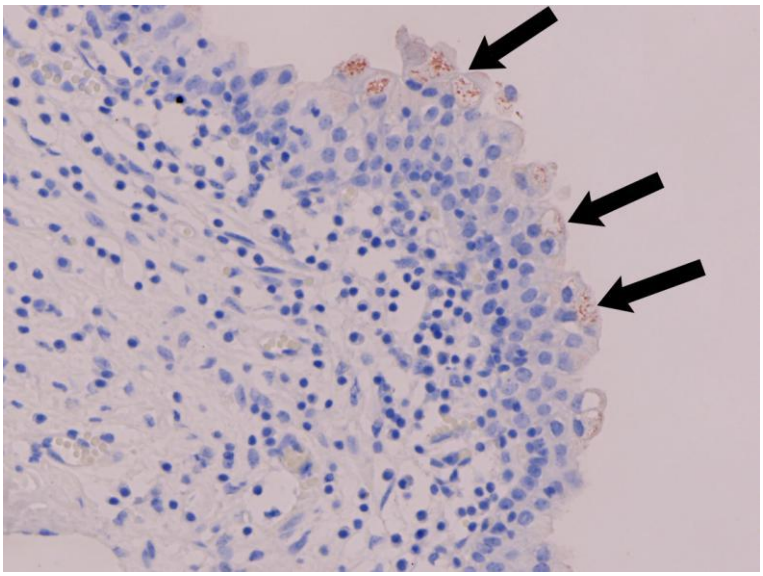
Twelve samples (92.3%) were positive for *E. coli* by immunohistochemistry; all of them had exclusively mononuclear inflammatory infiltrate, thus compatible with chronic cystitis. The only negative case was the one that presented neutrophilic infiltrate associated, corresponding to a subacute cystitis. All positive cases had cytoplasmic dilations, not present on the only negative case, but such dilations were not always readily evident; sometimes they were small and scarce.

We verified that the superficial pods observed in HE staining seem to be positively marked for *E. coli* by immunohistochemistry, although we could not track if exactly the same structures were represented on HE and IHC slides; positive staining was also observed on the surface of urothelium (figures 5 and 6). Even though marking was predominantly intracytoplasmic, multifocal and restricted to the superficial cells, five cases (38.4%) also had foci of positively staining cells along intermediate urothelial layer (figure 6).



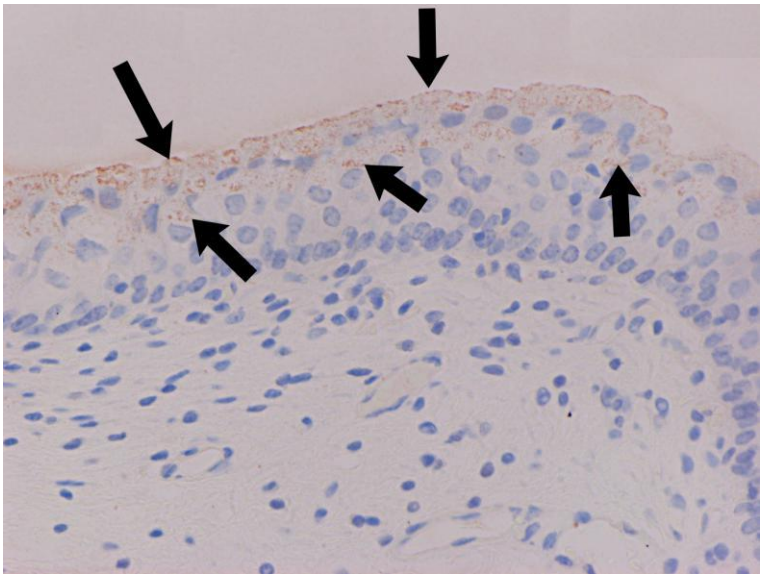
SOURCE: The author (2017)

Figure.4 – Swine. Urinary bladder. Hematoxylin and eosin. There are multifocal cytoplasmatic dilations affecting superficial epithelial cells (arrows), filled with basophilic material. Lamina propria is infiltrated by lymphocytes, plasma cells and histiocytes.



SOURCE: The author (2017)

Figure.5 - Swine. Urinary bladder. Immunohistochemistry. Intracytoplasmic "pods" positively marked on superficial urothelial cells.



SOURCE: The author (2017)

Figure.6 – Swine. Urinary bladder. Immunohistochemistry. Positive marking on the surface of urothelium and inside cytoplasmic vesicles on superficial and intermediate layer cells (arrows).

3.6 DISCUSSION

Escherichia coli IBC formation mechanism is already described in humans and was experimentally reproduced in mouse models (Justice et al. 2004). This is the first report of such findings in swine. Existence of IBCs started to be investigated from the study of chronic unresponsive cystitis in women (Scott et al. 2015), and further studies found increasing evidence of IBC formation on human urothelium as well as it's role for persistence of the *E. coli* on the urinary tract (Martinez et al. 2000, Mulvey et al. 2001, Anderson et al. 2003). Although it was not performed tests to identify the specific pathotype of the *E. coli* identified in this research, there is no evidence of *E. coli* other than UPEC involved in such type of infection to the urothelium. Therefore UPEC will be used as a model for further discussion.

Adherence of UPEC to the urothelium is essential for development of infection. Bacteria bonds to cells by interaction between FimH adesin, placed on the terminal extremity of type 1 pili, and glycoproteic receptors or uroplakins on the host cell (Flores-Mireles et al. 2015). This connection avoids UPEC elimination due to urine flow but interaction with bacterial lipopolysaccharides triggers a Toll-like 4-CD14 receptor mediated immune response which culminates with neutrophil infiltration, affected cells apoptosis and increased epithelial regeneration (Anderson et al. 2004a).

Bacteria is internalized by host cell 1 to 3 hours after adhesion by means of phagocytic system. Once in the citosol it has access to an environment rich in nutrients, protected from immune response, antibiotics and from friction on the mucosa. At this point, three stages of infection, based on mouse models, take place: early, intermediate and late (Mulvey et al. 2001, Justice et al. 2004).

First stage is characterized by detection of scarce rod-shaped microorganisms, measuring about 3μm long inside superficial urothelial cells about 6 hours after inoculation. There are reports of intracellular bacteria visualized in hematoxylin and eosin stained sections in mice during this stage, similar to the ones observed on the present paper (figure 4) (Anderson et al. 2003). A rapid bacterial replication starts with the population doubling its size every 30 to 35 minutes for up to 8 hours after host cell invasion (Justice et al. 2004).

Eight hours after the beginning of infection process, the intermediate phase takes place. Doubling time raises to 60 minutes, bacteria decreases to 0.7μm long, assume a coccoid shape and forms dense communities that occupy the whole cell, forming pods. In this stage colony has biofilm characteristics, making them resistant to antibiotic therapy (Kau et al. 2005, Scott et al. 2015).

With the microscopy techniques and sample collection used in this paper it is not possible do determine which phases are taking place on our observations but we conclude, by morphological similarity, that the protrusions observed on superficial urothelial cells are compatible either with initial or intermediate stages of infection.

About 12 hours after infection, UPEC placed at the periphery of IBC are released from colony, with high motile capacity, 2μm long, capable of leave host cell and reach new hosts cells nearby, restarting the cycle. The whole mass of UPEC can also leave the cell simultaneously. It is estimated that each IBC has 100,000 individuals. After releasing UPEC can turn into a 70μm filament that undergoes successive

septation, releasing new bacteria. Acute inflammatory response neutrophils are ineffective against UPEC in this shape (Anderson et al. 2004a, Scott et al. 2015).

Invasion of urothelial cells and IBCs formation continues but UPEC replication rate reduces after second cycle of reinfection. After some days only a few small IBCs are found inside epithelial cells and can remain in such form for months. These quiescent structures are constituted by four to 10 non-replicating bacterias (Flores-Mireles et al. 2015). Urinary bladder epithelium is normal in this stage, but bacteriuria episodes may occur, probably due to reactivation of IBCs for colonization of new cells. Such episodes might be interpreted as recurrence of infection but there will not always be an active inflammatory process going on. Factors that can lead to reactivation of infection are not well known yet but it is suggested that the normal process of urothelium reorganization and maturation can trigger IBCs activation and bacterial releasing into bladder lumen (Justice et al. 2004, Blango et al. 2014). Whether such factors and what factors at all are responsible for triggering IBC reactivation in sows are yet to be understood.

UPEC ability in invading intermediate and basal urothelial cells is controversial. Though authors agree that UPEC can invade such cell layers, some of them did not verify quantitative differences of bacteria inside basal cells while others say such cells host only a few UPEC when compared to superficial cells (Mulvey et al. 2001, Anderson et al. 2004a, Anderson et al. 2004b, Blango et al. 2014). In this paper, we found five cases with positive marked cells of the intermediate and basal layers but intensity of marking was significantly lower compared to superficial cells, although quantification was not performed.

A direct correlation between presence of basophilic material inside pods and IHC marking could not be established, given the fact that not all positive samples had this characteristic, making HE insufficient for acknowledging IBC presence. On the other hand, cystic dilations are easily observed, were present on all positive cases and appear to be positively marked by IHC therefore presence of such structures appears to be a consistent indicator of IBC presence. However, other factors and agents causing similar structures must be further investigated.

We deduce that our samples were of chronic cases due to the absence of neutrophilic inflammation, which was reported to be intense on the lamina propria and submucosa up to 48h after infection (Schilling et al. 2001, Anderson et al. 2003, Rosen et al. 2007). The fact that many cytoplasmic dilations were small and not filled with basophilic bacterial colonies also indicates the chronicity of such cases. Association of such dilations and dilations filled by bacteria possibly indicates that some replication cycles already took place in one individual, a hypothesis to be further investigated.

In mice, evaluation of IBC four to 12 days after infection revealed colonies similar to the initial stage, while urine of such mice was negative for bacterial growth, indicating establishment of a latent infection (Scott et al. 2015).

Presence of translucent intracellular dilations or sometimes visibly filled with basophilic bacterial colonies, may suggest that these are an indication of different stages of the infection cycle, given the fact that the initial stage is characterized by rod-shaped 3µm long individuals and later stages are characterized by 0.7µm coccoid bacterias that may not be observed in light microscopy. Acknowledgment of such hypothesis would beg the information of the exact time of bladder infection and more refined microscopy techniques.

Therefore, IBCs are a UPEC persistence mechanism in the urinary tract for allowing immune response, epithelial exfoliation and prolonged antibiotic therapy evasion. It also makes possible for one host to have multiple episodes of subclinical bacteriuria, spreading UPEC through the environment, increasing the possibility of infecting new hosts (Mulvey et al. 2001, Schiling et al. 2002, Anderson et al. 2004b). UPEC spreading by infected hosts is certainly a potential source for persistence of UTI in farms with high prevalence of the disease.

3.7 CONCLUSIONS

Swine are susceptible to formation of intracellular bacterial communities due to uropathogenic *E. coli*.

IBC mechanism allows urinary bladder itself to be a reservoir for bacterias that will reactivate infection and not only the intestines as previously thought, what poses a major risk for chronification and recurrence of cystitis in swines. Although probable, epidemiological importance of IBC as recurrent UTI source in sows must be better studied, as well as the molecular mechanism involved for formation and reactivation of such colonies, which will support development of better prophylaxis and treatments for the disease.

3.8ACKNOWLEDGMENTS

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4 CONSIDERAÇÕES FINAIS

Este trabalho tem o mérito de enriquecer a literatura disponível acerca das causas de morte de porcas e leitoas, tema que carece de estudos atualizados e, principalmente, que tenham foco nos aspectos patológicos das mortes, além de poder ser utilizado como uma base para trabalhos futuros que visem a avaliar a mortalidade destes animais do ponto de vista diagnóstico.

As causas de morte de matrizes suínas deveriam ser estudadas nas diversas regiões de criação de suínos e durante os períodos de tempo mais longos possíveis, com objetivo não apenas de elencar as doenças que acometem o rebanho, mas verificar a distribuição dos diagnósticos em função das estações do ano, do efeito de fatores locais como manejo, presença de agentes etiológicos específicos, qualidade de instalações, quantidade de animais, tipo de alimentação e da evolução dos diagnósticos com o passar dos anos.

Criar o hábito de realização de necropsias nas granjas é essencial para a melhoria da monitoria das condições sanitárias do plantel, assim como treinar os funcionários para identificarem animais doentes, visto que frequentemente são encontrados animais mortos, sem que houvessem sido notados sinais clínicos. Além disso, as causas de mortalidade são compiladas apenas com base em sinais ou suspeitas clínicas e quase sempre foram provadas erradas por meio da necropsia, evidenciando que não são confiáveis os registros de mortalidade emitidos pela administração das granjas. Isto impede uma avaliação correta da situação sanitária dos animais e consequentemente a possibilidade de melhorias no plantel.

A comprovação da ocorrência de CBI por ECUP na bexiga de porcas permite inferir que o mecanismo, por sí só, seja parcialmente responsável pelas altas taxas de ITU relatadas em porcas. Além disso, abre caminho para uma miríade de trabalhos que busquem descobrir o papel destas colônias no desenvolvimento, persistência e reativação das ITU. Também coloca questões sobre como e quais técnicas de diagnóstico aplicar na rotina de produção para detectar animais acometidos, ou mesmo se é viável tal detecção.

4.1 RECOMENDAÇÕES PARA TRABALHOS FUTUROS

São necessários trabalhos subsequentes que avaliem a mortalidade de porcas em granjas ao longo de todas as estações do ano e em granjas com diferentes condições de manejo e ambientais por meio de necropsias. Para tal trabalho, recomenda-se a participação de ao menos duas pessoas, a depender da quantidade de animais a serem monitorados, de forma a melhorar a detecção das mortes na rotina, acelerar o procedimento de necropsia e melhorar a coleta de informações acerca de cada animal. Também é necessário que seja dado enfoque aos aspectos patológicos das mortes como forma de se obter alguma padronização e comparabilidade entre diferentes estudos, além de aumentar a literatura disponível sobre o tema.

A realização de exames complementares às necropsias também é altamente recomendável, não só por meio do exame histopatológico mas também com exames voltados a identificação de agentes etiológicos, como a reação em cadeia da polimerase e isolamento bacteriano, para melhor detalhamento dos diagnósticos. Para tal, é necessário ter um meio ágil e seguro para o envio de amostras para o laboratório responsável por realizar tais exames, o que não foi possível no presente estudo.

A colaboração dos funcionários das granjas envolvidas na pesquisa também é crucial, pois frequentemente são eles que identificarão os animais doentes, além de fazer o transporte do cadáver até o local de necropsia e fornecer os dados clínicos sobre o animal; para tal é necessário manter um bom relacionamento com os trabalhadores do local e deixá-los cientes da importância do trabalho e da sua colaboração.

Com a comprovação da ocorrência de CBI no urotélio vesical de porcas surgem diversas questões sobre este mecanismo em suínos. Pesquisas subsequentes podem ter foco em:

a) Elucidar a importância deste mecanismo para a prevalência de ITU em porcas

b) Verificar se há uma relação de fatores individuais ou de rebanho para a formação das CBI

c) Se a sequência de eventos descritas em humanos e ratos para a formação e reativação das CBI é a mesma em suínos

d) Quais fatores podem estar implicados na reativação das CBI e desenvolvimento de ITU com importância clínica

e) Quais mecanismos moleculares relacionados as CBI podem ser alvo de drogas com objetivo de eliminar ou conter o desenvolvimento das colônias

f) Caracterizar detalhadamente, do ponto de vista histológico e clínico, qual a evolução da ITU por ECUP, principalmente para verificar quais as alterações histopatológicas do epitélio vesical com a progressão da infecção, a partir de quanto tempo as colônias podem ser detectadas por meio de coloração de hematoxilina e eosina e quando se tornam detectáveis apenas por meio de IHQ ou outros métodos

g) Métodos de diagnóstico, controle e tratamento de porcas afetadas por CBI

h) Identificar outros agentes cuja patogenia potencialmente envolva a formação de CBI ou processos similares

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