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

ASPECTOS PATOLÓGICOS DAS DOENÇAS NUTRICIONAIS EM SUÍNOS

MARINA PAULA LORENZETT

PORTO ALEGRE
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Tese apresentada como requisito parcial para obtenção do grau de Doutora em Ciências Veterinárias na área de concentração em Medicina Veterinária Preventiva e Patologia: Patologia Animal e Patologia Clínica

Orientador: Prof. Dr. Saulo Petinatti Pavarini

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MARINA PAULA LORENZETT

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DEDICATÓRIA

Este trabalho é dedicado aos meus pais, Adilson Antônio Lorenzett e Marlei Libra Secco Lorenzett.

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A Deus e a Maria, Nossa Mãe, por todas as graças concedidas.

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RESUMO

O diagnóstico das deficiências nutricionais na suinocultura deve ser realizado após estudo detalhado da alimentação fornecida aos suínos, envolvendo a mensuração quantitativa e qualitativa dos nutrientes das dietas, além de considerar alterações no manejo nutricional. As causas mais frequentes de deficiências nutricionais estão relacionadas a erros de formulação e preparo de premixes e/ou rações com excesso ou deficiência de algum nutriente. Nessa tese estão incluídos três manuscritos abordando casos clínicos de desequilíbrios nutricionais. O primeiro manuscrito teve o objetivo de descrever os achados epidemiológicos e clínico-patológicos de 22 surtos de urolitíase em suínos em crescimento e terminação. Os suínos afetados eram machos e a letalidade foi de 100%. Os urólitos foram submetidos ao método de determinação qualitativa dos componentes minerais, os quais foram compatíveis com carbonato de cálcio e fosfato de amônio magnesiano. A análise da ração de crescimento revelou baixos níveis de cálcio, em relação ao fósforo, perfazendo uma relação Ca:P de aproximadamente 0,35:1. A principal causa da morte dos suínos foi relacionada à desidratação e ao uroperitônio. O segundo manuscrito teve o objetivo de descrever dois eventos de mielopatia degenerativa em leitões de quatro a 27 dias de idade. A taxa de mortalidade atingiu 40%. A cromatografia líquida de alta eficiência indicou baixos níveis de ácido pantotênico nas amostras de premix e nas rações das fases de gestação e lactação fornecidas às porcas. Os leitões apresentavam depressão severa, fraqueza, anorexia, diarreia, ataxia sensorial e déficits posturais e paresia, sendo mais pronunciada nos membros pélvicos. A necropsia de vinte e dois leitões não demonstrou lesões macroscópicas significativas. Histologicamente, houve degeneração e necrose de neurônios da medula espinhal, principalmente no núcleo torácico e neurônios motores alfa no corno ventral na intumescência lombar e neurônios motores alfa no corno ventral na intumescência cervical. Degeneração axonal e de mielina mínima a moderada foi observada no funículo dorsal da medula espinhal e nas raízes nervosas dorsal e ventral. O terceiro manuscrito teve o objetivo de descrever os achados patológicos e toxicológicos de um quadro de fragilidade óssea generalizada compatíveis com osteoporose de origem nutricional. Quarenta e seis porcos apresentaram fraqueza muscular, paresia e paralisia de membros posteriores, emagrecimento, decúbito lateral e óbito, com evolução clínica de sete a 10 dias. Dois suínos foram submetidos à necropsia, na qual foram observadas formação de calo ósseo e múltiplas fraturas em membros, costelas e vértebras. A histologia revelou uma diminuição difusa e acentuada da espessura e do número de trabéculas. Sobrecarga significativa de zinco e consequente deficiência de cobre foram determinadas por espectrometria de absorção atômica.

Palavras-chave: urólitos, desbalanço mineral, cálcio, fósforo, cromatólise, ácido pantotênico, ataxia proprioceptiva, sobrecarga de zinco, deficiência de cobre, fragilidade óssea.

ABSTRACT

The diagnosis of nutritional deficiencies in pig farming must be carried out after a detailed study of the feed provided to pigs, involving quantitative and qualitative nutrient measurement in the diets, in addition to considering changes in nutritional management. The most frequent causes of nutritional deficiencies are due to errors in the formulation and preparation of premixes and/or rations with excess or deficiency of some nutrient. This thesis includes three manuscripts with clinical cases of nutritional imbalances. The first manuscript aimed to describe the epidemiological and clinical-pathological findings of 22 outbreaks of urolithiasis in growing and finishing pigs. The affected pigs were male, and the lethality was 100%. The uroliths were submitted for qualitative determination of the mineral components, which were compatible with calcium carbonate and magnesium ammonium phosphate. The analysis of the grower ration showed low levels of calcium when compared with phosphorus levels, making a Ca: P ratio of approximately 0.35:1. The main cause of death was related to dehydration and uroperitoneum. The second manuscript aimed to describe two events of degenerative myelopathy in piglets from four to 27 days old. The mortality rate reached 40%. High-performance liquid chromatography indicated low levels of pantothenic acid in the premix samples and the rations of gestation and lactation phases provided to the sows. Piglets presented with severe depression, weakness, anorexia, diarrhea, sensory ataxia, and postural deficits and paresis, which were more pronounced in the pelvic limbs. Necropsy of 22 piglets demonstrated no significant gross lesions. Histologically, there was neuronal degeneration and necrosis in the spinal cord, primarily in the thoracic nucleus and alpha-motor neurons in the ventral horn in the lumbar intumescence, as well as in the alpha-motor neurons in the ventral horn in the cervical intumescence. Minimal to moderate axonal and myelin degeneration were observed in the dorsal funiculus of the spinal cord, as well as in the dorsal and ventral nerve roots. The third manuscript aimed to describe the pathological and toxicological findings of an outbreak of osteoporosis of nutritional origin. Forty-six pigs presented with muscle weakness, hind limb paresis and paralysis, weight loss, lateral recumbency, and death, with a clinical course of seven to 10 days. Two pigs were submitted to necropsy, in which bone callus formation and multiple fractures in the limbs, ribs, and vertebrae were observed. Histology revealed a diffuse and marked decrease in thickness and the number of bone trabeculae. Significant zinc overload and consequent copper deficiency were determined by atomic absorption spectrometry.

Keywords: *uroliths, mineral imbalance, calcium, phosphorus, chromatolysis, pantothenic acid, proprioceptive ataxia, zinc overload, copper deficiency, bone fragility.*

SUMÁRIO

1. INTRODUÇÃO

O Brasil é atualmente o quarto maior produtor e exportador de carne suína no mundo, e os estados de Santa Catarina, Paraná e Rio Grande do Sul concentram a maior produção de suínos do país (ABPA. 2020). A produção nacional de suínos cresce a uma taxa anual de 2,15%, e as exportações de carne suína pelo Brasil apresentam um aumento médio de 6,6% ao ano (MARTINS, F. M.; SANTOS, J. I. F.; TALAMINI, D. J. D. 2018). A suinocultura brasileira está entre as cadeias produtivas mais avançadas do mundo. O implemento de alta tecnologia nas áreas de genética, nutrição, instalações e manejo, possibilitou a produção de carne suína com elevados padrões de qualidade (ABPA. 2020). Os índices produtivos alcançados pelos rebanhos brasileiros tecnificados são semelhantes aos de outros países com atividade suinícola desenvolvida (ZANELLA, J. R. C.; MORÉS, N.; BARCELLOS, D. E. S. N. 2016).

Neste contexto, os avanços constantes na suinocultura brasileira tornaram as deficiências nutricionais cada vez mais raras nos sistemas atuais de produção. As causas mais frequentes de deficiências nutricionais estão relacionadas a erros de formulação e preparo de premixes e/ou rações com excesso ou deficiência de algum nutriente. Nos casos em que ocorre o excesso de algum nutriente especifico pode haver a interferência na absorção de outros minerais. Além disso, nas misturas racêmicas pode haver a presença de agentes quelantes que poderiam interferir na absorção e biodisponibilidade dos nutrientes. Nos suínos, os fatores determinantes que poderiam favorecer o desenvolvimento de deficiências nutricionais estão relacionados à redução do consumo alimentar, pois a absorção dos diferentes nutrientes acrescentados à ração depende da quantidade de ração consumida por animal ao dia, além das variações nas exigências nutricionais entre as diferentes fases de criação dos suínos (MORENO, A. M.; SOBESTIANSKY, J.; BARCELLOS, D. 2012).

O diagnóstico das deficiências nutricionais na suinocultura deve ser realizado após estudo detalhado da alimentação fornecida aos suínos, envolvendo a mensuração quantitativa e

qualitativa dos nutrientes das dietas, além de considerar elementos do manejo nutricional (MORENO, A. M.; SOBESTIANSKY, J.; BARCELLOS, D. 2012).

O objetivo deste estudo é realizar a caracterização epidemiológica, clínica e patológica de surtos de doenças nutricionais em suínos diagnosticadas pelo Setor de Patologia Veterinária da UFRGS.

2. ARTIGOS CIENTÍFICOS

Os materiais e métodos aplicados, os resultados e as discussões da pesquisa serão a seguir apresentados em três artigos científicos.

Artigo 1: Obstructive urolithiasis in growing-finishing pigs.

Artigo 2: Motor and somatosensory degenerative myelopathy responsive to pantothenic acid in piglets.

Artigo 3: Swine metabolic bone disorders: outbreak of osteoporosis and literature review.

2.1. ARTIGO 1

Obstructive urolithiasis in growing-finishing pigs

Marina P. Lorenzett, Raquel A.S. Cruz, Bianca S. Cecco, Claiton I. Schwertz, Márcia E. Hammerschmitt, Daniela T. Schu, David Driemeier and Saulo P. Pavarini

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Original Article
Livestock Diseases

Obstructive urolithiasis in growing-finishing pigs[1]

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ABSTRACT.- Lorenzett M.P., Cruz R.A.S., Cecco B.S., Schwertz C.I., Hammerschmitt M.E., Schu D.T., Driemeier D. & Pavarini S.P. 2019. **Obstructive urolithiasis in growing-finishing pigs.** *Pesquisa Veterinária Brasileira 39(6):382-387.* Setor de Patologia Veterinária, Departamento de Patologia Clínica Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, Prédio 42505, Porto Alegre, RS 91540-000, Brazil. E-mail: sauloppvet@yahoo.com.br

Obstructive urolithiasis is a disease characterized by the presence of uroliths in the urinary tract, with consequent obstruction of excretion pathways. This paper described the epidemiological and clinical-pathological findings of 22 outbreaks of urolithiasis in growing-finishing pigs in Southern Brazil. All affected pigs were male and clinical presentation consisted of lethargy, dysuria, rectal prolapse, abdominal distention, peripheral cyanosis and reluctance to move. Clinical progression course ranged from 12 hours to one week, and the lethality rate was 100%. Gross changes were characterized by urinary bladder rupture associated with marked amount of yellowish liquid with ammoniacal odor (urine) in the abdominal cavity (uroperitoneum), as well as mild fibrin deposition on the surface of abdominal organs and hydronephrosis. Urinary uroliths ranging from 0.3 to 1cm in diameter were often observed obstructing the lumen of the penile urethra and sometimes those were free in the abdominal cavity. Histopathological findings included diffuse and marked urinary bladder edema and hemorrhage associated with inflammatory infiltrate of lymphocytes, plasma cells, and macrophages. Diffuse and marked necrosis of the mucosal epithelium was observed in the penile urethra. Intense fibrin deposition and inflammatory infiltrate of neutrophils were noted in the peritoneum, as well as in the serosa of the organs in the abdominal cavity. Uroliths were submitted to the method of qualitative determination of the mineral components, and were compatible with calcium carbonate and magnesium ammonium phosphate. Growing pigs ration analysis revealed low levels of calcium in relation to phosphorus, resulting in a Ca:P ratio of approximately 0.35:1. Histological findings and mineral analysis suggest that outbreaks of urolithiasis were related to a nutritional imbalance in the proportions of dietary calcium and phosphorus. The main cause of mortality was related to dehydration and uroperitoneum.

INDEX TERMS: Swine, urinary system, uroliths, uroperitoneum, mineral imbalance, calcium, phosphorus.

RESUMO.- [Urolitíase obstrutiva em suínos de crescimento e terminação.] Urolitíase obstrutiva é uma enfermidade caracterizada pela presença de urólitos no trato urinário, com consequente obstrução das vias de excreção. Este artigo descreve os achados epidemiológicos e clínico-patológicos de 22 surtos de urolitíase em suínos de crescimento e terminação no Sul do Brasil. Os suínos afetados eram machos e clinicamente apresentavam letargia, disúria, prolapso retal, abaulamento do abdômen, extremidades cianóticas e relutância em movimentar-se. A duração dos sinais clínicos variou de 12 horas a uma semana, e a letalidade foi de 100%. As alterações macroscópicas caracterizaram-se por ruptura da bexiga com acentuada quantidade de líquido de coloração amarelada e odor amoniacal (urina) livre na cavidade abdominal

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(uroperitônio), além de discreta deposição de fibrina sobre os órgãos e hidronefrose. Frequentemente obstruindo o lúmen da uretra peniana e por vezes livre na cavidade abdominal, era possível observar urólitos urinários que variavam de 0,3 a 1cm de diâmetro. Os achados histopatológicos incluíram edema e hemorragia difusos e acentuados na bexiga, associado a infiltrado inflamatório predominante de linfócitos, plasmócitos e macrófagos. Na uretra peniana havia necrose difusa e acentuada do epitélio da mucosa. No peritônio e nas serosas dos órgãos da cavidade abdominal havia intensa deposição de fibrina e infiltrado neutrofílico. Os urólitos foram submetidos ao método de determinação qualitativa dos componentes minerais, os quais foram compatíveis com carbonato de cálcio e fosfato de amônio magnesiano. A análise da ração de crescimento revelou baixos níveis de cálcio, em relação ao fósforo, perfazendo uma relação Ca:P de aproximadamente 0,35:1. Os achados histológicos e as dosagens minerais sugerem que os surtos de urolitíase foram relacionados a um desequilíbrio nutricional nas proporções de cálcio e fósforo dietético. A principal causa da morte dos suínos foi relacionada à desidratação e ao uroperitônio.

TERMOS DE INDEXAÇÃO: suíno, sistema urinário, urólitos, uroperitônio, desbalanço mineral, cálcio, fósforo.

INTRODUCTION

Obstructive urolithiasis is a disease characterized by the presence of uroliths in the urinary tract, with consequent obstruction of excretion pathways (Radostits et al. 2000, Cianciolo & Mohr 2016). Uroliths or calculi are macroscopic mineral concretions, which are composed by precipitated urinary solutes, associated with small amounts of organic matter (Drolet 2012, Cianciolo & Mohr 2016). Urolithiasis affects similarly males and females; however, urinary obstruction is a condition exclusively reported in males, mainly in castrated hogs (Radostits et al. 2000).

Case descriptions of urolithiasis affecting pigs are scarce when compared with other domestic species (Drolet 2012). Nevertheless, similarly to other species, males are more frequently affected due to specific features regarding their urinary tract morphologic anatomy (Maes et al. 2004). The condition is sporadically detected in pigs of all age groups and is occasionally observed as an incidental finding in pigs at the slaughter. Urinary calculi found in pigs may present various compositions, including calcium carbonate, calcium apatite, magnesium ammonium phosphate, uric acid, and urate (Drolet 2012).

Urinary calculi formation frequently results from the interaction of physiologic and nutritional factors with husbandry practices (Loretti et al. 2003), and is mainly related to the excessive or imbalanced ingestion of minerals present in the drinking water and feed (McIntosh 1978, Larson 1996, Radostits et al. 2000, Drolet 2012). Imbalance in the calcium and phosphorus ratio leads to high excretion of urinary phosphate, which is an important factor in the formation of phosphate calculi. Diets presenting high mineral concentration, associated with high levels of mucoproteins in the urine of fast growing animals, are likely the most important factor for calculogenesis (Radostits et al. 2000). Urinary pH, reduced water intake, urinary stasis, treatment with certain drugs, and preexisting urinary tract disease are also factors associated with the occurrence of urolithiasis. Such predisposing factors may act synergistically for calculi formation or play a role individually (Drolet 2012, Sobestiansky 2012). In the present study, the epidemiological and clinical-pathological findings of outbreaks of urolithiasis in growing-finishing pigs in Southern Brazil are reported.

MATERIALS AND METHODS

Clinical and epidemiological data were obtained directly with the farm owner and the referring field veterinarian. Out of forty finishing male pigs showing clinical signs of lethargy and dysuria submitted for necropsy during the outbreaks, four animals underwent histopathological examination. During the necropsy procedures, samples of various organs were collected for histological examination, fixed in 10% formalin, routinely processed and stained by hematoxylin and eosin (HE). In one pig submitted for necropsy, serum samples were collected in order to obtain total serum calcium and phosphorus values. Ration samples (ration for growing pigs, phases 1 and 2) were collected to determine the levels of calcium, phosphorus, magnesium, fluoride, sodium and moisture. In addition, a group of pigs with history of urolithiasis was monitored at the slaughterhouse. In this occasion, 20 urinary bladders were collected and urine samples were obtained for urinalysis. Uroliths collected during the necropsy procedures were tested using the qualitative determination technique for regular components of renal calculus (kit Cálculo Renal Biodiin®, Quibasa Química Básica Ltda, Belo Horizonte/MG, Brasil).

RESULTS

From April 2016 to September 2017, 22 growing-finishing pig farms integrated to the same company in the municipality of Harmonia, Rio Grande do Sul State, Brazil, reported the occurrence of obstructive urolithiasis in pigs ranging from 73 to 163 days of age. During the outbreaks, 44 pigs from 22 different growing-finishing sites died. Groups of pigs ranged from 400 to 600 hogs, with an average of 500 pigs allocated in the same farm. During the outbreaks, these pigs, originally from 3 farrowing farms, were fed the same ration. Feed and water access ad libitum were provided. The types of drinking systems varied from farm to farm, and were represented by drinking bowl (16/22), drinking bowl with nipple (4/22) and nipple drinkers (2/22).

All affected pigs were male, and the clinical manifestation included lethargy and dysuria, which was characterized by vigorous abdominal movements showing an attempt to urinate, causing marked rectal prolapse (Fig.1A), and progressing to distended abdomen (Fig.1B), peripheral cyanosis (distal extremities), and reluctance to move. Clinical signs were observed in pigs ranging from 15 days after allocation in the growing-finishing facilities until the animals reached age for slaughter. Clinical progression lasted from 12 hours to one week. Disease morbidity varied from 0.2 to 1% among different pig herds (average 0.6%), and lethality rate was 100%.

During the outbreaks, 40 growing-finishing crossbred castrated male pigs were submitted for necropsy in order to confirm the diagnosis of urolithiasis. Gross changes were

Fig.1. Obstructive urolithiasis in growing and finishing pigs. (A) Pig showing rectal prolapse. (B) Pig showing markedly distended abdomen and presenting persistent sternal decubitus position.

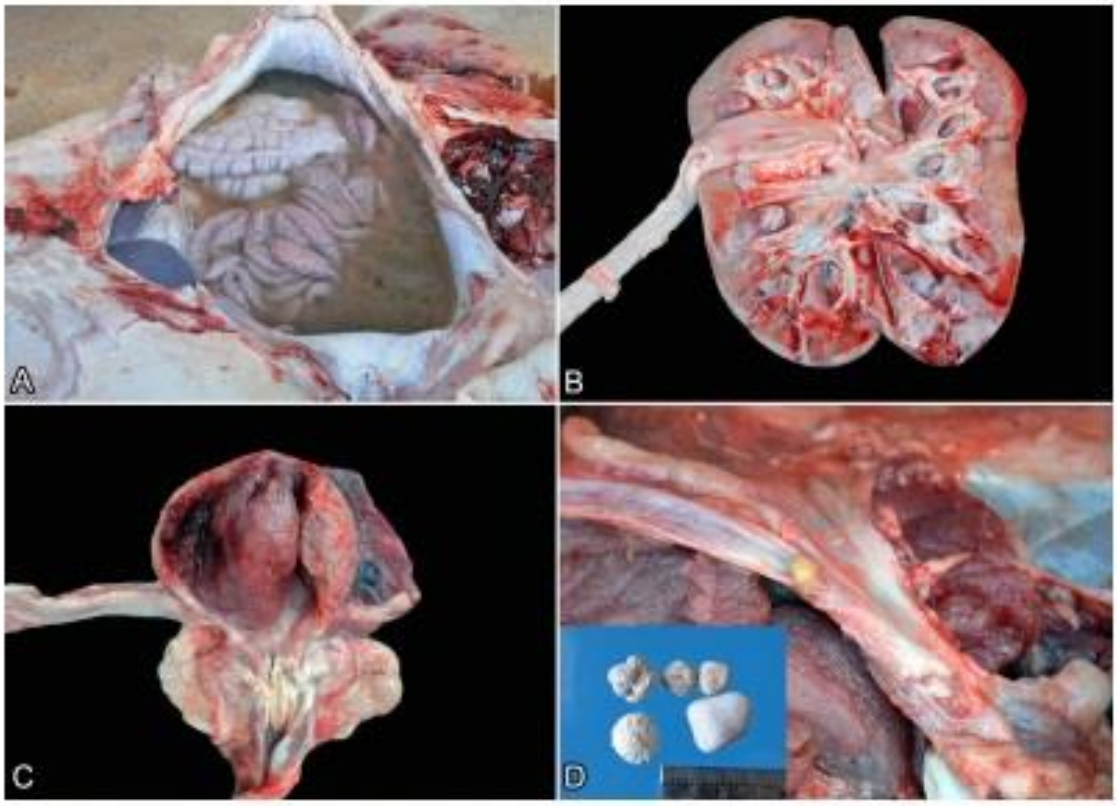

Fig.2. Obstructive urolithiasis in growing and finishing pigs. (A) Abundant amount of free yellowish fluid, presenting ammoniacal odor (urine) in the abdominal cavity due to urinary bladder rupture. (B) Marked dilatation of ureter (hydroureter), and moderate renal pelvis dilation (hydronephrosis). (C) Diffusely reddened, thickened, and irregular urinary bladder mucosa. In the urethra, a focally extensive area of fibrin deposition is noted. (D) Urethral luminal obstruction due to a 0.5cm in diameter calculus. Inset (left): size of uroliths.

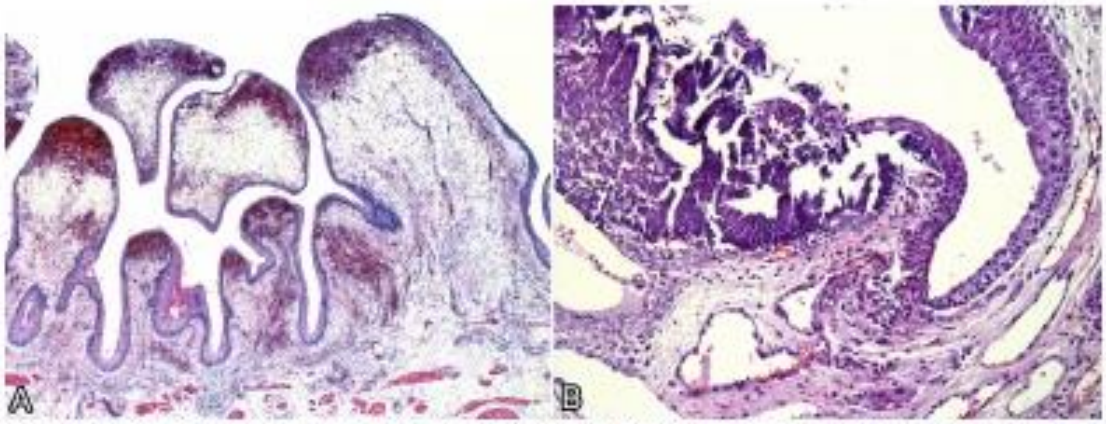

Fig.3. Obstructive urolithiasis in growing and finishing pigs. (A) Urinary bladder. Marked diffuse edema of the submucosa, associated with mucosal projections, as well as extensive areas of hemorrhage and multifocal inflammatory infiltrate. HE, obj.4x. (B) Urethra. Multifocal necrosis of the mucosal lining epithelium, associated with multifocal mild inflammatory infiltrate of neutrophils, lymphocytes and macrophages, as well as deposition of necrotic cell debris, foci of mineralization and bacillary basophilic bacterial aggregates. HE, obj.20x.

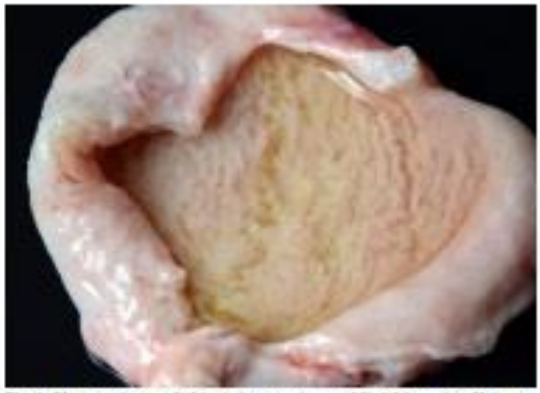

Fig.4. Obstructive urolithiasis in growing and finishing pigs. Urinary bladder presenting multiple uroliths ranging from 0.1 to 0.3cm in diameter. Sample collected during slaughterhouse monitoring conducted in a group of swine with the previous history of urolithiasis.

characterized by bladder rupture associated with abundant amount of free yellowish fluid presenting ammoniacal odor (urine) in the abdominal cavity, as well as mild fibrin deposition on the surface of abdominal organs (Fig.2A), marked bilateral dilatation of ureters (hydroureter), and moderate renal pelvis dilatation (hydronephrosis) (Fig.2B). The urinary bladder mucosa was diffusely reddened, thickened, and irregular and presented mild yellowish fibrillar material (fibrin) deposition. In the distal urethral segment, a focally extensive area of fibrin deposition was noted (Fig.2C). Uroliths ranging from 0.3 to 1cm in diameter were frequently observed obstructing the penile urethral lumen, and sometimes those were free in the abdominal cavity (Fig.2D). The urethral mucosa adjacent to the obstruction site was reddened and ulcerated.

Samples from four pigs were microscopically evaluated and showed diffuse marked edema in the urinary bladder submucosa, as well as extensive areas of hemorrhage and polypoid proliferation in the mucosa (Fig.3A). These lesions were associated with moderate inflammatory infiltrate of lymphocytes, plasma cells, and macrophages. In the penile urethra, marked multifocal necrosis of the mucosal lining epithelium, associated with deposition of necrotic cell debris, foci of mineralization, and bacillary basophilic bacterial aggregates were observed (Fig.3B). Mild to moderate fibrin deposition and infiltrate of neutrophils were noted in the peritoneum and in the serosa of the organs in the abdominal cavity. Marked mesothelial cell hypertrophy and mild fibrous connective tissue proliferation were evidenced in the serosa of the abdominal cavity organs, for instance, in the small and large intestine.

Ration samples of growing pigs (phases 1 and 2) showed magnesium, fluoride, sodium, and moisture levels within reference values recommended for the swine species. Nonetheless, the ration for growing pigs (phase 1) showed calcium and phosphorus values of 1589mg/Kg and 4513/Kg respectively, which corresponds to a Ca:P ratio of approximately 0.35:1 (1.2:1). Ration for growing pigs (phase 2) presented calcium and phosphorus values of 4404mg/Kg and 4169mg/Kg respectively, representing a Ca:P ratio of 1:0.95.

During the slaughterhouse monitoring, 20 urinary bladders were evaluated, of which only one presented uroliths (Fig.4). Six urine samples were submitted for analysis, and no significant alterations regarding density, pH, cellularity, and presence of crystals were noted except for one sample, which presented a small amount of triple phosphate crystals (magnesium ammonium phosphate).

Uroliths collected during the necropsy procedures were submitted to a mineral component qualitative test through the Cálculo Renal Bioclin® kit, which indicated that uroliths were composed of calcium carbonate and magnesium ammonium phosphate. Serum samples were collected from one of the pigs submitted for necropsy. Seric calcium and phosphorus levels were measured, revealing values of 7.05mg/dL (7.1mg/dL) and 13.6mg/dL (9.6mg/dL) respectively. After adjusting the dietary levels of calcium and phosphorus in the ration for growing-finishing pigs in the affected farms, clinical cases of obstructive urolithiasis were no longer observed.

DISCUSSION

The diagnosis of urolithiasis was based on the epidemiological, clinical, and pathological findings, along with the observation of uroliths in the urinary tract of affected animals. In the present study, obstructive urolithiasis was attributed to an imbalance in the ratio of calcium and phosphorus in the diet of growing pigs, since animals were fed the same ration, and the availability of drinking water in the facilities of affected farms was considered adequate in all outbreaks. Furthermore, after dietary levels of calcium and phosphorus in the ration for growing pigs were corrected in the affected farms, cases of obstructive urolithiasis were no longer reported.

Predisposing factors for the occurrence of urolithiasis include diet composition, mainly related to excess or imbalance of minerals, urinary pH, reduced water intake, urinary stasis, metabolic disturbances, and preexistent urinary tract disease. Such predisposing factors may act synergistically or play a role individually in calculi formation (Drolet 2012, Sobestiansky 2012). Excessive consumption of minerals may be associated with artesian wells, and with unbalanced diets, which contain particularly high phosphate concentrations. Sheep fed on diets with high phosphorus concentration present hyperphosphatemia, and consequently show increased phosphorus urinary excretion, which may favor the precipitation of the excess of such mineral in the urinary tract (Radostits et al. 2000).

The ration fed to the growing pigs presented low calcium levels in its composition, which resulted in a calcium to phosphorus ratio of approximately 0.35:1, while the adequate proportion for the swine species is approximately 1.2:1; however, increased calcium proportions, ranging from 1.5 to 2.0, have also been recommended (Radostits et al. 2000, Moreno et al. 2012). Serum levels of calcium (7.05mg/dL) were slightly below the recommended levels for the species, which is 7.1mg/dL, and seric levels of phosphorus (13.6/dL) were above the maximum reference values described for pigs, which is 9.6mg/dL (Radostits et al. 2000, Jackson & Cockcroft 2007).

Calcium carbonate uroliths are originated from calcium salts, which is the mineral type commonly found in pigs (Osborne et al. 1989, Maes et al. 2004), and horses (Neumann et al. 1994). However, differently from horses, which present large amounts of calcium carbonate crystals in the urine and frequently develop uroliths (Neumann et al. 1994), pigs rarely present spontaneous mineral precipitation (Maes et al. 2004). The formation of phosphate uroliths is directly related to the consumption of grain-based rations and mineral supplements, which lead to an increased excretion of phosphorus and magnesium through urine when compared with calcium. Increased levels of the referred minerals along with the availability of ammonia ions in the urine may act synergistically to promote calculogenesis (Manning & Blaney 1986). Uric acid and urate uroliths are frequently observed in dehydrated neonatal piglets, and piglets presenting negative energetic balance, which results from an increase in the catabolism of tissue proteins and purines. Such calculi are observed as orange precipitated mineral deposits, which are noted in the kidneys, ureters, and in the urinary bladder (Kakino et al. 1998, Cianciolo & Mohr 2016). Calculi originated from urinary tract infection are occasionally observed in sows (Drolet 2012). In these situations, crystalluria, characterized by abnormal microscopic precipitation, is considered an important risk factor for the development of urinary tract diseases, such as cystitis and pyelonephritis of bacterial origin (Carr et al. 1995, Drolet 2012, Chigerwe et al. 2013).

Although the occurrence of obstructive urolithiasis is considered to be sporadic, outbreaks affecting large numbers of animals have been reported (Manning & Blaney 1986, Radostits et al. 2000). Pigs rarely develop urolithiasis comparatively with other animal species. Sporadic cases of urolithiasis have been described in pigs of all age groups and occasionally as an incidental finding in swine at the slaughterhouse (Drolet 2012). Even though urinary sediment is frequently observed in both male and female adult pigs, obstructive urolithiasis leading to death of affected pigs is a disease exclusively reported in castrated males. This condition is probably related to the great length and small diameter presented by the urethra in the referred swine category (Carr et al. 1995, Maes et al. 2004).

Typical clinical signs of urinary obstruction by uroliths include reduced feed intake, oliguria or anuria, and abdominal distention and pain (Drolet 2012) similar to the findings described in the reported outbreaks. Lethality rate was high in all studied farms, since all pigs presenting clinical signs of obstructive urolithiasis died. In these cases, death usually results primarily from urinary bladder rupture and secondarily from complete urethral obstruction by calculi, which leads to progressive urine leakage to the abdominal cavity, culminating in a markedly distended abdomen (Radostits et al. 2000, Loretti et al. 2003). Hypertonic urine associated with uroperitoneum promotes the leakage of large amounts of extracellular fluid to the abdominal cavity, leading to severe dehydration, abdominal distention, as well as cardiovascular alterations (Gasthuys et al. 1993, Loretti et al. 2003). Urethral or urinary bladder rupture usually takes place within 2 to 3 days, and death results from uremia or secondary bacterial infection (Radostits et al. 2000, Maes et al. 2004), however, none of the pigs from this study had uremic lesions.

The treatment of pigs with obstructive urolithiasis is mainly surgical (Larson 1996, Van Meter et al. 1996); however, it is not considered economically feasible (Drolet 2012). The main preventive measure for urolithiasis is the supplementation of calcium and phosphorus in the ratio of 2:1 respectively,

in the complete diet (Radostits et al. 2000). Other measures include the addition of 4% sodium chloride in the diet, to stimulate water consumption and increase urine production (Radostits et al. 2000, Loretti et al. 2003), and to ensure that pigs raised in confinement facilities have access to adequate water supply and balanced diets, as well as to avoid urinary tract infections. After adjusting the dietary levels of calcium and phosphorus in the ration for growing-finishing pigs in the affected farms, clinical cases of obstructive urolithiasis were no longer observed.

CONCLUSIONS

In this study, the cause of obstructive urolithiasis was attributed to a nutritional imbalance in the ratio of calcium and phosphorus in the diet. The ration for growing animals fed to the affected pigs presented low calcium levels, which may have led to elevated phosphate excretion through the urinary tract. All affected animals were male and presented clinical signs of reduced feed intake, oliguria or anuria, abdominal distention and pain, with consequent death due to bladder rupture. Lethality rate reached 100%.

Mineral components which predominated in the uroliths found in the present study were calcium carbonate and magnesium ammonium phosphate. The chemical composition of uroliths and environmental risk factors must be considered to determine adequate preventive measures for urolithiasis. It is also crucial to point out the importance of appropriate nutritional management practices to prevent the occurrence of such condition in swine production systems.

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2.2. ARTIGO 2

Nesse item é apresentado o artigo intitulado:

Motor and Somatosensory Degenerative Myelopathy Responsive to Pantothenic Acid in Piglets

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Artigo “accept with major revision” no periódico **Veterinary Pathology.**

Motor and Somatosensory Degenerative Myelopathy Responsive to Pantothenic Acid in Piglets

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Abstract

Two events of a degenerative myelopathy in four to 27 days old piglets are described. Mortality rate reached 40%. Sows were fed with ration containing low levels of pantothenic acid (PA). Sintomes reversed and no new cases occurred after PA levels were corrected in the ration and piglets received parenteral administration. Piglets presented severe depression, weakness, sensory ataxia and paresis, which were more pronounced in the pelvic limbs. There was no significant gross lesions. Histologically, there was degeneration and necrosis of neurons of the spinal cord, primarily in the thoracic nucleus and alpha-motor neurons in the ventral horn in the lumbar intumescence, and alpha-motor neurons in the ventral horn in the cervical intumescence. Minimal to moderate axonal and myelin degeneration were observed in the dorsal funiculus of the spinal cord and in the dorsal and ventral nerve roots. Immunohistochemistry, demonstrated depletion of acetylcholine neurotransmitter in alpha-motor neurons and accumulation of neurofilament in the perykarion in neurons of the thoracic nucleus and alpha-motor neurons. Ultrastructurally, thoracic nucleus neurons and alpha-motor neurons presented dissolution of the Nissl granulation. The topographical distribution of the lesions points out to a damage of the second-order neuron of the spinocerebellar tract and first-order axon cuneocerebellar tract and dorsal column-medial lemniscus pathway as the cause of the conscious and unconscious proprioceptive deficit, and the damage of the alpha motor neuron as the cause of the motor deficit. This study highlights the important and the practical use of a detailed neuropathological analysis to refine the differential diagnosis.

Keywords: Motor neurons, chromatolysis, pantothenic acid, proprioceptive ataxia, paresis, swine, thoracic nucleus, Wallerian degeneration

Disorders of the nervous system in swine may occur as a result of infectious or noninfectious etiologies or may have a multifactorial basis. Diagnosis of neurological conditions may become difficult in the absence of clinical history, detailed physical evaluation, and effective diagnostic workup. In swine, neurological disorders may frequently affect a specific age category or a large number of pigs of different ages.[19]

A variety of infectious agents has been associated with diseases of the nervous system. Among viruses infecting suckling piglets (0 to 28 days) are classical swine fever virus, pseudorabies virus, porcine reproductive and respiratory syndrome virus, eastern equine encephalomyelitis, Japanese encephalitis virus, malignant catarrhal fever associated with ovine herpesvirus type 2, encephalomyocarditis, teschovirus A and sapelovirus A. Most of these viral infections lead to nervous clinical signs as a result of inflammatory processes in the central nervous system. Bacterial agents and their toxins are able to affect young pigs. *Escherichia coli* is a common cause of septicemia in neonates. Congenital and neonatal diseases affecting suckling piglets include conditions such as splayleg, congenital tremor, hypoxia, hypoglycemia, hydrocephalus, cerebellar hypoplasia, and cerebellar abiotrophy.[19]

Less commonly, nutrient deficiency or toxicity are associated with primary neurological disease in piglets. Calcium, magnesium and phosphorus deficiency can produce hyperesthesia, tremors, tetany, or posterior paralysis in the absence of significant morphological lesions. Copper, Vitamin B5 and B6 deficiency can cause proprioceptive deficit, ataxia, paresis, and paralysis in pigs in the presence of mild lesions.[14,19] In contrast, selenium poisoning that causes similar clinical sings, is characterized by a marked segmental poliomyelomalacia.[19,28]

Pantothenic acid (vitamin B5) is a crucial precursor for the biosynthesis of the phosphopantetheine moiety of coenzyme A (CoA) and of the acyl carrier protein (ACP).[12,20] CoA is a fundamental enzyme cofactor in all organisms,[12] which is important for acetic acid to enter the Krebs cycle for energy production.[12,20] CoA is also essential for cell control and signaling, the synthesis of various important molecules, and the regulation of inflammation and the immune response.[12] This vitamin is formed through a condensation reaction of β-alanine, pantoic acid, and an amide bond. In addition to the physiological importance of CoA metabolism, pantothenic acid acts on lipid metabolism and energy production. This vitamin acts in the catabolism and synthesis of two-carbon units produced during the process.[23] Pantothenic acid is a component of the fatty acid synthesis complex and is present at the active site of ACP.[12]

Pantothenic acid is usually found in corn and soybean meal.[29] Despite the natural bioavailability of this nutrient in these ingredients, pantothenic acid is added to commercial diets fed to pigs of all ages and categories to mitigate the risk of deficiency.[9] Pantothenic acid supplementation is usually performed through the addition of calcium pantothenate, a salt that is more stable than pantothenic acid and contains 46% of vitamin B5 in its active form.[23]

The onset of clinical signs of pantothenic acid deficiency may be observed from approximately seven to 10 days after the introduction of a vitamin B5-deficient diet.[6] The disease is characterized by incoordination and ataxia affecting the forelimbs and hind limbs.[31] The main clinical manifestation of pantothenic acid deficiency in pigs is described as “goose-stepping gait”, which is characterized by gait changes, including hyperextension of the pelvic limbs associated with short steps.[6,31]

Case descriptions of pantothenic acid deficiency are scarce,[20] likely because synthetic vitamin is supplemented.[9] Nonetheless, the occurrence of outbreaks may be related to ration formulation errors, as well as operational issues involved in ration production, since in these cases, a large number of animals may be affected. In addition, outbreaks may occur in association with noncommercial diets in which pantothenic acid is not added.[6]

Neurodegenerative disorders that affect more specifically or severely the spinal cord than the brain are relatively uncommon in swine, compared to other domestic animal species.[24] Thus a systematic examination of the CNS is necessary for the accurate diagnosis of degenerative disorders.[4,31] A meticulous characterization of the nature of a lesion, affected neuroanatomical structures and, affected neurophysiological system, are of great value in the diagnosis and comprehension of poorly understood degenerative, metabolic or nutritional diseases.[7,28,35] For instance, the identification of neurological systems by immunohistochemistry is of great value highlighting sensory axonal degeneration tracts aiming the diagnostic of equine degenerative myelopathy associated with Vit E deficiency.[7]

Described herein is a neurodegenerative disorder with primary spinal cord involvement in suckling piglets. These piglets nourished from sows fed diets containing deficient levels of pantothenic acid. The objective of the current study is first to describe the neuropathological, immunohistochemical and ultrastructural findings of a antothenic acid-responsive myelopathy in piglets and second, based on the topographical distribution of the lesions in the nervous system, to identify the neurological system affected elucidating the pathophysiology of this condition.

Materials and methods

Clinical history and epidemiology

Clinical and epidemiological information were obtained directly with field veterinarians and swine farm owners during on-site visits. Twenty-two piglets, including males and females, aged between six hours and 27 days, presenting severe neurological signs were necropsied. In the first outbreak, pigs were referred from two farms (farms 1 and 2) located in the state of Santa Catarina, Brazil. In the second outbreak, pigs were referred from a single farm (farm 3) located in the state of Goiás, Brazil.

The first outbreak occurred from June to July 2016. These two sow farms were integrated to the same company, which provided premix to the formulation of the ration fed to all pig age categories. These farms reported increased mortality in piglets in the first week after farrowing and gait abnormalities affecting suckling, weaned, and growing-finishing pigs. Pigs in the affected farms presented varied genetic makeup, and were represented by several commercial breeds and crossbreeds. The onset of clinical signs was observed in suckling piglets independently of age, including newborn piglets (first six hours after farrowing) to piglets aged 27 days of life. Most affected animals died within 48 hours after the onset of clinical signs. The total combined number of sows and gilts in each of these farms (farms 1 and 2) ranged from 700 to 1000. Prior to the onset of the outbreak, suckling piglets experienced a mortality rate of approximately 5%. The outbreak lasted approximately 45 days in these farms, and during this period the mortality rate of suckling piglets was around 40%, although in some litters, the mortality reached 100%. Due to the likelihood of a nutritional disease, the premix company was

contacted, and it confirmed that to reduce production costs, vitamin B5 had been removed from the premixes shortly before the outbreak happened.

The second outbreak (farm 3) occurred from June to July of 2018 in a sow farm with wean-to-finish facilities. The referred farm had a total of 1,300 sows. During the outbreak, which lasted 60 days, the average mortality rate of suckling piglets increased from 6% to 40%. Some litters presented 100% mortality rate in piglets up to the fifth day after farrowing. Clinical signs were observed in suckling piglets as young as six hours of life, and these signs were seen in piglets of all ages throughout this phase until weaning (27 days of life). Litters of gilts and multiparous sows were affected. Approximately 15% of the suckling piglets started presenting gait abnormalities at weaning. Gait abnormalities observed were similar to those of the first outbreak. In this outbreak, it was investigated and determined by the referral that pantothenic acid-deficient levels in lactation and gestation premixes were due to a formulation error owing to a failure in an operational process.

The clinical manifestation presented by the piglets was documented through a detailed history, observation of the animals, sequential photographs and videos. Neurological signs were evaluated using parameter defined by two references[1,4] Neurological examination for reflexes, postural reactions, touch and pain abnormalities were not available for this study. The gathered clinical information was analyzed in conjunction with the topographical distribution of the lesion in the central and peripheral nervous system (CNS, PNS).

Piglets were euthanized and necropsy was performed on the farms. The study was approved by the Federal University of Rio Grande do Sul, Veterinary Research Commission (No. 33526).

Postmortem examination

Twenty-two affected piglets were necropsied. Portions of the following tissues were sampled at necropsy and fixed in 10% buffered formalin: adrenal glands, bladder, brachial plexus, esophagus, heart, kidneys, large intestine, liver, lungs, mediastinal lymph nodes, mesenteric lymph nodes, pancreas, sciatic nerves, skeletal muscle, small intestine, spleen, stomach, thyroid, and tonsils. Additionally, the skull, vertebral column, brain, cerebellum, spinal cord, and peripheral nerves were evaluated in great detail for gross abnormalities. The entire brain and spinal cord from cervical to *cauda equina* were removed and placed into 10% buffered formalin within 2 hours after euthanasia. CNS and PNS tissue were fixed in formalin for a minimum of 48 hours. Tissue samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Additionally, selected sections of spinal cords were stained with Bielschoswky.

Neuropathology

A generic evaluation to determine the nature and distribution of the lesions in the CNS and PNS was performed. Sections of diseased animals were examined for inflammatory, circulatory and degenerative changes. Examined areas of the CNS encompassed telencephalon (coronal section at 3 levels), basal nuclei (coronal section at 1 level), thalamus (coronal section at 1 level), cerebellum (horizontal section at 1 level), mesencephalon (coronal section at 1 level) and pons and medulla oblongata (coronal sections at 2 levels). Cross sections of the spinal cord were examined at the following regions/segments: cervical (C1, C2, C5-7), thoracic (T2, T10, T13), lumbar (L1, L3, L4-6), sacral (3 levels), and coccygeal (1 level). All dorsal and ventral nerve roots of the lumbosacral segments and dorsal root ganglia were

examined. In addition, transverse and longitudinal sections of the sciatic nerve, brachial plexus, muscles semimembranosus, and semitendinosus and gastrocnemius were examined.

After determining that the primary pathological process was degenerative in nature, a systematic evaluation of neural centers and pathways associated with somatosensory and motor control was performed. Two age-matched pigs were used as controls. For each spinal cord segment, cervical, thoracic and lumbar, two to six histological sections were examined. The evaluation of the CNS was performed by two authors (MPL and AGA).

Qualitative and semiquantitative neuronal degeneration and/or necrosis and, axonal and myelin degeneration were assessed in the following areas:

- cerebrum: including the gyri sigmoideus, marginalis, ectomarginalis, suprasylvius, ectosylvius, and cinguli;
- nucleus basalis, caudatus, and putamen;
- thalamus, globus pallidus, capsula interna, externa, extrema, and ventral thalamic nuclei;
- mesencephalon: tectum, nucleus ruber, and substantia nigra, oculomotor nuclei and formatio reticularis;
- cerebellum: all folia of the cerebellar vermis and hemispheres, nucleus lateralis, interpositus, fastigii and vestibular;
- brainstem: nuclei cuneatus lateralis, gracilis, olivae, vestibularis, and formatio reticularis;
- gray and white matter of the cervical, thoracic, lumbar, sacral, and coccygeal spinal cord.

Histological changes were graded according to the level of severity: minimum (+), mild +, mild/moderate +(+), moderate ++, moderate/severe ++(+) and severe +++.

This initial qualitative and semiquantitative screening of the CNS and PNS permitted the identification of the primarily affected areas of the spinal cord. Subsequently, due to the variability of neuronal population in individual cervical, thoracic, lumbar and sacral spinal cord segments, a semiquantitative evaluation assessing specifically the nucleus IX of the ventral horn and thoracic nucleus was performed. The scale here developed considers the average number of degenerate neurons viewed in both right and left thoracic nuclei and nucleus IX of the ventral horn. In the ventral horns of the spinal cord, an average of 30 neurons were visualized in each side. In the thoracic nucleus in control cases as well as in diseased piglets, in each side (right and left), the average of neurons observed was 11. According to the number of degenerated neurons, the lesion scale was determined as the following: lesions in nucleus IX of the ventral horn were considered minimal when up to 3 degenerated neurons were affected, mild when 4-5 degenerated neurons were found, mild to moderate when 6-7 degenerate neurons were detected, moderate when 8-10 degenerate neurons were observed, moderate to severe when 11-14 degenerate neurons were found, and severe when 15 or more degenerated neurons were presented. In the thoracic nucleus a lesion was considered minimal when one degenerate neuron was detected, mild when 2-3 degenerate neurons were observed, mild to moderate when 4-5 degenerate neurons were found, moderate when 6-7 affected neurons were detected, moderate to severe when 8-10 degenerate neurons were presented, and severe when 11 or more neurons observed. Axonal and myelin degeneration scale was set according to the

number of axonal spheroids and digestion chambers observed in the nerve roots and in the white matter of the spinal cord. Minimal axonal lesions were less than 5, mild with 6 to 8, mild to moderate with 9 to 11, moderate with 12 to 14, moderate to severe with 15 to 17, and severe above 20. Six out of the 22 piglets with neurological signs submitted for necropsy did not present histological changes. Thus, these six piglets were not included in the study due to the lack of validated tests to detect and measure levels of pantothenic acid deficiency in animal tissue.

Immunohistochemistry (IHC)

Immunohistochemical staining using monoclonal and polyclonal antibodies was performed on selected sections from the thoracic and lumbar spinal cord in two severely affected piglets (details provided in Supplementary Table 1). Staining for the neuronal cytoskeletal proteins nonphosphorylated neurofilament (NF) and phosphorylated neurofilament, for the calcium-binding protein involved in neuronal calcium signaling calretinin, and the neuromuscular junction neurotransmitter enzyme choline acetyltransferase. In addition, to determine astroglial and microglial responses, immunostaining for the glial fibrillary acidic protein (GFAP), and for the microglial ionized calcium binding adaptor molecule 1 (Iba1) were performed. Immunohistochemistry was completed with an automated slide stainer (Dako, Carpenteria, CA) and a peroxidase-labeled polymer conjugate system (Dako) was used as a secondary antibody. Sections 4 µm thick were deparaffinized and rehydrated in a decreasing graded alcohol series. Antigens were unmasked by the heat-induced epitope retrieval method via a Biocare Decloaking Chamber (Biocare Medical, Concord, CA) and a retrieval buffer of pH 6.0 or 9.0. Endogenous peroxidase was blocked with 3% H_2O_2 for 15 minutes. Nonspecific binding sites were

blocked with normal goat serum, 1:10 in tris buffered saline, for 15 minutes. Slides were incubated with the primary antibody. Thereafter, sections were incubated with the horseradish peroxidase-conjugated secondary antibody. Immunoreactivity was detected with 3-amino-9-ethylcarbazole+ for 5 to 15 minutes. Slides were lightly counterstained with Mayer's hematoxylin for 5 minutes.[7] For Iba-1 detection, heat-induced antigen retrieval was performed prior to incubation in primary antibodies in a premade buffer (catalog no. DV2004 MX; Biocare).[34]

Electron microscopy

For electron microscopy evaluation, brain, spinal cord, and spinal ganglia fragments of two piglets from farm 3 were fixed in 2% glutaraldehyde. Fragments of 1 to 3 mm were postfixed in 2.5% glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA) in 0.1 M sodium cacodylate buffer (Electron Microscopy Sciences). Tissue samples were postfixed in 1% osmium tetroxide (Electron Microscopy Sciences) in 0.1 M sodium cacodylate buffer, dehydrated, and embedded in resin as previously described.[35] Thin sections (60–70 nm) were stained with 5% uranyl acetate and lead citrate. Samples were visualized using a JEOL 1400 Plus transmission electron microscope (JEOL LTD, Tokyo, Japan). Images were obtained using an AMT Capture Engine Version 7.00 camera and software (*Advanced Microscopy Techniques Corp.* Woburn, MA, USA). Image analysis was carried out using ImageJ (NIHR public domain).

Determination of pantothenic acid concentration

Samples of lactation premix and ration (farm 1) from outbreak 1 were collected. Premix and rations of gestation and lactation phases from outbreak 2 (farm

3) were also sampled, aiming to assess and measure calcium pantothenate. Both, samples form outbreak 1 and 2 were submitted to the CBO laboratory analysis, São Paulo, Brazil. High-performance liquid chromatography was used to measure vitamin B5 levels. The limit of detection of vitamin B5 of this technique is 5.0 mg/kg, and the limit of quantification is 7.0 mg/kg.[17] Tests for the determination of pantothenic acid in tissues were not available.

Results

The clinical manifestations were characterized mainly by locomotion deficits occasionally evolving to tetraparesis (Fig. 1-8 and Supplementary Videos 1, 2 and 3). Locomotion deficits were more severe in the pelvic limbs. However, there was great variation between front and posterior legs and among animals (Table 1). Clinical signs in their first week of life were dominated by severe depression and weakness. Piglets showed dropped head and neck, knuckling over, hypermetry of hind limbs, prolonged periods in sternal recumbence with splayed legs or legs in forward and in backward position (paraparesis and tetraparesis). Piglets in their second week of life exhibited prominent clinical sings. Animals presented knuckling, which in most animals was observed in all four legs and exhibited severe weakness (paresis). Piglets supported the body weight on the tarsus-metatarsus region, presenting light to marked “hock weight-bearing” and “hock walking” (Supplementary Fig 1). Further, piglets showed “goose-stepping gait. Animals also developed hypermetry, instability and incoordination, which commonly led to falls (sensory ataxia; Supplementary Videos 1 and 2). Some animal demonstrated abnormal and irregular alternation of movements (dysdiadochokinesia). Piglets with a longer clinical course, developed an inability to stand up and support weight, adopting dog siting position or sternal

recumbence with splayed legs or legs in forward and in backward position (paraparesis and tetraparesis). Some piglets performed continuous movements of all four limbs in an attempt to walk, as they were swimming in a pool (tetraparesis; Supplementary Videos 3). Other clinical signs presented were anorexia, depression, drowsiness, and diarrhea, which was not responsive to therapy with antibiotics in piglets in the first week.

At necropsy, gross findings were nonspecific and included yellow liver (fatty degeneration) and watery contents in the intestinal lumen.

The most important microscopic findings were in the spinal cord (Table 1). Microscopic lesions were observed in 16/22 (72.73%) piglets and varied from discreet to severe. In addition, scattered necrotic neurons (ranging from one to eight neurons) were also observed in the reticular formation of the brainstem in four out of 16 piglets. No microscopic changes were evidenced in the remaining portions of the brain cortex, basal nuclei and thalamus, mesencephalon and cerebellum, spinal ganglion, brachial plexus, sciatic nerves, and muscles.

The lesions in the spinal cord were characterized by mild to severe neuronal degeneration and necrosis and axonal and myelin degeneration. Degenerated neurons were swollen with pale and eosinophilic cytoplasm in part due to loss of the Nissl bodies, which was more evident in the central perikaryon (chromatolysis). The nucleus in a number of these neurons was peripherally displaced (Figs. 9 and 10). Pyknosis or absence of the nucleus indicated unequivocal neuronal cell death. Occasionally, some necrotic neurons were shrunken, hypereosinophilic, with lack of the nucleus (Fig. 11). These necrotic cells were surrounded by microglia (neuronophagia), as confirmed by IHC. Few necrotic neurons were vacuolated (Fig. 12). On Bielschoswky histochemical staining, degenerated and necrotic neurons

were swollen, with disintegration of the “neurofibrils”, which remained accumulated in the periphery of the soma (Figs. 13 and 14). Degenerated and necrotic neurons were found primarily in the thoracic nucleus at the base of the dorsal horns and in the nucleus IX in the ventral horns of the spinal cord (Figs. 15-17, Table 1).

Additionally, white matter lesions, which were characterized by variable degrees of axonal and myelin degeneration with macrophages infiltration (gitter cells) were observed in the dorsal and ventral roots, and root entry zone of the spinal cord. Axonal spheroids along with myelin degeneration and gitter cells were more prevalent in the dorsal funiculus especially in the thoracic and lumbar spinal cord segments (Figs. 18-21); however, these changes were not consistently observed in all the sections.

Although the lesions in the spinal cord were bilateral, these were asymmetrical. The number of degenerated and necrotic neurons detected on H.E. stained preparations, varied among spinal cord segments, as shown in Table 1. Degeneration or necrosis of neurons in nucleus IX was observed in 11 out of 16 piglets (68.75%). The lesion varies from discreet to moderate. Eight out of 16 (50%) piglets showed minimal to mild/moderate degeneration or necrosis of neurons in nucleus IX, in the cervical spinal cord segments. Five out of 16 (31.25%) piglets showed minimal to mild degeneration or necrosis of neurons in nucleus IX, in the thoracic spinal cord segments. Eight out of 16 (50%) piglets showed minimal to moderate degeneration or necrosis of neurons in nucleus IX, in the lumbar spinal cord segments. Four out of 16 (25%) piglets showed minimal to mild degeneration or necrosis of neurons in nucleus IX, in the cervical and lumbar spinal cord segments concomitantly. Degeneration or necrosis of neurons in thoracic nucleus including thoracic and lumbar portion, was observed in 15 out of 16 piglets (93.75%). The

lesion varies from discreet to severe. Eight out of 16 (50%) piglets showed minimal to severe degeneration or necrosis of neurons in both thoracic and lumbar portion of the thoracic nucleus. Two out of 16 (12.5%) piglets showed minimal degeneration or necrosis of neurons only in the thoracic segments of the thoracic nucleus. Four out of 16 (25%) piglets showed minimal to moderate degeneration or necrosis only of neurons in the lumbar portion of the thoracic nucleus. One out of 16 (6.25%) piglets showed no lesion in the thoracic nucleus.

Axonal and myelin degeneration in the dorsal funiculus was present in 7 out of 16 (43.75%) piglets (Table 1). The lesion varied from discreet to moderate. Five out of 16 (31.25%) piglets showed discreet to moderate axonal and myelin degeneration in the dorsal funiculus in all three, cervical, thoracic and lumbar segments of the spinal cord. Five out of 16 (31.25%) piglets showed discreet axonal and myelin degeneration in the lateral funiculus in the cervical and thoracic segments of the spinal cord. Nine out of 16 (56.25%) piglets showed no lesion in the dorsal funiculus in neither three, cervical, thoracic and lumbar segments of the spinal cord. Five out of 16 (31.25%) piglets showed degeneration or necrosis of neurons in the nucleus IX, thoracic nucleus and, axonal and myelin degeneration in the dorsal funiculus concomitantly (Table 1). Further analysis of the dorsal funiculus showed that all three proper, gracilis and cuneate fasciculi were affected (Table 2). At the thoracic and cervical spinal cord segments, the proper and cuneate fasciculi displayed slightly more severe axonal and myelin degeneration than the gracilis fasciculus. The lateral funiculus displayed only discreet lesions at all levels of the spinal cord. At the thoracic and cervical spinal cord segments, axonal and myelin degeneration seemed to be restricted to the spinocerebellar tract.

Mild to moderate gliosis was observed in all piglets, predominantly in areas with more severe neuronal and axonal degeneration.

A moderate number of axonal spheroids in the roots of the dorsal and ventral nerves and in the dorsal funiculus were diffusely immunoreactive for calretinin (inset Supplementary Fig. 21). Compared to unaffected neurons, an accumulation of phosphorylated and non-phosphorylated neurofilaments in necrotic neurons of the nucleus IX (Figs. 22 and 23) and thoracic nucleus (Figs. 24 and 25) was detected by IHC. As demonstrated by choline acetyltransferase IHC, low expression of the acetylcholine neurotransmitter was observed in alpha-motor neurons (α-MN) of nuclei IX. Degenerate α-MN have clear cytoplasm with no granulations compared to internal controls (Figs. 26-28). Iba1 highlighted that the predominant cell population of glial cells response were represented by microglial cells (Supplementary Figs. 2 and 3).

Ultrastructural changes were detected in the lumbar segment of the spinal cord in one of the two animals (piglet 15). Neurons of the thoracic nucleus and α-MN of nucleus IX presented dissolution of the Nissl substance with a redistribution of organelles (Figs. 29-32). In the center of the neuron cell body, there was paucity of the endoplasmic reticulum cisterna and polyribosomes that compound the Nissl substance. Between scant organelles mostly mitochondria there were sparsely distributed intermediary filaments. Remaining rough endoplasmic reticulum aggregates were displaced to the periphery of the soma. Degenerate axons were sparsely distributed and were characterized by accumulation of residual body, mitochondria and vesicles among sparse intermediary filaments. Frequently these axons were surrounded by a thin myelin sheath that showed segmental decompaction. In the center of the degenerate myelinated axonal tube there was

often macrophages phagocytizing axonal and decompacted myelin debris (digestion chamber, Supplementary Figs. 4 and 5). No significant ultrastructural changes were observed on spinal ganglia or nerves.

The determination of pantothenic acid concentration demonstrated that the lactation ration samples (outbreak 1) presented calcium pantothenate levels lower than 7 mg/kg, and lactation premix samples presented 35.73 mg/kg of calcium pantothenate. The analysis of premix and ration samples of gestation and lactation phases (outbreak 2) indicated pantothenic calcium levels lower than 7 mg/kg, making the quantification unfeasible.

After the identification of dietary pantothenic acid deficiency (lack or low concentration levels) in the rations fed to the affected piglets, injectable and oral (by drinking water) vitamin supplementation was performed using commercial preparations containing vitamin B5. In addition, calcium pantothenate was added to the rations. After the correction of pantothenic acid dietary deficiency, a marked reduction in neonatal mortality was noted. Furthermore, the observation of new cases of proprioceptive deficit ceased within 10 days of vitamin supplementation.

Discussion

In this study, we describe an outbreak of a neurological condition in suckling piglets characterized by degenerative myelopathy. The distribution and severity of degenerate changes in spinal cord neurons and tracts, and dorsal and ventral spinal nerve roots of affected piglets were consistent with proprioceptive and motor deficits.[1,4] Common circumstances reported in the two outbreaks were: 1) feeding pregnant and lactating sows with ration containing deficient levels of pantothenic acid (Vitamin B5) due to ration formulation errors and, 2) after adjusting dietary

requirements of pantothenic acid in the ration fed to swine, piglets presenting proprioceptive and motor deficits recovered and mortality ceased.

Pantothenic acid deficiency has been reported in pigs fed diets based exclusively on corn and in pigs fed diets based on self-made rations with human food leftovers with fish, meat and bone. In both situations pantothenic acid was not supplemented.[30,31] In the current outbreaks, pantothenic acid deficiency has been noted in farms in which corn and soybean meal are the main ration ingredients, showing that naturally occurring bioavailable pantothenic acid is not sufficient to cover the metabolic demand and to prevent clinical signs.[9] Suckling piglets represented the most affected age category. This was attributed to the fact that gestating sows were fed pantothenic acid-deficient diets for half of their gestation period due to error in the ration formulation. Furthermore, sows on the deficient diets nursed piglets during the lactation period when pigs have a high demand for pantothenic acid. In swine, the highest requirement for pantothenic acid is presented in piglets up to 15 kg of body weight (BW).[15] The pantothenic acid requirement of piglets weighing two to 10 kg is 15.0 mg/kg of BW.[23] This requirement is significantly higher than the levels detected in the rations fed to gestating and lactating sows in the reported outbreaks, which were below 7 mg/kg. A lower level of pantothenic acid available is however expected for this newborn piglet, due to intestinal absorption losses as they also developed diarrhea. After adjusting dietary levels of pantothenic acid in the ration fed to swine of all age categories in the affected farms, clinical cases of pantothenic acid deficiency were reversed and no new cases were reported. In addition to dietary supplementation, pantothenic acid was added to the drinking water. Piglets presenting clinical signs were also injected, via intramuscular route, with Vitamin B5. After parenteral therapy and diet supplementation, an immediate

reduction in neonatal mortality was evident. Response of clinical diseased pigs to daily supplementation with calcium pantothenate had been also reported elsewhere. Those pigs presented rapid and complete recovery and a great improvement in feed conversion after the supplementation.[36] The clinical disease observed in suckling piglets likely favored increased mortality rates associated with secondary causes of noninfectious origin, including severe dehydration, increased piglet crushing rates, hypothermia and hypoglycemia, all of which represent critical survival and viability points for newborn piglets in swine production systems.[33] In the present cases, the lower viability and reduced birth weight of piglets may be related to inadequate energetic intake and consequent ineffective cellular metabolism associated with CoA deficiency in the gestation period.[20]

Important limitations in our study were the impossibility of determining the levels of vitamin B5 in the pig's tissue, and the consequent lack of reference values for tissue measurements of pantothenic acid in swine. Furthermore, α-tocopherol (Vit E) and tissue minerals levels such as selenium and copper, were not measured. Viral infectious disease investigation was not performed. The epidemiological clinical data and the absence of characteristic microscopical lesions, however, rule out aforementioned noxas as a potential causal differential diagnosis as further discussed.

The pathogenesis of neuron cell damage due to pantothenic acid deficiency remains to be elucidated. It is recognized that the absence or low level of vitamin B5 interferes directly with the synthesis of ubiquitous CoA, which will negatively affect the entry of acetic acid to the Krebs cycle for energy production, synthesis of multiple neurotransmitters and steroid hormones, synthesis of cholesterol, amino acids, phospholipids and fatty acids.[18,12,20]

In the current cases of pantothenic acid responsive degenerative myelopathy (PARDM), the lesions were characterized by degeneration and death of neurons in the thoracic nucleus and in α-MN of nucleus IX in the spinal cord. At light microscopy on HE preparation, neurons were pale, eosinophilic, with nucleus located at the periphery of the cell body or absent, and loss of the Nissl granulation, which is confirmed on Toluidine blue preparations. This process of neuronal degeneration is known as chromatolysis, which is a term applied in a large variety of animal conditions, including neurodegenerative disease, toxic, metabolic, virus infection and axonal injury.[31] In our study at electron microscopy, chromatolytic neurons displayed changes similar to those observed in neurons after axonal injury.[22] Chromatolysis after axonal injury is induced by a disruption of the protein synthesis infrastructure due to action of several ribonucleases.[22] Changes involve degradation of stacks of rough endoplasmic reticulum leaving clear areas and disaggregation of poly-and-monoribosomes.[22] Further, not observed in piglets in this study, ribosomes and rough endoplasmic reticulum may also be degraded in autophagic vacuoles by ribophagy and reticulophagy, respectively.[22]

In piglets with PARDM in our study, neuronal chromatolysis and death could be seen in the absence of axonal and myelin degeneration in the corresponding somatosensory tracts and in motor nerve roots and nerves. This evidence indicates that neuronal chromatolysis and death are the results of a primary insult to the cell bodies rather than a consequence of primary axonal damage.[22] In contrast, axonal and myelin degeneration in first-order somatosensory tracts in the absence of corresponding ganglionic neurons showing chromatolysis and death shed evidence that axonal degeneration is a consequence of primary axonal damage.[22] Yet, studies show that the site of neuronal degeneration is not a reliable indicator of where the

initial injury occurred and that the sequences of events that follow are difficult to establish. For instance, a populations of axons, synapsis and cell bodies showed different vulnerability and degenerated asynchronously.[3]

An important finding in piglets with PARDM was the accumulation of intermediary filaments in the perikaryon of neurons in the thoracic nuclei and in α-MN in nucleus IX, as was demonstrated by neurofilaments immunohistochemistry. Accumulation of phosphorylated neurofilaments and non-phosphorylated neurofilaments in the perikaryon of chromatolytic α-MN is a feature of neurodegenerative diseases in humans and in an variety of animals, including pigs.[2,5,16,26,31] Neurofilaments belong to the family of cytoskeletal intermediate filament proteins that give cells their shape; they determine axonal caliber, which controls signal conduction, and they regulate the transport of synaptic vesicles and modulate synaptic plasticity by binding to neurotransmitter receptors.[2,5,11] Aggregation of phospho-NFs is a hallmark in various neurodegenerative diseases such as Alzheimer's disease, Amyotrophic lateral sclerosis, Parkinson's disease, diabetic neuropathy, Charcot-Marie-tooth disease and giant axonal neuropathy.[2,5,11] Motor neuron diseases in animals have received attention because of theirs similarity with Amyotrophic lateral sclerosis in humans.[16,21,27,31] The mechanisms for accumulation of neurofilaments is not completely understood. Deregulation of protein kinases, such as hyperactivation of Cdk5 due to neuronal insults such as oxidative stress, αβ toxicity, glutamate toxicity, leads to intraperikaryal accumulation of hyperphosphorylated cytoskeletal proteins, aggregated phosphorylated neurofilaments and subsequential neuronal death. The phosphorylated-NF aggregates are found in the cell body and proximal parts of the axons, whereas normally phosphorylated-NFs only reside in the distal parts of the axons. The

accumulation of neurofilaments closely correlates with mitochondrial accumulation and bioenergetic stress.[5] Accumulation of phosphorylated and non-phosphorylated neuron filament in neurons in the thoracic nucleus, is a distinctive finding in piglets with PARDM, compared to lower motor neuron diseases described in pigs and all other species.[16,21, 27]

In this study, the majority of piglets with PARDM had degeneration and necrosis of neurons in the thoracic nucleus in both thoracic and lumbar portion of the spinal cord and, α-MN of nucleus IX in the ventral horn of the cervical, thoracic and lumbar spinal cord segments. Further, axonal and secondary myelin degeneration (Wallerian degeneration) were evident in the dorsal and ventral spinal nerve roots and in the dorsal funiculus. In an experimental study in pigs fed with pantothenic acid deficient rations, lesions were confined to peripheral sciatic and brachial nerves, neurons in the sensory spinal ganglia and nerve roots and dorsal funiculus.[32] These changes were characterized by chromatolysis of neurons and Wallerian degeneration of myelinated nerve fibers. A fundamental distinction between previous experimental studies[32] and our report, was the involvement of neurons (thoracic nucleus) and α-MN (nucleus IX in the ventral horn) in piglets with PARDM. Gait abnormalities were presented after eight weeks of continued feeding with the deficient diet.[32] In contrast to piglets with PARDM, pigs in experimental studies were older at the beginning of the trials; between four to eleven weeks of age. [32] It is possible that this difference in the distribution of the lesions in our study, was due to a major metabolic susceptibility of suckling pigs to the pantothenic acid insufficiency.

As a differential diagnosis in piglets with PARDM in our outbreak, we must take into consideration the age class, nature and pathogenesis of the neuronal degeneration/necrosis, topography of the lesion and affected functional system(s).

Herein, disease in pigs that are pertinent to discussion are pyridoxine deficiency (Vit. B6), copper (Cu) deficiency, motor neuron diseases, hereditary porcine neuronal system degeneration, (HPNSD) selenium (Se) toxicity and α-tocopherol (α-TP; Vitamin E) deficiency. Pyridoxine and pantothenic acid deficiency are indistinguishable. Both deficiencies induce sensory ataxia, due to lesions in proprioceptive pathways.[10,32] While degeneration of the afferent axons is the initial and most prominent feature in pyridoxine deficiency, chromatolysis seems to be the first evidence of damage to the afferent neurons in pantothenic acid-deficient animals.[10] Copper deficiency has been described in new born piglets and in pigs up to 21 weeks of age. In contrast to piglets with PARDM, in Cu deficiency the lesion is characterized by a primary axonal degeneration affecting the ventral and lateral funiculi of the thoracic and lumbar spinal cord and occasionally in the brainstem, and ventral peripheral nerve roots. Chromatolysis and necrosis of neurons in the brain, midbrain and brainstem are not observed in pigs. [25,31] A motor neuron disease in pigs, which presumably has a hereditary base, has been described in six 5 week old Yorkshire and in six 6 week old Hampshire breeds.[16,21] Yorkshire pigs presented bilateral posterior ataxia and weakness which rapidly progressed to tetraplegia by 10 weeks of age. Bilaterally, there was chromatolysis, degeneration and neuronal loss restricted to motor nuclei in the ventral horns of the spinal cord, in the medulla oblongata and in the midbrain. Additionally, there was diffuse Wallerian degeneration in the ventral and lateral funiculi and ventral peripheral nerve roots, and prominent atrophy of skeletal muscles, which does not present in piglets with PARDM. Ultrastructurally, the perikaryon and processes of affected neurons contained massive accumulations of neurofilaments.[21] No lesion is present in the dorsal funiculi and dorsal nerve root.[21] The hereditary porcine neuronal system degeneration

(HPNSD),[27] also results from a progressive degeneration of lower motor neurons. In contrast to Yorkshire and Hampshire breeds[16,21] HPNSD pig breeding colony is characterized by vacuolation and deposition of osmiophilic lipid droplets in alpha motor neurons in the spinal cord.[27] Wallerian degeneration affects the sulcus marginalis and spinocerebellar tract. Axonal degeneration is solely observed in ventral spinal nerve roots and is accompanied by atrophy in skeletal muscles.[27] Further, cytoplasmic accumulation of phosphorylated and non-phosphorylated neurofilaments demonstrated by immunohistochemistry is not a feature of this condition as it is in motor neuron diseases in horses and in humans.[31] In PARDM in the current study, affected pigs of the various farms presented a diverse genetic make-up ruling out a hereditary disease base.

Another important differential diagnosis in piglets of this age class includes a toxic myelopathy caused by selenium poisoning. Reported neurological signs in selenium poisoning are similar to those seen in piglets with PARDM, which were mainly characterized by proprioceptive and motor deficits. However, in selenium poisoning, clinical signs are the result of extensive areas of pannecrosis of gray matter of the ventral horns (focal symmetrical poliomyelomalacia) of the cervical and lumbar intumescences.[13,28]

Lesions described in piglets with PARDM resemble a concurrent onset of neuroaxonal dystrophy/degenerative myeloencephalopathy (NAD/EDM) and equine motor neuron disease (EMND) recently reported in three young horses.[8] Based on the age, clinical signs and histological lesions, NAD/EDM and EMND are categorized as distinct conditions, despite the common association with an α-tocopherol deficiency.[8] NAD/EDM develops in genetically susceptible individuals if α-TP deficiency occurs during the first year of life whereas EMND occurs in adult horses

after an extended period of α-TP deficiency. NAD/EDM-affected horses have general proprioceptive symmetric ataxia of all four limbs beginning at a few months of age whereas EMND-affected horses have generalized lower motor neuron weakness, muscle atrophy, trembling, low head carriage, and tail head elevation, with a peak risk at 16 years of age. The histologic lesion associated with NAD/EDM is central axonal degeneration, which is most pronounced in the somatosensory tracts (spinocuneocerebellar and dorsal spinocerebellar tract).[7,8] Lesions associated with EMND include chromatolysis of lower motor neurons with perikaryal accumulation of neurofilaments as well as peripheral axonal degeneration and associated neurogenic atrophy of muscle fibers.[8] We were not able to measure the levels of Vitamin E in piglets with PARDM. Conditions associated with a temporal α-TP deficiency such as NAD/EDM or EMND have not yet been described in pigs, even though they have been reported in several other animal species.[19,31]

Despite pigs with PARDM in the current report presenting clinical signs similar to previous studies,[10,31,32] the topographic distribution of affected neurons points to fundamental differences. Neurological signs in piglets with PARDM cannot be explained by lesions in the somatosensory pathways alone, since prominent lesions affect motor neurons of the spinal cord ventral horn as well. While neurological deficits due to lesions affecting motor neurons can be explained in a more direct manner, neurological impairments due to lesions affecting somatosensory nuclei and pathways are more complex and as a result, more difficult to elucidate.[1,4]

Somatosensory areas affected in piglets with PARDM were the dorsal nerve rootlets, dorsal root entry zone, dorsal funiculus (dorsal column) and thoracic nucleus; these areas are related to proprioceptive functions. Lesions affecting the dorsal funiculus of cervical, thoracic and lumbar spinal cord segments, were present

in 6 out of 16 piglets. In comparison, lesions affecting the thoracic nucleus were present in 15 out of 16 piglets. Based on the affected pathways and nucleus in piglets with PARDM, it can be suggested that unconscious proprioception rather than conscious proprioception, was the primary impairment.[1,4] The unconscious proprioceptive information to the cerebellum is conveyed by the spinocerebellar tract and cuneocerebellar tract. In contrast, the conscious proprioceptive information is transmitted by the dorsal column-medial lemniscus system to the somatosensory telencephalic cortex.[1,4]

The spinocerebellar tract conveys proprioceptive information about the activity of the effector muscles or motor neuron pools to the cerebellum. This unconscious proprioceptive information is critical for the maintenance of the station and gait. The first-order neurons of the spinocerebellar tract are located in the dorsal root ganglia. The dorsal nerve root enters in the dorsolateral aspect of the spinal cord and splits into small rootlets. Large myelinated axons of first order neurons, conveying information from skin mechanoreceptors and proprioceptors, form medial bundles that enter the dorsal column. The spinocerebellar tract is further divided in dorsal and ventral spinocerebellar tracts. The dorsal spinocerebellar tract conveys ipsilateral axons from the second-order neurons localized in the thoracic nucleus. The thoracic nucleus is found in most animal species between the spinal cord segments T1 and L3. These axons ascend through the dorsolateral region of the lateral funiculus to the cerebellum through the caudal cerebellar peduncle.[1,4,7] The ventral spinocerebellar tract conveys contralateral axons from the second-order neurons localized in the intermediary gray matter in the cervical and lumbar segments. These second-order neurons receive simultaneous ascending and descending information affecting motoneurons and interneurons. Second-order axons transmit the information of the

front and hind limbs ascending through the ventrolateral region of the lateral funiculus to the cerebellum through the rostral cerebellar peduncle.[1,4] Both dorsal and ventral spinocerebellar tract neurons act as comparison between the action of inhibitory and excitatory input to the spinal α-MN and interneurons.[1] Similar to the spinocerebellar tract, the first-order neurons of the cuneocerebellar tract, are located in the dorsal root ganglia. Large first order myelinated axon conveys information from skin mechanoreceptors and proprioceptors located in the front limbs. Via cuneate fasciculus in the dorsal funiculus (dorsal column), first-order axons make synapse with second-order neurons in the lateral cuneate nuclei located in the posterior brainstem. The second order axons from the lateral cuneate nuclei ascend in the ipsilateral caudal cerebellar peduncle.[1,4] In piglets with PARDM, the presence of axonal degeneration in the dorsal nerve rootlets, dorsal root entry zone and dorsal funiculus of the posterior midthoracic and lumbar spinal cord, and in the thoracic nucleus (second-order neuron) suggests that both the first-order (in 7 out of 16 pigs) and second-order neurons (in 16 out of 16 pigs) of these segments are primarily impaired and, that the unconscious proprioceptive information conveyed from the hind limbs is markedly disrupted. The presence of axonal degeneration in the dorsal nerve rootlets, dorsal root entry zone and dorsal funiculus of cervical spinal cord segment and the absence of lesions in the lateral cuneate nucleus (second-order neuron) suggests that the first-order neurons (spinal ganglia neurons), are primarily impaired, and unconscious proprioceptive information conveyed from the front limbs is disrupted.

The dorsal column-medial lemniscus system is critical for conscious proprioception associated with complex motor activities such as touch, pressure and joint proprioception, and these are controlled through low-threshold

mechanoreceptors. Similar to the tracts related to unconscious proprioception pathways, the first-order neurons of the dorsal column-medial lemniscal system are located in the dorsal root ganglia. Large myelinated axons of first order neurons located in spinal ganglia form medial bundles that enter the dorsal column. At cervical and anterior midthoracic levels, the dorsal column consists of a medially located gracile fasciculus, which conveys input from hind limbs to the gracile nucleus and, the cuneate fasciculus, which conveys input from the front limbs to the cuneate nucleus. Posterior to the midthoracic level, the dorsal column consists only of fasciculus gracile. The second order axon from the gracile and cuneate nuclei decussates in the medulla and ascends in the contralateral lateral medial lemniscus forming the dorsal column-medial lemniscus system. In piglets with PARDM, the presence of axonal degeneration in the dorsal nerve rootlets, dorsal root entry zone and dorsal funiculus of the cervical spinal cord segment and the absence of lesions in the nerve ganglia (first-order neuron) and in gracile and cuneate nucleus (second-order neuron) suggest that the axon of first-order neurons are affected and impair the conscious proprioception.

The central nervous system must constantly be apprised of position, tone, and movements of the limbs and trunk. This is accomplished by proprioception input integration (primarilyy in the cerebellum) and by transmission of these data back to the motor neurons. Sensory information is essential for smooth, coordinated motor activity.[1,4] Although proprioceptive pathways are physiologically important, it is extremely difficult to distinguish abnormalities resulting from lesions in this system. For instance, the clinical sign manifested in diseases impairing the proprioceptive pathways is ataxia. Ataxia due to impairment of the somatosensory system is classified as proprioceptive ataxia. In piglets with PARDM, clinical signs were

knuckling over, hypermetria, goose stepping gait, and incoordination. These neurological signs were more severe in the hind limbs, which coincided with a more severe and widespread lesion in the second-order neuron of the spinocerebellar tract.

The ventral horn motor neurons also referred to as lower motor neurons (LMN) are the final common pathway for segmental motor control. All motor neurons have acetylcholine as their neurotransmitter. Alpha motor neurons are large neurons arranged in the lamina IX of the ventral horn of the cervical and lumbar enlargements. The α-MN and its axon and all muscle fibers that it innervates, constitute one motor unit. Normal movements involve the coordination up to thousands of motor units in many muscles. The segmental motor apparatus of the spinal cord is involved in: 1) reflex activity that control posture and voluntary movements and 2) complex motor synergies such as locomotion. A lesion affecting the α-MN impairs muscle to contract fully, inducing weakness or paralysis, loss of the muscles stretching reflex, and muscle tone, and fasciculation of the corresponding myotome. Muscle atrophy occurs after a lesion has persisted for sufficient time.[1,4] In our study, pigs were not neurologically examined. However, on site observation of animals and analysis of videos permitted us to conclude that impairment of the reflex activity that controls posture and voluntary movements and locomotion is likely a consequence of the chromatolysis and necrosis in the α-MN of the cervical and lumbar enlargements in piglets with PARDM. In addition, the lack of or low expression of acetylcholine in affected α-MN, as was demonstrated by immunohistochemistry with enzyme choline acetyltransferase offers solid support for this theory. Interestingly, acetylcholine decreases have also been found in humans with pantothenic acid deficiency.[12,18] Because both sensory pathways inputs and α-

MN are involved in the normal functioning of motor units, damage to either system will affect reflex activity that controls posture and voluntary movements and the contribution of either to the neurological deficit in pigs with PARDM is difficult to determine.

In conclusion, the neurological signs in the current piglets were attributed to pantothenic acid responsive degenerative spinal cord disorder. All affected piglets developed sensory ataxia and paresis. Pathological lesions were characterized by necrosis of neurons in the thoracic nucleus and α-MN of nuclei IX of the spinal cord. Axonal degeneration was presented in the roots of the dorsal and ventral spinal nerves and in the dorsal funiculus. The distribution of the lesion indicated that the conscious and unconscious proprioception and the motor final common pathway were primarily affected. This study highlights the importance and the practical use of a detailed neuropathological analysis to refine the differential diagnosis. To the author's knowledge, there are no previous descriptions of degenerative myelopathy responsive to pantothenic acid in suckling piglets.

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Declaration of conflicting interests

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Figure legends

Figures 1-8. Pantothenic acid responsive degenerative myelopathy in pigs. **Figures 1-3**. Pig 16. Animal with tetraparesis. The ventral abdominal surface is in contact with the floor, and the forelimbs are flexed or laterally positioned. Yet, the orientation of the head and ears maintains a normal special orientation. **Figures 4-8**. Pig 14. Sequence of photographs showing a pig with severe sensory ataxia and paresis; in sternal recumbence evolving to “sitting position” and to stance; the piglet can stand up with limbs wide open, followed by falls.

Figures 9-14. Pantothenic acid responsive degenerative myelopathy, spinal cord, pig, case 15, and 16. **Figure 9**. Alpha-motor neuron within the normal limit. Hematoxylin and eosin (HE). **Figure 10**. Degenerate neurons displaying swollen, pale, homogeneous, and eosinophilic cytoplasm. Nissl granules are dissolved in the center of the cell body, and the remaining granules are in the periphery of the neuronal soma. The nucleus is peripherally displaced (HE). **Figure 11**. Necrotic neuron, shrunken, eosinophilic with nucleus showing pyknosis (HE). **Figure 12**. Necrotic neuron with multiple vacuoles (HE). **Figure 13**. Normal (control) neuronal body. Bielschoswky. **Figure 14**. Necrotic neurons swollen with neurofibrils accumulation predominantly on the periphery of the soma. Bielschoswky.

Figures 15-17. Pantothenic acid responsive degenerative myelopathy, spinal cord, pig, case 15. **Figure 15**. Left, a diagram of the central nervous system (CNS) depicts the location of the thoracic nucleus, which extends from the thoracic vertebra T1 to the lumbar vertebra L3 (red bracket). Right top picture shows L1 transverse section. The white rectangles show the location of thoracic nuclei. Right bottom picture shows

L5 transverse section. The white rectangle shows the location of lower motor neuron nuclei in the ventral horns. **Figure 16**. Necrosis and degeneration of sensory neurons located in the thoracic nucleus in the L1 thoracic segment (HE). *Central spinal cord canal. **Figure 17**. Necrosis and degeneration of motor neurons IX located in the ventral horns of the spinal cord in the L5 lumbar segment (HE).

Figures18-21. Pantothenic acid responsive degenerative myelopathy, spinal cord, pig, case 15. **Figure 18.** L1 spinal cord segment; the box delimits the dorsal nerve root, dorsal root entry zone and dorsal funiculus. Bielschoswky. **Figure 19.** Dorsal nerve root, between arrows, there is a distended myelin sheath containing a presumptive gitter cell (macrophages). **Figure 20.** Dorsal root entry zone, the arrow shows a distended myelin sheath containing a presumptive Gitter cells. **Figure 21.** Dorsal funiculus displaying distended myelin sheath with swollen axon (arrow) and presumptive gitter cells (arrowheads). HE. Inset: Immunohistochemistry for Calretrin (brown labeling) highlighting axonal spheroids.

Figures 22-25. Pantothenic acid responsive degenerative myelopathy, L5 spinal cord segment, pig, case 15. Immunohistochemistry for Phosphorylated and non-phosphorylated neurofilament (brown labeling). **Figure 22.** Necrotic neurons of the thoracic nucleus displaying accumulation of phosphorylated neurofilament within the cell bodies as well as within cell processes. Black arrow denotes an unaffected neuron. **Figure 23.** Necrotic neurons of the thoracic nucleus demonstrating accumulation of non-phosphorylated neurofilament within cell body. Black arrow denotes an unaffected neuron. **Figure 24.** Necrotic α-motor neurons in the nucleus IX displaying accumulation of phosphorylated neurofilament within the cell bodies as

well as within cell processes. Black arrow denotes control neuron, unaffected. **Figure 25.** Necrotic neurons of the motor nucleus IX displaying accumulation of non-phosphorylated neurofilament within the cell bodies. Black arrow denotes control neuron.

Figures 26-28. Pantothenic acid responsive degenerative myelopathy, L5 spinal cord segment, pig, case 15. Choline acetyltransferase immunohistochemistry (brown labeling). **Figure 26**. Degeneration and necrosis of α-motor neurons in the nuclei, in the ventral horns of L5 (magnification from the box in the Inset); the black arrow indicates an affected neuron and the white arrow indicates an unaffected neuron. **Figure 27**. Magnification of a degenerate/necrotic α-motor neuron from figure 26, demonstrating marked depletion of choline acetyltransferase. Compared with an unaffected motor neuron **Figure 28**, while the degenerate/necrotic α-motor displayed light grey-brown homogeneous cytoplasm and unaffected showed abundant course brown granulation.

Figures 29-32. Pantothenic acid responsive degenerative myelopathy, L5 spinal cord segment, pig, case 15. **Figure 29**. Affected ventral horn motor neuron presenting dissolution of the Nissl bodies and absence of the nucleus. **Figure 30**. Unaffected neuron; nucleus (N). Toluidine blue. **Figure 31**. Ultramicrophotography of neuron in figure 29, demonstrating degranulation of rough endoplasmic reticulum, and disaggregation of polyribosomes. Few small cisterns of endoplasmic reticulum are presented (black arrows) along with relative increased number of mitochondria (m), increased amount of intermediary filaments and few lysosome (L). **Figure 32**. Approximated view of the Nissl substance in an unaffected neuron. The white border

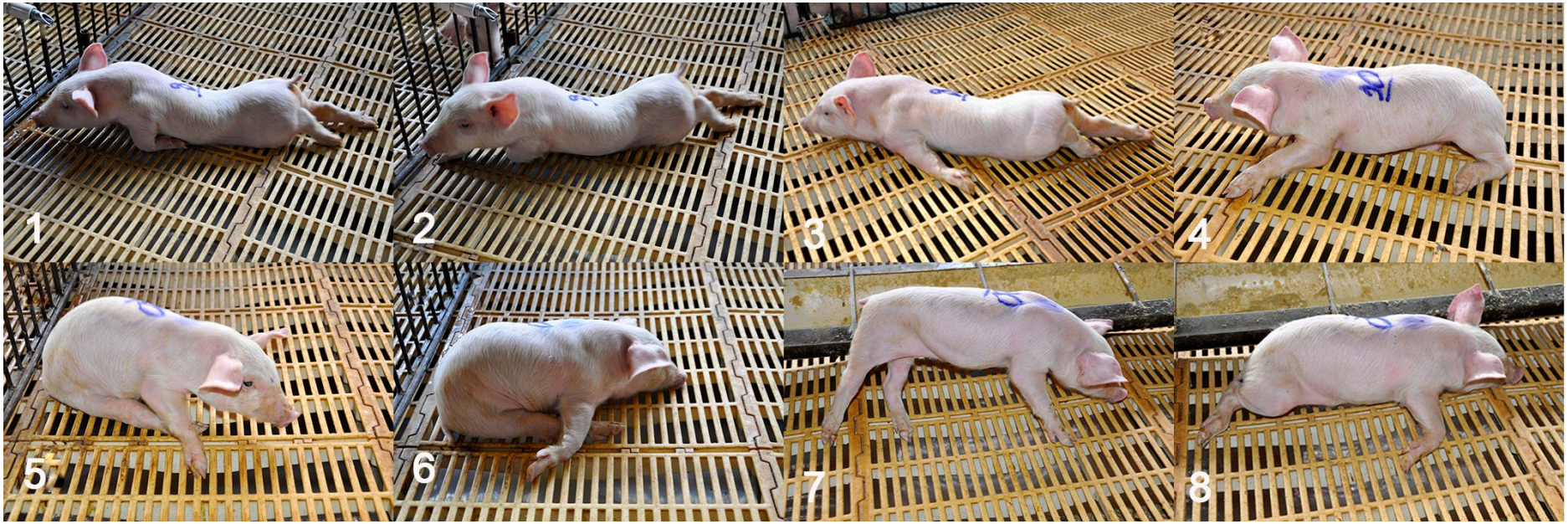
of the square delineates aggregates of rough endoplasmic reticulum with parallel cisterns (white arrow) surrounded by abundant polyribosomes, mitochondria (m) and lysosome (L). Transmission electron microscopy.

Supplementary Figure 1. Pantothenic acid responsive degenerative myelopathy, pig, case 08. The piglet supported the body weight on the tarsus-metatarsus region, presenting light to marked “hock weight-bearing” and “hock walking”.

Supplementary Figure 2 - 3. Pantothenic acid responsive degenerative myelopathy, pig, case 15. Glial fibrillary acidic protein (GFAP) and ionized calcium-binding adaptor molecule 1 (Iba1) immunohistochemistry. Figure 2. Necrotic neurons of the thoracic nucleus showing positive immunohistochemical staining in macrophages (arrow). Immunohistochemistry for GFAP. Figure 3. Iba1 highlighted that the predominant cell population of glial cells response were represented by microglial cells.

Supplementary Figure 4 - 5. Pantothenic acid responsive degenerative myelopathy, L5 spinal cord segment, pig, case 15. Toluidine blue and transmission electron microscopy. Figure 4. Cross sections of axons in the gracile fasciculus of the dorsal funiculus. The arrow indicates a distended myelin sheath containing a gitter cell. The arrowhead indicates a preserved axon. Figure 5. Approximate cross section of a degenerate axon (black asterisk) surrounded by myelin sheath (white arrow) that shows focal decompacted myelin (arrowhead). The axon showed swelling and axoplasmic vesicles (V) and myelin fragments (my). Within the distended myelinated

axon, there is a macrophage displaying nuclear pyknosis, (N) and phagolysosomes (SL). Adjacent, a normal axon (Ax) is highlighted for comparison.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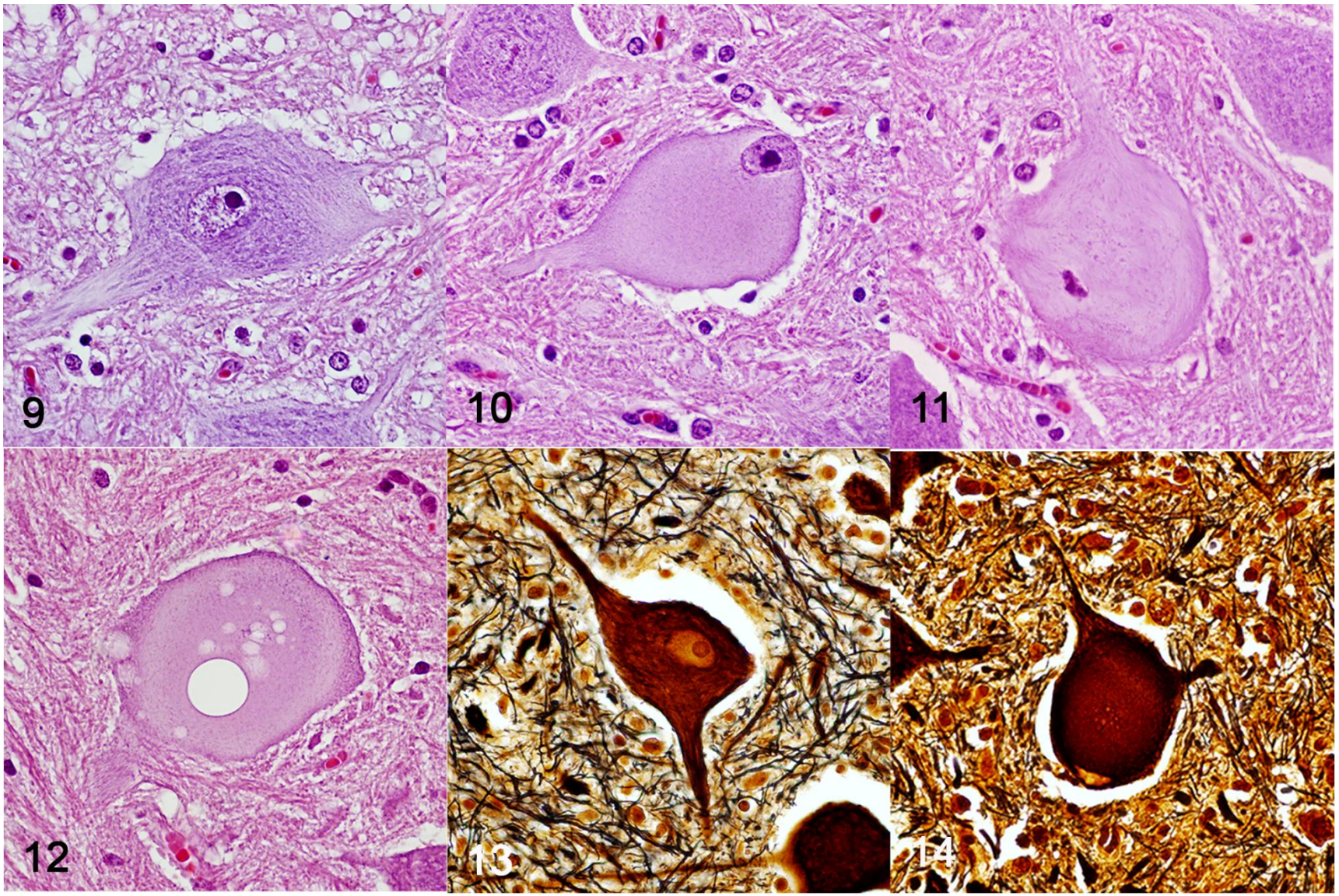
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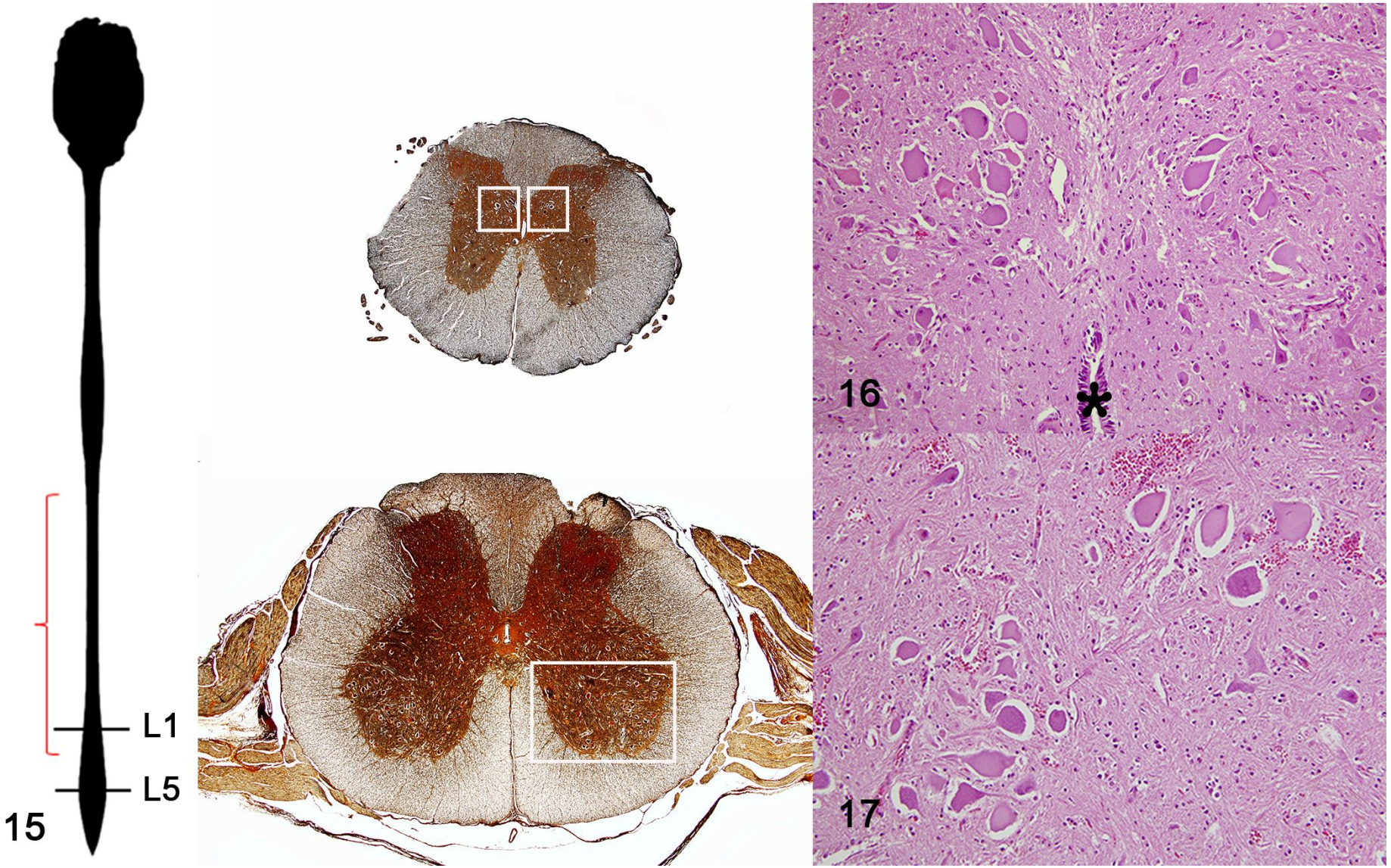
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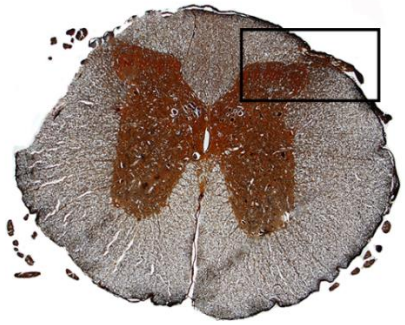

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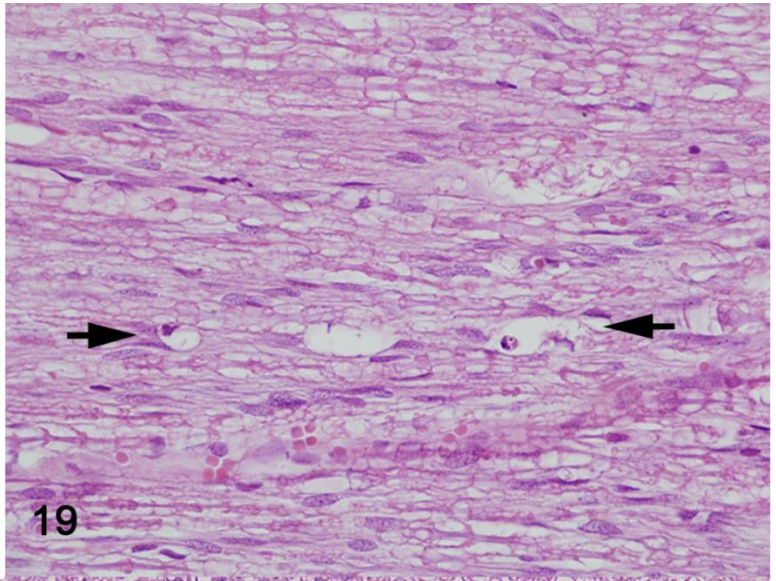

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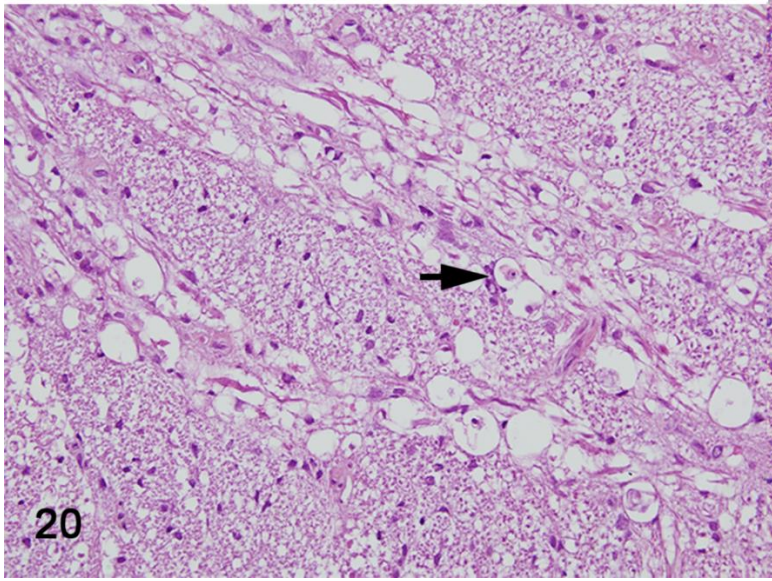

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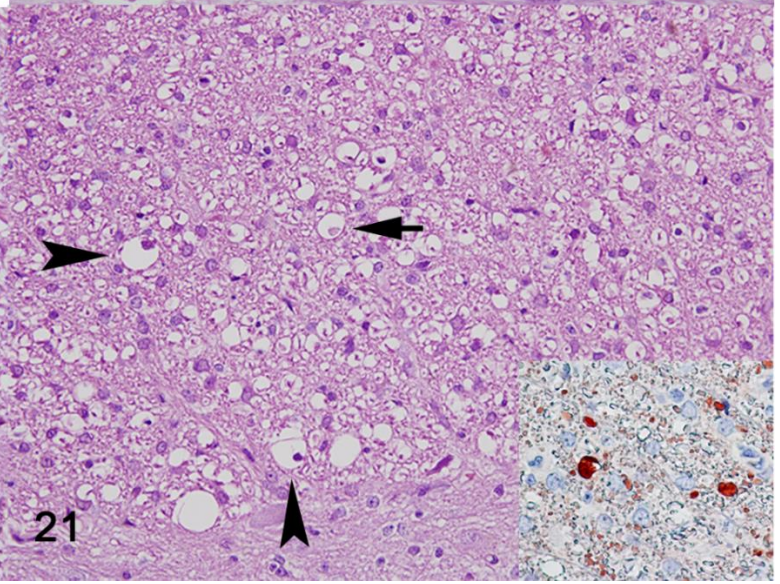

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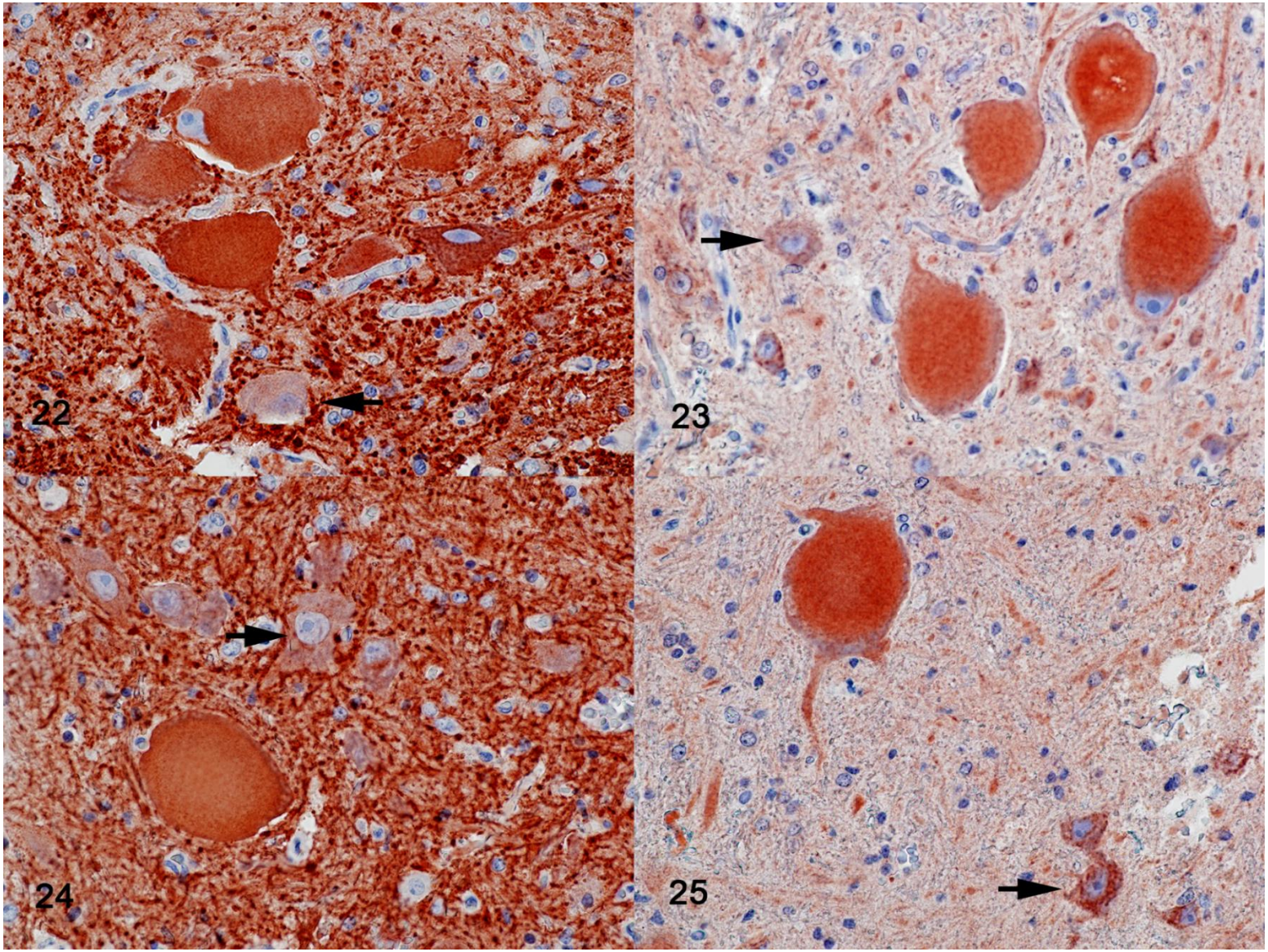
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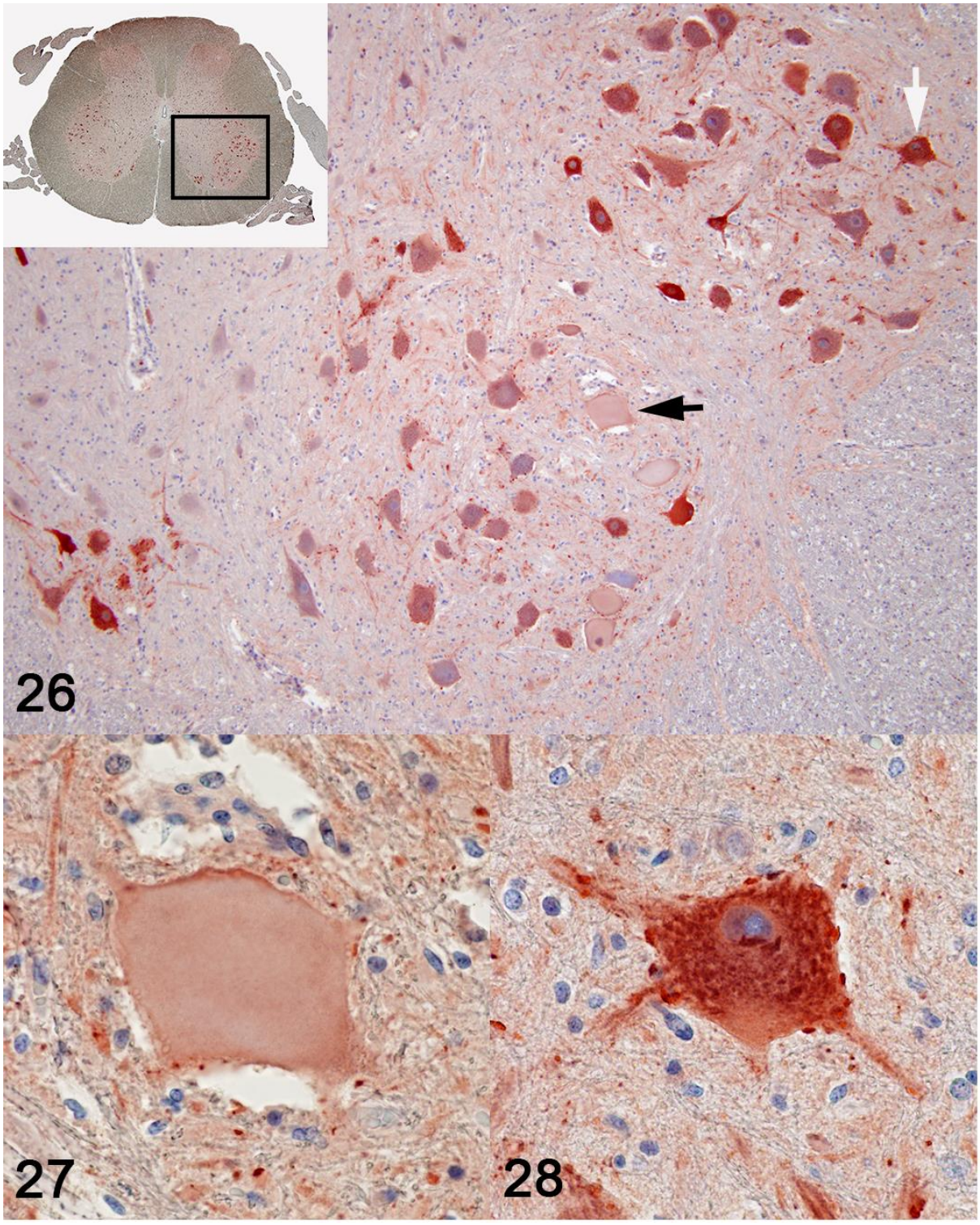
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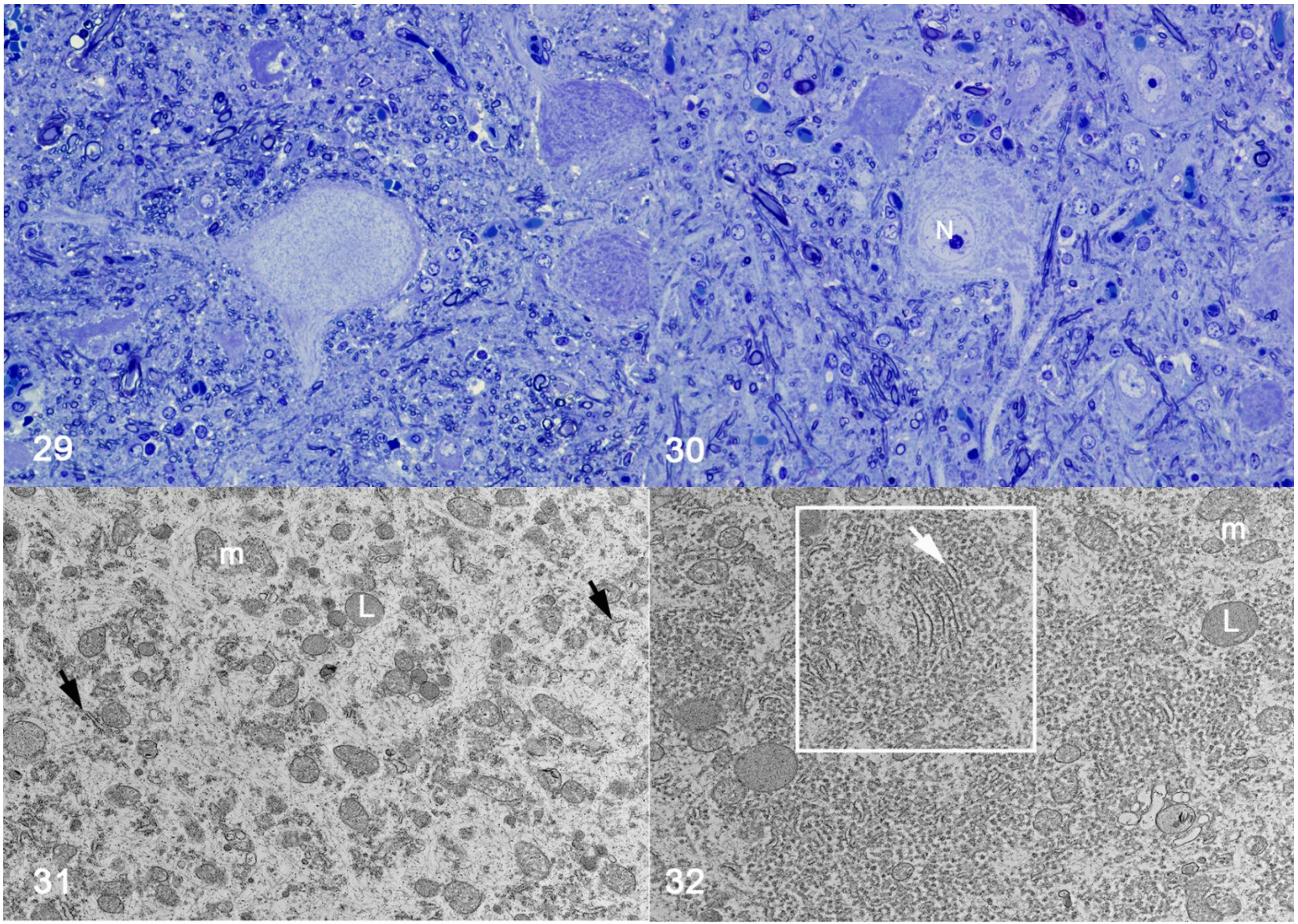
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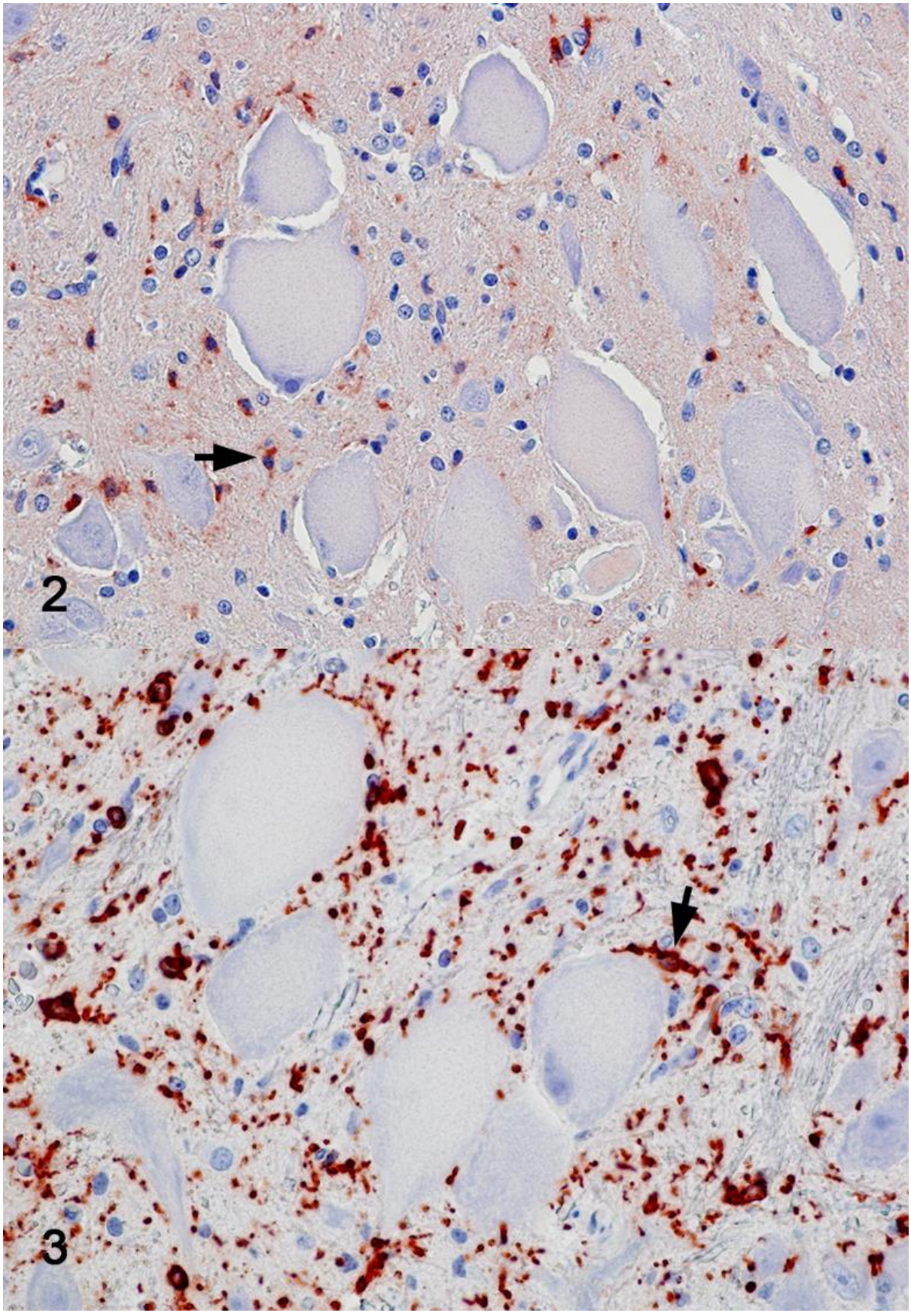
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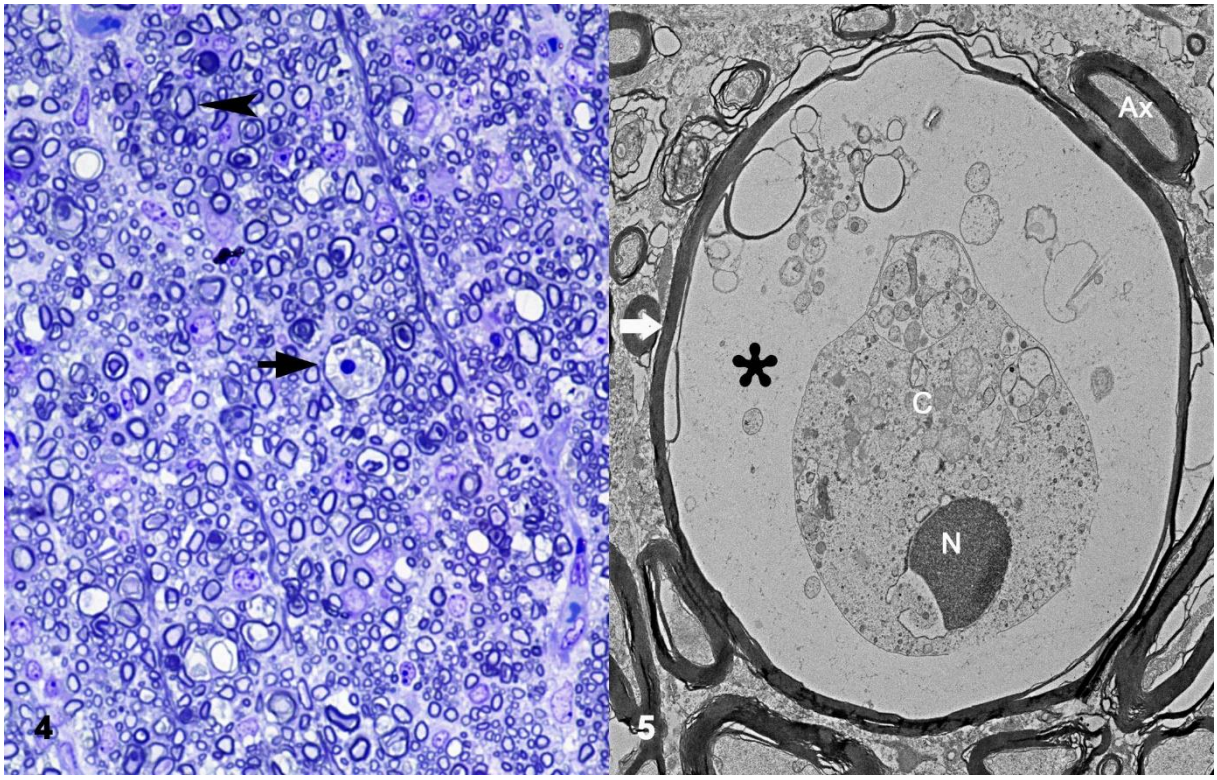
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Table I. Neurological sings and topography of lesions affecting motor and somatosensory nuclei and pathways of the spinal cord in piglets with pantothenic acid responsive myelopathy.

Pig ID no.	Age (days)	Locomotion deficits		Spinal cord									
				Motor system			Somatosensory system						
		Front limbs	Hind limbs	Nuclei IX			Thoracic nucleus		Dorsal funiculus			Lateral funiculus[c]	
				Cervical	Thoracic	Lumbar	Thoracic	Lumbar	Cervical	Thoracic	Lumbar	Cervical	Thoracic
1	5	-[a]	+	-	-	-	(+)	-	-	-	-	-	-
2	6	-	++	-	-	-	(+)	-	-	-	-	-	-
3	7	+	++	-	-	+(+)	-	+	-	-	-	-	-
4	15	++	++	-	-	-	-	+	-	-	-	-	-
5	10	++	++	-	(+)	+(+)	+(+)	+	-	-	-	-	-
6	11	++	+++	(+)	-	++	-	+	+(+)	-	+(+)	-	-
7	8	+	++	(+)	-	+	-	+	-	-	(+)		-
8	15	+++	+++	+(+)	+	-	+	++	+(+)	+(+)	+(+)	(+)	(+)
9	4	++	+	-	-	-	(+)	n/a[b]	(+)	(+)	(+)	(+)	(+)
10	5	+	-	(+)	(+)	+	-	-	+	+	+(+)	(+)	(+)
11	20	+	+++	(+)	-	(+)	+	+++	-	-	-	-	-
12	21	++	+++	(+)	+	-	+	++	-	-	-	-	-
13	4	+	++	(+)	-	-	+	++	-	-	-	-	-
14	5	+	+	-	-	-	(+)	(+)	-	-	-	-	-
15	11	+++	+++	(+)	(+)	+	+(+)	+++	(+)	(+)	+	(+)	(+)
16	27	+++	+++	-	-	+	+	++(+)	(+)	++	++	(+)	(+)

[a] +++ = severe; ++ = moderate; + = mild; (+) = discreet; - = no lesion

[b] n/a = not analyzed

[c] Spinocerebellar tract

Table 2. Neurological sings and topography of lesions affecting motor and somatosensory nuclei and pathways of the spinal cord in piglets with pantothenic acid responsive myelopathy.

Piglet no.	Somatosensory pathway													
	Cervical					Thoracic					Lumbar		Sacral	
	Dorsal funiculus			Lateral funiculus		Dorsal funiculus			Lateral funiculus		Dorsal funiculus	Lateral funiculus	Dorsal funiculus	Lateral funiculus
	FP	FG	FC	DSCT	VSCT	FP	FG	FC	DSCT	VSCT				
6	+(+)	(+)	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	-	-
7	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	-	-	-	-
8	+(+)[a]	(+)	+(+)	(+)	(+)	+	(+)	++	(+)	(+)	-	-	-	-
9	(+)	(+)	(+)	(+)	(+)	+	(+)	+	(+)	(+)	-	-	-	-
10	+	+	+(+)	(+)	(+)	(+)	(+)	+(+)	(+)	(+)	+(+)	(+)	(+)	(+)
15	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	-	-	-	-
16	(+)	(+)	(+)	(+)	(+)	(+)	(+)	+	(+)	(+)	+(+)	(+)	(+)	(+)

Abbreviations: FP, fasciculus proprius; FG, fasciculus gracilis; FC, fasciculus cuneatus; SCT, spinocerebellar tract; DSCT, dorsal spinocerebellar tract; VSCT, ventral spinocerebellar tract.

[a] +++ = severe; - = no lesion; ++ (+) = moderate/severe; ++ = moderate; + (+) = mild/moderate; + = mild; (+) = minimum.

Supplemental Table 1: Antibody specificity, dilution and isotype

Antibody Specificity	Source	Clone Isotype	Dilution Primaty antibody (working Ig conc.)	Incubation time/temp	Secondary antibody	Secondary antibody source
Glial fibrillary acidic protein (GFAP)	AbDSerotec/ MCA4733	1B4/ IgG2b	1:3200 mouse -anti-GFAP (0.3ug/ml)	30 min; Room temp	EnVision+ goat anti-mouse	Dako
Nonphosphorylated Neurofilament (NF)	Abcam/ ab28029	SMI-32/ IgG1	1:500 mouse -anti-NF Nonphos (no conc. Data)	Overnight; 4^{o}C	EnVision+ goat anti-mouse	Dako
Calretinin (CR)	Abcam/ ab16694	IgG	1:50 rabbit-anti-Calretrine (no conc. Data)	Overnight; 4^{o}C	EnVision+ goat anti-rabbit	Dako
Neurofilament protein	Dako/ M0762	2F11 IgG	1:2000 mouse -anti-NF (0.16ug/ml)	30 min; Room temp	EnVision+ goat anti-mouse	Dako
Iba1	Biocare/ CP 290A	IgG	1:400 rabbit-anti- (no conc. Data)	45 min; Room temp	EnVision+ goat anti-rabbit	Dako
Choline Acetyltransferase (ChAT)	Millipore/ AB144P	IgG	1:1000 goat-anti-ChAT (no conc. Data)	Overnight; 4 ^{o}C	ImmPRESS+ horse anti-goat	Vector

Abbreviations: IgG = immunoglobulin G; working Ig conc. = working dilution of immunoglobulin G concentration; Dako = Dako Agilent pathology Solutions, Carpinteria, California CA 93013, US; Vector = Vector Laboratories, Burlingame, California CA 94010, US.

2.3. ARTIGO 3

Nesse item é apresentado o artigo intitulado:

Swine metabolic bone disorders: outbreak of osteoporosis and literature review

Marina P. Lorenzett, Bianca S. de Cecco, Matheus V. Bianchi, Raquel AS. Cruz, Daniel CL. Linhares, Roberto MC. Guedes, David Driemeier, and Saulo P. Pavarini

Artigo a ser submetido ao periódico **Journal of Veterinary Diagnostic Investigation**

Brief communication

Swine metabolic bone disorders: outbreak of osteoporosis and literature review

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Running head: Osteoporosis in growing-finishing and breeding pigs

Abstract: 155 words, Main text: 1699 words, Figures: 2, Tables: 1.

Abstract: Forty-six pigs presented muscle weakness, hind limb paresis and paralysis, weight loss, lateral recumbency, and death, with a clinical course of 7 to 10 days. There was generalized bone fragility, bone callus formation, and multiple fractures in the limbs, ribs, and vertebrae of two necropsied pigs. Microscopically there was a diffuse and marked decrease in the thickness and the numbers of trabeculae. The trabeculae were disconnected, with the appearance of "free-floating trabeculae". The cortex of long bones was severely thinned, characterized by the increase of the cortical porosity by the enlargement of Haversian canals and endosteal erosion. A low level of osteoblastic activity was evident. Flame atomic absorption spectrometry in liver samples revealed significant zinc overload and copper deficiency. The pathological findings were compatible with osteoporosis. In this report, we present the first pathologic description of an outbreak of osteoporosis in pigs. We also provide a brief review of metabolic bone diseases in pigs.

Key-words: swine, bone disease, bone fragility, metabolic disorder, pathological fractures

Metabolic bone disease broadly categorizes disturbances related to bone formation and remodeling (Madson et al., 2019). The process of bone formation and maturation is complex and involves the direct interaction between genetic factors, local and systemic hormones, dietary nutrients, and mechanical forces (Craig et al., 2016). Metabolic bone disease in swine is usually related to ration formulation or feed mixing errors, leading to deficiency or toxicity of specific nutrients, such as calcium, phosphorus, copper, and vitamin D and A (Fox et al., 1985). Other primary causes include parathyroid hormone disturbances, starvation, lactation, or increased stress (Thompson, 2007, Spencer, 1979, Doige 1982, Madson et al., 2019). The expression of a bone abnormality depends on the phase of skeletal development when it occurs, the severity of the defect, the age of the animal at the time of the insult, and how long it persists (Craig et al., 2016). Metabolic bone diseases in animals include rickets, osteomalacia, fibrous osteodystrophy, and osteoporosis (Craig et al., 2016). In swine, rare osteoporosis cases have been described, usually associated with lactation (Doige, 1982). The objective of the current study is to describe an osteoporosis outbreak in growing-finishing and breeding pigs. We also provide a brief review of other metabolic bone diseases that affect pigs.

The outbreak occurred in one swine growing-finishing and breeding farm, located in Minas Gerais, southeast Brazil (18° 24' 56" S, 46° 25' 17" W), from November 2014 to January 2015. We obtained clinical and epidemiological information directly from the field veterinarians and swine farm owners. Piglets were weaned at 21 days old and sent to the growing-finishing site at 60 days of age. All pigs housed had the same nursery origin. These pigs were fed with the same ration during this period, based on corn and soybean, added with macro and micro minerals, vitamins, and lactose. Pigs had free access to

water and food. The feed formulation was modified before the outbreak began, and an excessive amount of zinc oxide was added to the formulation.

Forty-six of 744 growing pigs, including males and females, ages between 73 to 79 days, presented clinical signs, and died in the period of three months were affected. Clinical signs were characterized as hind limb paresis and paralysis, weight loss, muscle weakness, and recumbency over a clinical course of 7 to 10 days. Two pigs (pigs 1 and 2) were selected and referred to systematic *postmortem* examination. Grossly, both pigs had similar lesions, but more severe in pig 1. A complete fracture was observed in pig 1 in the following bones: scapula, humerus, femur, ribs, and vertebrae (thoracic, lumbar, and sacral). In pig 2, complete fractures were observed in the ribs, sternum, and vertebrae. Marked depletion of cancellous bones and thinner cortical bones were observed in the long bones. In some areas, it was possible to observe the mild formation of transverse reinforcement trabeculae. The fractured bones were surrounded by moderate swelling and hemorrhage in adjacent soft tissue. In both pigs, the ribs exhibited beside the fractures, areas of callus formation associated with multifocal hemorrhage and edema. In the spine, shortening of vertebral bodies and multiple microfractures were observed in thoracic, lumbar, and sacral segments. No gross lesions were observed in organs.

Tissue samples were collected, fixed in 10% formalin, routinely processed for histology, and stained with hematoxylin and eosin. Pigs used as a control for bone abnormalities had similar age to affected ones (52 days), and were euthanized due to mild kyphosis. Histopathological analysis was performed in many long bones, ribs, and vertebrae. Bone trabeculae in the central part of the metaphysis were decreased, both in thickness and also in the numbers of trabeculae. The trabeculae were disconnected, with

the appearance of “free-floating trabeculae”. The cortex of long bones was severely thinned, characterized by the increase of the cortical porosity by the enlargement of Haversian canals and by endosteal erosion. A moderate decrease in osteoblasts (low level of osteoblastic activity), with areas of hemorrhage and deposition of eosinophilic fibrillar material was seen in the medullary region. In the ribs, the callus formation exhibited focally extensive areas with a proliferation of hyaline cartilage, surrounded by a moderate proliferation of connective tissue. The areas of microfractures were characterized by discontinued lamellar bone, with a focally extensive area of hemorrhage and moderate proliferation of connective tissue. In the bone marrow, a moderate decrease of hematopoietic cells was observed. Fragments of the spinal cord adjacent to microfractures of the vertebrae presented mild Wallerian degeneration. No lesions were observed in organs, including the gastrointestinal tract, and pancreas.

Samples of liver from these two pigs were also collected in formalin, aiming to determine the zinc and copper levels using flame atomic absorption spectrometry after tissue digestion by microwave pretreatment. The determination of zinc on the liver samples showed values of 2,856 ppm and 2,321 ppm, more than four times higher than found in zinc intoxication outbreaks (Komatsu et al., 2020). The copper (Cu) dosage on the liver was 22.7 ppm and 33.1 ppm, below the reference range of 60-117 ppm (Cancilla et al., 1967). After identifying of dietary zinc overload in the rations, zinc levels in the diets were corrected, and a marked reduction in pathological fractures was noted and new cases of pathological fractures ceased to occur.

Based on the clinical and pathological findings, the diagnosis of nutritional osteoporosis was made. Osteoporosis is a skeletal disorder characterized by reduced

bone mass and altered trabecular microarchitecture that leads to bone fragility and fractures (Bonucci and Ballanti, 2013). This condition is one of the most frequent metabolic bone diseases in humans (Bonucci and Ballanti, 2013), and can also occur in lambs (Suttle et al., 1972), pigs (Doige, 1982; Craig et al., 2016), goats (Rosa et al., 2013), and dairy cows (Yoshida, 2015). Classically, osteoporosis is characterized by a negative imbalance between bone formation and bone resorption, resulting in a bone structurally normal but with reduced breaking strength caused by excessive resorption of bone, resulting in an endosteal thinning of the trabeculae and cortices (Spencer, 1979; Madson et al., 2019, Craig et al., 2016).

Osteoporotic bones are weak and fracture easily due to the decrease in bone mass and changes in the organic matrix. Human patients with osteoporosis frequently suffer vertebral compression fractures, hip and distal radius fractures (called Colles' fractures). Almost half of the vertebral compression fractures occur spontaneously (McCarthy and Frassica, 2015). In the case described here, pigs had spontaneous fractures and microfractures of long bones, vertebrae, and ribs. The occurrence of pathologic bone fractures without evidence of excessive trauma may be the first indication that these pigs developed osteoporosis related to increased bone fragility. In the swine industry, growing-finishing pigs exhibit a high rate of growth and weight gain, which significantly increases the prevalence of fractures when associated with bone weakness (Craig, 2016).

Frequently, osteoporosis is caused by nutritional imbalance, related to a mineral deficiency or excess, or starvation (Pepper, et al., 1978; Craig et al., 2016). In the outbreak described here, it is suggested that the overload of ZnO in the feed provided to the pigs before the outbreak could contribute to the low levels of Cu detected in the liver

of the affected pigs. The possibility of a direct toxic effect of zinc on developing bones and cartilages must be considered since it has been described that Zn can prevent normal deposition of phosphorus in the bones, and high levels of dietary Zinc interferes in the calcium/phosphorus metabolism (Thompson et al., 1959). ZnO overload in pigs is reported to cause a reduced rate of gain, anorexia, gastroenteritis, and lameness (Brink et al., 1959; Puhl, 1994). The zinc and copper values detected in the liver samples demonstrated a zinc overload and Cu deficiency, suggestive of ZnO-induced copper deficiency caused by competitive absorption-inhibition.

Copper deficiency may be primary, caused by an inadequate diet with low levels of this compound, or secondary to the increase of the dietary levels of copper antagonists, like zinc (Craig et al., 2016). Zinc and other divalent cations, such as iron and cadmium, compete with Cu for a common transport mechanism, therefore decreasing the copper absorption and availability (Craig et al., 2016; Madson et al., 2019; Burrough et al., 2019). Copper deficiency can occur in many domestic species, and a feature of this deficiency is a skeletal deformity since Cu is required for the cross-linkage of collagen molecules, which influences bone formation, skeletal mineralization, and the integrity of the connective tissue (Palacios, 2006; Craig et al., 2016). Also, in the outbreak described here, moderate depletion of hematopoietic precursors was observed in the bone marrow, which is in accordance with previous studies regarding copper deficiency in piglets, where the main finding was severe anemia (Lahey et al., 1952).

Currently, commercial diets for pigs are specifically adapted for the production of lean muscle mass and growth, and nutritional programs provide sufficient calcium, phosphorus, and vitamin D supplementation for normal bone growth and mineral

homeostasis (Madson et al., 2012). Therefore, metabolic bone diseases are uncommon in swine production and account for 0.2% to 1.0% of causes of death (Coelho, 2017; Brum et al., 2013). The differential diagnosis for osteoporosis in pigs must include other metabolic bone diseases, especially rickets, and fibrous osteodystrophy, although other metabolic bone diseases are described in the swine species. Clinical signs for all metabolic bone diseases can be similar and include lameness, pathological fractures, sudden death, reluctance to rise, and limb weakness (Madson et al., 2019). The differentiation among these conditions can be performed through epidemiological aspects, especially the age of the affected pig, added to gross and histological features (Table 1). In this study, the main lesions were characteristic of osteoporosis, with the severe thickness and decrease in the number of trabeculae, increased porosity of the cortical bone due to enlargement of the Haversian canal, and low level of osteoblastic activity, making it possible to differentiate from other metabolic bone diseases.

Investigations of multiple spontaneous fractures, muscle weakness, paresis, and sudden death in swine should include metabolic bone disease conditions such as osteoporosis in the differential diagnosis. It is essential to perform the gross and histological evaluation of the bones and growth plates in order to correctly diagnose the underlying condition involved in skeletal disorders.

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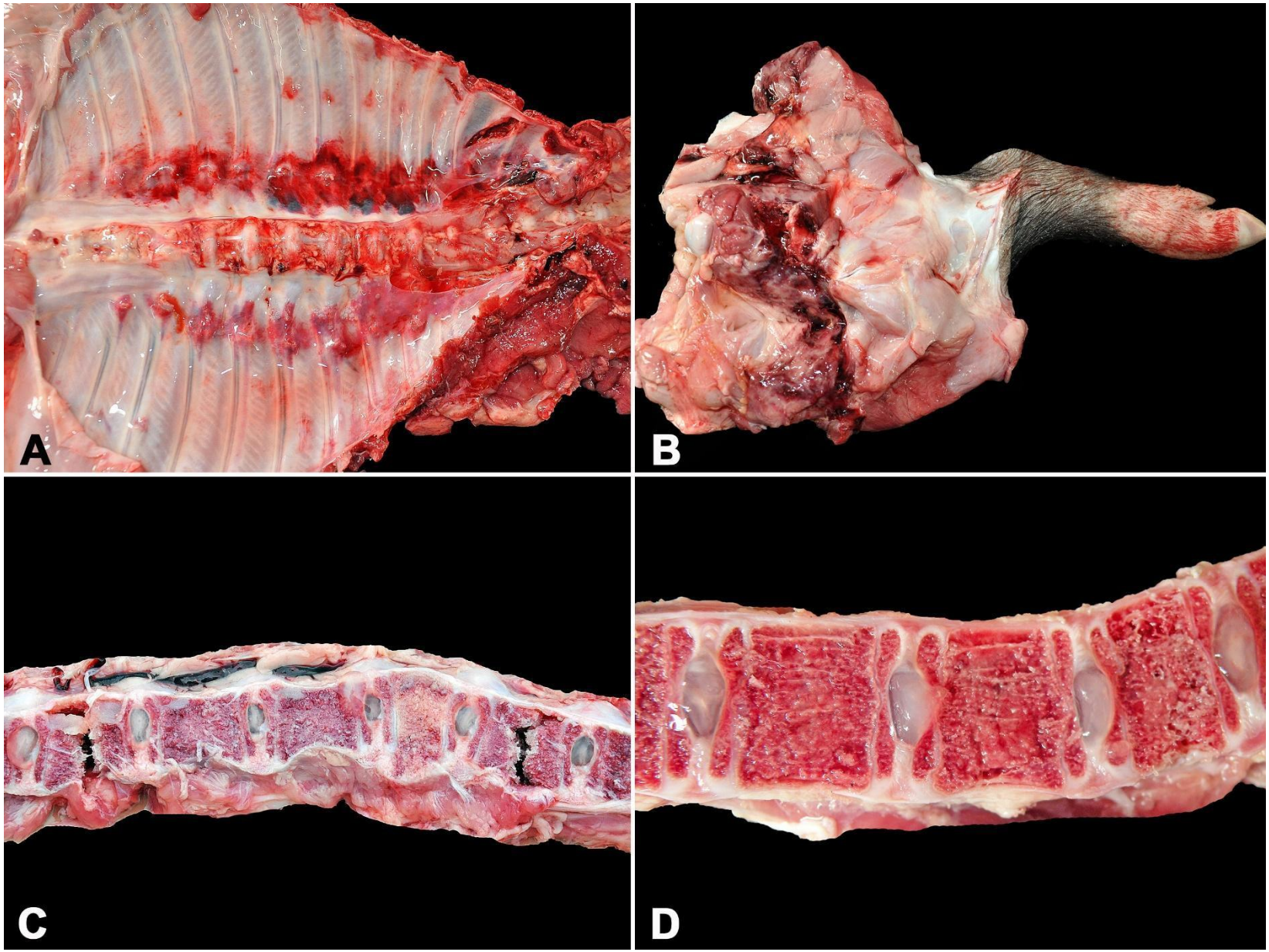
Figure legends:

Figure 1. Osteoporosis in pigs. **A,** several ribs present fractures near the costochondral junction and are surrounded by hemorrhage. **B,** the femur exhibits comminuted complete fracture and is surrounded by hemorrhage and edema (pig 1). **C,** two vertebrae present complete fracture, and some vertebrae exhibit severe shortening with an indication of the previous fracture. **D,** the vertebrae show marked depletion of cancellous bone and formation of transverse reinforcement trabeculae.

Figure 2. Osteoporosis in pigs. **A,** microscopically, in the vertebrae the bone trabeculae are severely decreased, both in thickness and in number. The trabeculae were disconnected, with the appearance of "free-floating trabeculae". There is a moderate decrease of hematopoietic cells. H&E. Original objective 10x. **B,** normal bone (vertebrae) appearance. H&E. Original objective 10x. **C,** in the vertebrae, the cortex of long bones is severely thinned, with an increase of the cortical porosity by the enlargement of Haversian canals and by endosteal erosion. H&E. Original objective 20x. **D,** normal bone (vertebrae) appearance. H&E. Original objective 20x. **E,** in the rib, a moderate decrease in osteoblasts (low level of osteoblastic activity). H&E. Original objective 20x. **F,** normal bone (rib) appearance. H&E. Original objective 20x.

Table 1. Metabolic disease in growing/adult pigs

Disorder	Age of pigs affected	Cause	Lesion distribution	Gross features	Histopathology
Osteoporosis (Doige 1982, Craig 2016)	Growing and adult pigs	Cu/Ca deficiency, starvation, vitamin A toxicity, increased stress	Vertebrae, ribs, long bones, flat bones of the skull	Reduced breaking strength of ribs. Marked depletion of cancellous bone in the metaphyses and epiphyses. Reduced cortex thickness.	Bone trabeculae decreased in thickness and number, disconnected, "free-floating trabeculae", increased porosity of the cortical bone due to enlargement of Haversian canal, low level of osteoblastic activity
Fibrous osteodystrophy (Thompsom, 1989)	Growing and adult pigs	Ca and vitamin D deficiency, excess P	Skull bones, especially mandibles and maxilla. Also, scapula and vertebral column	Bilateral enlargement of the bones of the skull, movable teeth, jaw swelling. Soft, pumice-like bones, and decrease in bone density. Ribs may bend or snap with little effort	Increased osteoclastic bone resorption, marked fibroplasia, and increased osteoblastic activity with formation of immature woven bone, frequently poorly mineralized
Rickets (Pepper, 1978; Madson, 2012; Gris et al., 2020)	Growing pigs	Deficiency of Ca, P, vitamin D. Ca/P imbalance	Sites of rapid growth, metaphyseal and epiphyseal regions of long bones, and costochondral junctions of the large middle ribs.	Weak bones that bend before breaking with a weak snap, and have enlarged growth plates giving a clinical appearance of swollen joints, increased volume of costochondral joints (rachitic rosary), kyphosis	Decreased mineralization of the growing bone, evident persistence of hypertrophic chondrocytes at sites of endochondral ossification
Osteomalacia (Craig, 2016)	Late finishing and adult pigs	Deficiency of Ca, P, vitamin D. Ca/P imbalance	Vertebrae, femur, pelvis, and ribs	Bones with reduced resistance to pressure and tension. In severe cases the marrow cavity is expanded, the cortex is thin, spongy, and soft	Decreased mineralization with the significant remodeling of the mature bone. Localized accumulation of osteoid at sites of mechanical stress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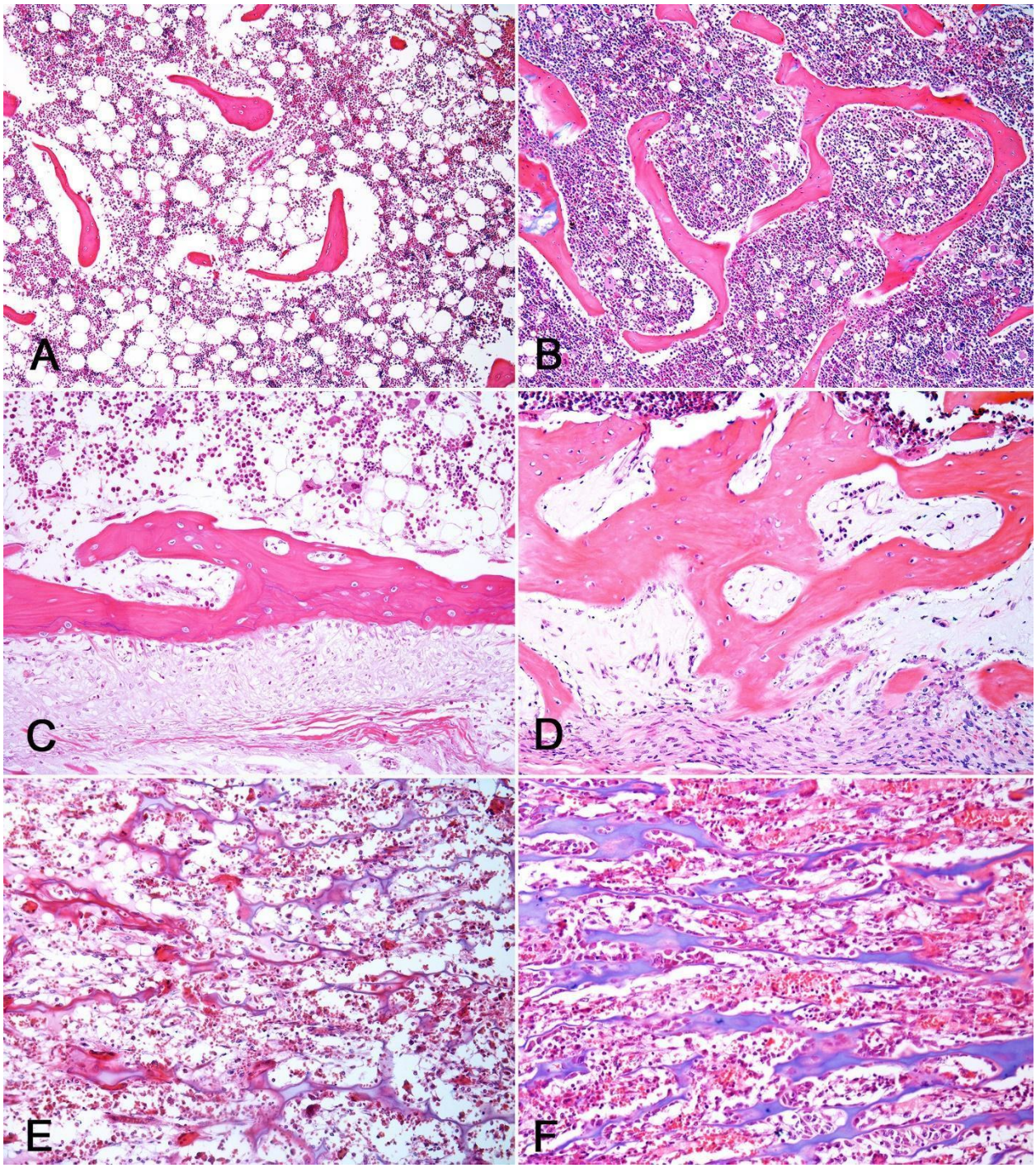
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3. CONSIDERAÇÕES FINAIS

- A urolitíase obstrutiva, em suínos de crescimento e terminação, foi atribuída a um desequilíbrio nutricional na proporção de cálcio e fósforo na dieta. A ração fornecida aos suínos apresentava baixos níveis de cálcio, o que pode ter levado a uma elevada excreção de fosfato pelo trato urinário.
- Todos os suínos que desenvolveram urolitíase eram machos e apresentavam sinais clínicos de redução do consumo de ração, oligúria ou anúria, distensão e dor abdominal, com consequente óbito por ruptura vesical. A taxa de letalidade atingiu 100%.
- Os componentes minerais que predominaram nos urólitos encontrados no presente estudo foram carbonato de cálcio e fosfato de amônio magnesiano.
- Leitões nascidos de porcas que receberam dietas deficientes em ácido pantotênico durante a gestação e lactação desenvolveram sinais clínicos neurológicos. Os sinais neurológicos observados nestes leitões, foram atribuídos à lesões degenerativas na medula espinhal responsiva a suplementação com ácido pantotênico.
- Todos os leitões afetados desenvolveram ataxia sensorial e paresia. As lesões patológicas foram caracterizadas por necrose de neurônios do núcleo torácico e neurônios motores alfa dos núcleos IX da medula espinhal. A degeneração axonal foi observada nas raízes dos nervos espinhais dorsal e ventral e no funículo dorsal.
- Sobrecarga de zinco e consequente deficiência de cobre foram associadas com osteoporose nutricional em suínos. Na necropsia constatou-se fragilidade óssea generalizada, formação de calo ósseo e múltiplas fraturas em membros, costelas e vértebras. A histologia revelou uma diminuição difusa e acentuada da espessura e do número de trabéculas.

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