

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

**ENFERMIDADES PULMONARES E INTESTINAIS DIAGNOSTICADAS EM
EQUINOS NO SUL DO BRASIL**

MATHEUS VIEZZER BIANCHI

PORTO ALEGRE, 2019

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ENFERMIDADES PULMONARES E INTESTINAIS DIAGNOSTICADAS EM EQUINOS
NO SUL DO BRASIL

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DEDICATÓRIA

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RESUMO

Doenças relacionadas ao sistema alimentar são tidas como a principal causa de morte de equinos, enquanto doenças respiratórias são a quarta a quinta maior causa de morte na espécie. Todavia, os aspectos patológicos de ambas são pobremente descritos na literatura. Dessa forma, nessa tese estão incluídos quatro manuscritos abordando esse tema. O primeiro manuscrito teve o objetivo de descrever os aspectos patológicos e microbiológicos de lesões pulmonares de equinos abatidos em matadouro-frigorífico. Um total de 84 amostras foi analisada, e um diagnóstico final conclusivo foi obtido em 74. Broncopneumonia supurativa foi o principal diagnóstico (50/74), seguido por pneumonia eosinofílica granulomatosa (9/74), obstrução aérea recorrente (7/74), fibrose pulmonar (4/74), hemorragia pulmonar (3/74) e pitiose pulmonar (1/74). As broncopneumonias foram causadas principalmente por *Streptococcus equi* subsp. *equi* (21/50). O segundo manuscrito teve o objetivo de descrever os aspectos macroscópicos, microscópicos e etiológicos relacionados a pneumonias e pleurites diagnosticadas em 50 equinos necropsiados de 2005 a 2017 no Sul do Brasil. Pneumonias supurativas foram as principais condições diagnosticadas (17/50), e foram subdivididas em agudas (8/17), subagudas (6/17) e crônicas (3/17) baseadas nos padrões morfológicos das lesões. *Streptococcus* sp. foi identificado em 11 desses casos através de cultivo bacteriano ou exame imuno-histoquímico. As outras lesões foram classificadas em pneumonias piogranulomatosas por *Rhodococcus equi* (14/50), pneumonias aspirativas (5/50), pneumonias micóticas por *Aspergillus* spp. (4/50), pneumonias broncointersticiais por *Influenza A* (3/50), pleurites (3/50) e pneumonias embólicas (3/50). Pneumonias são causas significativas de morte em equinos no Sul do Brasil, sendo as pneumonias supurativas e piogranulomatosas as principais condições. Os aspectos epidemiológicos, patológicos, bacteriológicos e imuno-histoquímicos são essenciais para a obtenção do diagnóstico final. O terceiro manuscrito teve o objetivo de descrever os achados patológicos de enterites e tiflocolites parasitárias fatais em equinos. Dez cavalos apresentavam enterite e/ou tiflocolite parasitária fatal, e as principais causas foram: tiflocolite por ciatostomíneos (6/10), enterite por *Eimeria leuckarti* (1/10), enterite por *Strongyloides westeri* (1/10), colite por *Balantidium coli* (1/10) e infecção por múltiplos agentes (1/10). A tiflocolite por ciatostomíneos exibia acentuado espessamento de mucosa, com nódulos multifocais elevadas contendo parasitas filiformes. Microscopicamente, a mucosa e submucosa exibiam estruturas parasitárias encistadas envoltas por inflamação eosinofílica e granulomatosa. A enterite por *E. leuckarti* exibia macrogamontes, microgamontes e oocistos no interior de células do hospedeiro. A enterite por *S. westeri* exibia atrofia de vilosidades com numerosas estruturas parasitárias encistadas na mucosa. O quarto manuscrito teve o objetivo de descrever os aspectos epidemiológicos e patológicos de doenças não infecciosas do trato gastrointestinal de equinos necropsiados entre 2005 e 2017 no Sul do Brasil. Durante esse período, 114 equinos morreram devido a doenças não infecciosas do trato gastrointestinal, e as principais causas foram: dilatação gástrica primária (27/114), vólvulos (27/114), enterolitíase (20/114), ruptura (colônica) retal (15/114), compactação gástrica ou cecocolônica (10/114), encarceramentos (6/114), intussuscepções (4/114) e outros (5/114). Doenças não infecciosas do trato gastrointestinal, assim como enterites e tiflocolites parasitárias, são importantes causas de morte em equinos. Os aspectos epidemiológicos, macroscópicos e microscópicos das condições devem ser considerados para que um diagnóstico conclusivo da causa da cólica seja obtido.

Palavras-chave: cólica, doenças infecciosas, doenças parasitárias, patologia de equinos, pneumonias, sistema alimentar, sistema respiratório.

ABSTRACT

Diseases related to the alimentary system are the main cause of death in horses, while respiratory diseases are fourth to fifth most common causes of death. Yet, the pathological features of both are poorly described. Thus, four manuscripts on this subject are included on this thesis. The first manuscript aimed to describe the pathological and microbiological features of lung lesions in slaughtered horses. A total of 84 samples were analyzed, and a final diagnosis was obtained in 74. Suppurative bronchopneumonia was the main diagnosis (50/74), followed by granulomatous eosinophilic pneumonia (9/74), recurrent airway obstruction (7/74), lung fibrosis (4/74), lung hemorrhage (3/74), and pythiosis (1/74). Bronchopneumonia was caused mainly by *Streptococcus equi* subsp. *zooepidemicus* (21/50). The second manuscript aimed to describe the gross, microscopic and etiologic findings related to pneumonia and pleuritis in 50 horses necropsied between 2005 and 2017 in Southern Brazil. Suppurative pneumonia was the main condition diagnosed (17/50), and was further divided into acute (8/17), subacute (6/17), and chronic (3/17) based on the morphological pattern of lesions. *Streptococcus* sp. was identified through bacterial culture or immunohistochemistry in eleven cases of these cases. The following lesions were pyogranulomatous pneumonia by *Rhodococcus equi* (14/50), aspiration pneumonia (5/50), mycotic pneumonia by *Aspergillus* spp. (4/50), bronchointerstitial pneumonia by Influenza A (3/50), pleuritis (3/50), and embolic pneumonia (3/50). Pneumonia is a significant cause of death in horses in Southern Brazil, with suppurative and pyogranulomatous pneumonia as the main conditions. Epidemiological, pathological, bacteriological, and immunohistochemical features are essential to obtain a final diagnosis. The third manuscript aimed to describe the pathological findings of fatal parasite-induced enteritis and typhlocolitis in horses. Ten horses had fatal parasitic enteritis and/or typhlocolitis, and the main causes were: cyathostominae typhlocolitis (6/10), *Eimeria leuckarti* enteritis (1/10), *Strongyloides westeri* enteritis (1/10), *Balantidium coli* colitis related to cyathostominae (1/10), and infection by multiple agents (1/10). Cyathostominae typhlocolitis showed marked mucosal thickening, with multifocal elevated nodules containing tangled filiform parasites. Microscopic examination revealed that the mucosa and submucosa had encysted parasitic structures surrounded by eosinophilic and granulomatous inflammation. *E. leuckarti* enteritis had macrogamonts, microgamonts, and oocysts inside the host cells. *S. westeri* enteritis had atrophy of the villi with numerous mucosal encysted parasitic structures. *B. coli* typhlocolitis showed severe diffuse mucosal reddening, with microscopic superficial mucosal necrosis associated with multiple protozoan trophozoites. The fourth manuscript aimed to describe the epidemiological and pathological features of noninfectious diseases of the gastrointestinal tract in horses necropsied between 2005 and 2017 in Southern Brazil. During this period, 114 horses died due to noninfectious diseases of the gastrointestinal tract, and the main causes were: primary gastric dilation (27/114), volvulus (27/114), enterolithiasis (20/114), rectal (colonic) perforation (15/114), gastric or cecocolonic impaction (10/114), incarcerations (6/114), intussusception (4/114), and others (5/114). Noninfectious gastrointestinal diseases, as well as parasite-induced enteritis and typhlocolitis, are important causes of death in horses. Epidemiological, gross and microscopical features of the conditions should be accounted to obtain a final diagnosis of the cause of the colic.

Keywords: alimentary system, colic, equine pathology, infectious diseases, parasitic diseases, pneumonia, respiratory system.

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1. INTRODUÇÃO

A equinocultura é um ramo economicamente importante da agropecuária brasileira com uma movimentação de cerca de 16 bilhões de reais ao ano e emprega direta e indiretamente cerca de 600.000 pessoas (BRASIL, 2007). O Brasil possui a quarta maior população de equinos mundial com cerca de 5,5 milhões de animais, atrás apenas de Estados Unidos da América, China e México (IBGE, 2016). Entre os estados, o Rio Grande do Sul possui a segunda maior população de equinos com cerca de 535.000 animais (IBGE, 2016).

Em medicina veterinária, estudos retrospectivos de causas de morte procuram determinar a prevalência de doenças e relacioná-la com os aspectos epidemiológicos de uma determinada região (PIEREZAN *et al.*, 2009). Há poucos estudos de prevalência de causas de morte e doenças em equinos (BAKER; ELLIS, 1981; MARCOLONGO-PEREIRA *et al.*, 2014; PIEREZAN *et al.*, 2009), os quais são também muitas vezes restritos a sistemas, como tegumentar (BIANCHI *et al.*, 2016; SOUZA *et al.*, 2011), sistema nervoso central (RECH; BARROS, 2015), oftálmico (REICHMANN *et al.*, 2008), e órgãos, como a cavidade nasal (TROTTE *et al.*, 2008).

Equinos apresentam características anatômicas no sistema digestivo que os predispõem a enfermidades no trato gastrointestinal, como menor capacidade do estômago em relação à capacidade digestiva total, intestino delgado extenso, maior mobilidade do cólon maior e ceco, locais com diminuição abrupta da luz intestinal, incapacidade de eructar e regurgitar (GODOY; NETO, 2007). Dessa forma, o sistema digestivo é o mais envolvido com a causa de morte de equinos, com alterações de posição, rupturas, obstruções e compactações como as principais afecções (BAKER; ELLIS, 1981; PIEREZAN *et al.*, 2009).

Apesar de as parasitoses gastrointestinais contabilizarem cerca de 4% das causas de morte em equinos (MARCOLONGO-PEREIRA *et al.*, 2014) e serem um dos principais problemas em equinos de países em desenvolvimento (SHEFERAW; ALEMU, 2015), os aspectos

patológicos das enterites parasitárias são pouco descritos, se limitando a diagnósticos parasitológicos através de exames coprológicos (MARCOLONGO-PEREIRA *et al.*, 2014), nos quais os parasitas mais comumente identificados incluem: *Strongylus* spp., ciatostomíneos, *Triodontophorus* spp., *Strongyloides westeri*, *Parascaris equorum*, *Dictyocaulus arnfieldi*, *Oxyuris equi* e *Gastrodiscus* spp. (SHEFERAW & ALEMU, 2015). Aspectos patológicos são restritos a descrições de surtos ou de pequenos grupos de equinos afetados por ciatostomíneos (PIEREZAN *et al.*, 2009b) ou *Strongyloides westeri* (LUCENA; FIGHERA; BARROS, 2012).

O sistema respiratório é a quarto mais envolvido na causa de morte em equinos (BAKER; ELLIS, 1981; PIEREZAN *et al.*, 2009). Todavia, é descrito que a principal causa de morte nesses casos é a depressão anestésica, seguida pelas broncopneumonias (PIEREZAN *et al.*, 2009), e os equinos envolvidos tem de 1 a 5 anos de idade (BAKER; ELLIS, 1981). As pneumonias com envolvimento bacteriano, por sua vez, são mais comuns em potros de 2 a 8 meses, os quais são predispostos à infecção devido a condições ambientais (temperatura, poeira), superlotação, parasitismo e infecções virais primárias (LAKRITZ *et al.*, 1993).

Todavia, os aspectos patológicos de lesões pulmonares em equinos são pouco descritos, e, comumente, restritos a pneumonias em potros (LAKRITZ *et al.*, 1993). Equinos adultos geralmente se infectam por bactérias oportunistas habitantes da nasofaringe que colonizam os pulmões quando as respostas imunes respiratórias estão comprometidas, como no estresse, má nutrição e doenças virais. Nessas condições *Streptococcus equi* subsp. *zooepidemicus* é o agente mais comumente isolado de broncopneumonias em adultos (REUSS; GIGUÈRE, 2015). As pneumonias podem ser classificadas ainda de acordo com a natureza do agente causador, a distribuição da lesão e sua persistência. Desses, os principais aspectos considerados são os padrões morfológicos macroscópicos e microscópicos, com lesões caracterizadas como broncopneumonias ou pneumonias supurativas, pneumonias granulomatosas, pneumonias

intersticiais, pneumonias broncointersticiais, e pneumonias embólicas (CASWELL & WILLIAMS, 2016).

A produção de carne equina no Brasil não constitui uma atividade de destaque quando comparada às exportações de carne bovina, suína ou de aves, todavia a sua produção é voltada quase que exclusivamente à exportação. O Brasil é o terceiro maior exportador mundial desse produto. Os equinos destinados ao abate no Brasil são animais descarte, compostos em sua maioria por animais senis ou fracos (ABRAHÃO, 2002). Esses animais já debilitados, quando submetidos a situações de estresse (longos deslocamentos, alimentação precária e dieta hídrica forçada no transporte), podem ter suas respostas imunológicas alteradas, favorecendo a ocorrência de outras enfermidades (HOFER *et al.*, 2000).

Dessa forma, este trabalho teve os seguintes objetivos: (1) caracterizar os aspectos patológicos e microbiológicos de lesões pulmonares observadas em equinos abatidos em matadouro frigorífico da região Sul do Brasil, bem como discutir possíveis diagnósticos diferenciais; (2) descrever os aspectos macroscópicos, microscópicos e etiológicos relacionados às pneumonias e às pleurites diagnosticadas em 50 equinos necropsiados de 2005 a 2017 no Sul do Brasil (3) descrever os aspectos patológicos macroscópicos e microscópicos de enterites e tiflocolites parasitárias fatais causadas por ciatostomíneos, *Eimeria leuckarti*, *Balantidium coli* e *Strongyloides westeri* em equinos; (4) descrever os aspectos epidemiológicos e patológicos de doenças não infecciosas envolvendo o trato gastrointestinal de equinos necropsiados de 2005 a 2017 no Sul do Brasil.

2. ARTIGO 1

Nesse item é apresentado o artigo intitulado:

Lung lesions of slaughtered horses in southern Brazil

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G.M. Snel, David Driemeier and Saulo P. Pavarini

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Lung lesions of slaughtered horses in southern Brazil¹

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Gustavo G.M. Snel², David Driemeier² and Saulo P. Pavarini^{2*}

ABSTRACT. Bianchi M.V., Mello L.S., De Lorenzo C., Lopes B.C., Snel G.G.M., Driemeier D. & Pavarini S.P. 2018. **Lung lesions of slaughtered horses in southern Brazil.** *Pesquisa Veterinária Brasileira* 38(11):2056-2064. Setor de Patologia Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brazil. E-mail: saulo.pavarini@ufrgs.br

Respiratory diseases cause significant veterinary costs, reduce performance and require withdrawal of horses. Yet, studies of the causes of pneumonia in horses are scant. This study aimed to describe the pathological and microbiological features of lung lesions in slaughtered horses in southern Brazil. In this study, 84 samples of lungs were examined, and a conclusive diagnosis was obtained in 74 cases. These were composed of bronchopneumonia in 50 cases, followed by granulomatous eosinophilic pneumonia (9/74), recurrent airway obstruction (7/74), lung fibrosis (4/74), lung hemorrhage (3/74) and pulmonary pythiosis (1/74). Bronchopneumonia had grossly firm focally extensive yellow to dark-red areas, which consisted microscopically of multifocal to coalescing infiltrate of degenerate neutrophils. *Streptococcus equi* subsp. *zooepidemicus* was identified in 21 of the 50 cases. Granulomatous eosinophilic pneumonia had multifocal pinpoint firm-hard yellow areas, which microscopically were composed of granulomas with a mineralized center surrounded by collagen fibers and severe infiltrate of eosinophils. Recurrent airway obstruction had mild multifocal pinpoint firm white areas that consisted microscopically of large amounts of mucus inside bronchi and bronchiole. Lung fibrosis had two patterns: focally extensive areas of consolidation and firm nodular areas. Microscopically, the first pattern had interstitial to peribronchial fibrosis, while the second had, in addition to the interstitial fibrosis, a severe pneumocyte hyperplasia and an alveolar infiltrate of neutrophils and macrophages with rare intranuclear inclusion bodies (equine herpesvirus 5, EHV-5). Pulmonary pythiosis presented a focal firm nodular area, with multiple kunkers observed in the cut surface, which corresponded microscopically to areas of necrosis surrounded by a mixed inflammatory infiltrate. At the periphery of the necrotic areas, multiple negatively stained hyphae were observed, which were evidenced through Grocott's stain and immunohistochemistry anti-*Pythium insidiosum*.

INDEX TERMS: Lung lesions, slaughtered horses, Brazil, equine multinodular pulmonary fibrosis, granulomatous eosinophilic pneumonia, pneumonia, pythiosis, recurrent airway obstruction, *Streptococcus equi*, horses, pathology.

RESUMO. [Lesões pulmonares de equinos de matadouro-frigorífico no Sul do Brasil.] Doenças respiratórias causam em equinos custos significativos com tratamento veterinário, redução de performance e descarte de animais. No entanto, estudos que abordem as causas de pneumonia em

equinos são escassos. O objetivo deste estudo foi descrever os aspectos patológicos e microbiológicos de lesões pulmonares em equinos abatidos em matadouro-frigorífico no Sul do Brasil. Neste estudo, 84 amostras de pulmões foram examinadas, e o diagnóstico conclusivo das condições foi obtido em 74 casos. Esses foram compostos por broncopneumonia em 50 casos, seguido por pneumonia granulomatosa eosinofílica (9/74), obstrução aérea recorrente (7/74), fibrose pulmonar (4/74), hemorragia pulmonar (3/74) e pitiose pulmonar (1/74). A broncopneumonia era caracterizada macroscopicamente por áreas focalmente extensas firmes de coloração amarelada a

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vermelho-escuras, as quais consistiam microscopicamente em infiltrado multifocal a coalescente de neutrófilos degenerados. *Streptococcus equi* subsp. *zooepidemicus* foi identificado em 21 dos 50 casos. A pneumonia eosinofílica granulomatosa era caracterizada por áreas multifocais puntiformes firmes a duras e amareladas, que microscopicamente eram compostas por granulomas com área central mineralizada circundados por fibras de colágeno e infiltrado acentuado de eosinófilos. A obstrução aérea recorrente era caracterizada por discretas áreas puntiformes firmes e brancacentas que consistiam microscopicamente em grande quantidade de muco no interior de brônquios e bronquíolos. A fibrose pulmonar exibia dois padrões: áreas de consolidação focalmente extensas e áreas nodulares firmes. Microscopicamente, o primeiro padrão exibia fibrose intersticial a peribronquial, enquanto no segundo padrão havia, além da fibrose intersticial, intensa hiperplasia de pneumócitos e infiltrado alveolar de neutrófilos e macrófagos com raros corpúsculos de inclusão intranucleares (herpesvírus equino 5, EHV-5). A pitiose pulmonar exibia uma área nodular firme focal com múltiplos kunkers ao corte, os quais correspondiam microscopicamente a áreas de necrose circundadas por infiltrado inflamatório misto. À periferia das áreas necróticas, múltiplas imagens negativas de hifas eram observadas, as quais foram evidenciadas através da coloração de Grocott e imuno-histoquímica anti-*Pythium insidiosum*.

TERMS DE INDEXAÇÃO: Lesões pulmonares, equinos, matadouro-frigorífico, Brasil, fibrose pulmonar multinodular equina, pneumonia eosinofílica granulomatosa, pneumonia, pitiose, obstrução aérea recorrente, *Streptococcus equi*, patologia.

INTRODUCTION

Brazil has a horse population estimated at a total of 5.5 million animals, which encompasses the fourth largest population worldwide after United States, China and Mexico (FAO 2016). Horses in Rio Grande do Sul state compose the second largest population in Brazil, with approximately 537,159 animals (IBGE 2016), and are widely employed for handling cattle herds (Bianchi et al. 2016), for sports (Costa et al. 2014), and occasionally they are slaughtered for meat consumption. Horse meat consumption became popular in Europe after World War II, among people with lower incomes, since cattle beef was scarce (Padalino 2015).

Knowledge of diseases that occur in the equine species is crucial for determining its economic importance as well as the establishment of control procedures. Numerous studies described the causes of death in horses in the United States (USDA 2017), Europe (Baker & Ellis 1981) and Brazil (Pierezan et al. 2009, Pereira et al. 2012, Marcolongo-Pereira et al. 2014). In these studies, the alimentary and locomotor systems were the most commonly affected (Baker & Ellis 1981, Pierezan et al. 2009, Pereira et al. 2012, USDA 2017), while the respiratory system has been indicated as one of the five leading causes of death in horses (Baker & Ellis 1981, Pierezan et al. 2009, Pereira et al. 2012, USDA 2017).

Respiratory diseases cause significant veterinary costs, reduce performance and require the withdrawal of racehorses (Wood et al. 2005, Carvalho et al. 2017). Bacterial pneumonia in foals is mainly caused by *Rhodococcus equi* and *Escherichia coli* (Lakritz et al. 1993), while in adult horses it has been associated

to upper respiratory tract normal flora and opportunist pathogens that reach the lower respiratory tract, such as *Streptococcus equi* subsp. *zooepidemicus* and *S. equi* subsp. *equi* (Carvalho et al. 2017). Still, data for the causes of pneumonia in slaughtered horses have not yet been described.

This study aimed to describe the pathological and microbiological features of lung lesions observed in slaughtered horses in the southern Brazil, and to provide and discuss the differential diagnosis of these conditions.

MATERIALS AND METHODS

From January 2014 to December 2016, lung samples from adult horses (more than 5 years-old) submitted to slaughter were received refrigerated by the pathology laboratory for gross and histopathological analysis. Samples were collected at a slaughterhouse, which is located 350 km distant from the pathology laboratory in the municipality of São Gabriel (30°20'11" S; 54°19'12" W), only when the veterinary inspector of the abattoir had a suspicion of pneumonia due the presence of gross lesions, while respiratory clinical signs were not reported by the veterinary inspector. These samples were then sent refrigerated or frozen to the pathology laboratory, where the lung lesions were grossly described.

Bacterial culture was performed when a bacterial etiology was suspected and it consisted in the inoculation of tissue fragments and purulent contents in Blood agar plates (5% sheep blood, Mueller Hinton, Kasvi®, Brazil) and MacConkey agar (Kasvi®, Brazil) followed by aerobic incubation at 37°C for 72 hours. *Streptococcus equi* subsp. *zooepidemicus*, *Streptococcus equi* subsp. *equi* and *S. equi* subsp. *ruminatorum* were identified by Gram staining, microscopic appearance, and by epidemiology, cultural and biochemical characteristics (Table 1) (Fernández et al. 2004, Markey et al. 2013).

Samples were fixed in 10% neutral buffered formalin for 24-48h, trimmed, processed routinely for histopathology and stained by hematoxylin and eosin (HE). Microscopically, when a granulomatous response was observed, the slides were submitted to the Ziehl-Neelsen (ZN) histochemistry exam, in addition to Grocott's methenamine silver (GMS) stain to evidence fungal structures. Cases of recurrent airway obstruction were submitted to alcian blue stain. In the cases where fibrosis was microscopically observed, tissue sections were submitted to Masson's trichrome stain.

Fungal-suspected cases were submitted to the immunohistochemistry anti-*Pythium insidiosum*, according to a protocol previously described (Konradt et al. 2016) with a polyclonal antibody. The reaction was revealed with 3-amino-9-ethylcarbazole (AEC; Biocare Medical, Pacheco/CA, USA), followed by counterstaining with Harris hematoxylin.

RESULTS

From January 2014 to December 2016, 84 samples of lungs (containing 1 to 2 lung lobules) from slaughtered horses were examined. Horses slaughtered in this slaughterhouse were originated from multiple sites in the state and from the neighboring state of Santa Catarina, with a maximum travel distance of 750km. A conclusive diagnosis was obtained in 74 cases, which were mainly composed of suppurative inflammation involving the bronchi, bronchioles and alveolar spaces (bronchopneumonia; 50/74), followed by granulomatous eosinophilic pneumonia (9/74), recurrent airway obstruction (7/74), lung fibrosis (4/74), lung hemorrhage (3/74) and pulmonary pythiosis (1/74).

Suppurative bronchopneumonia

Grossly, the lungs with suppurative inflammation had firm (36/50) focally extensive to multifocal yellow (24/36) to dark-red (17/36) (Fig.1A,B) areas in addition to purulent

content within the bronchi (22/36), occasionally extending to the parenchyma (7/36). Microscopically, a moderate to intense multifocal to coalescing inflammatory infiltrate of degenerate neutrophils involving the bronchi (36/50),

Table 1. Biochemical characteristics useful in distinguishing *Streptococcus equi* subsp. *equi* from *S. equi* subsp. *zoepidemicus* and *S. equi* subsp. *ruminatorum*

Test	Strain		
	<i>S. equi</i> subsp. <i>equi</i>	<i>S. equi</i> subsp. <i>zoepidemicus</i>	<i>S. equi</i> subsp. <i>ruminatorum</i>
CAMP reaction	-	-	+
Hydrolysis of hippurate	-	-	+
Hydrolysis of esculin	-	-	-
Production of acid from:			
Sorbitol	-	+	-
Trehalose	-	-	-
Lactose	-	+	+
Ribose	-	+	+
Maltose	+	+(-)	+
Sucrose	+	+	-

- Negative, + positive; (-) = a few strains are negative.

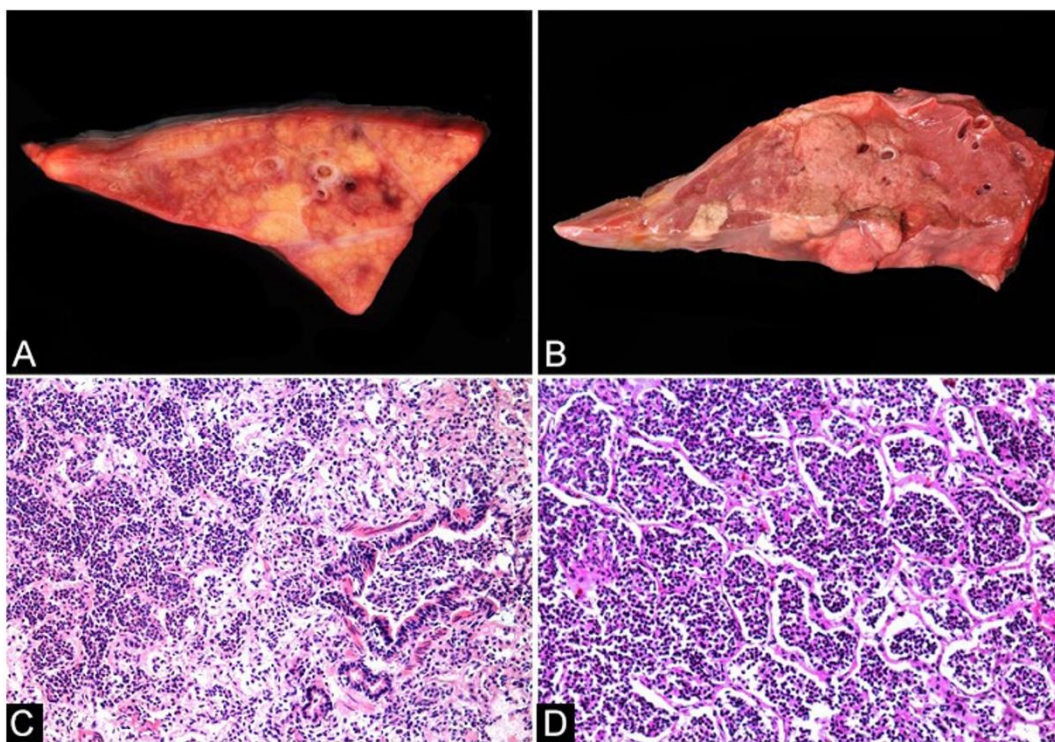


Fig.1. Suppurative inflammation involving the bronchi, bronchioles and alveolar spaces (bronchopneumonia) in slaughtered horses. (A-B) The lungs had focally extensive to multifocal yellow to dark-red areas in the parenchyma. (C) Intense multifocal to coalescent infiltrate of degenerate neutrophils involving the bronchioles and alveolar spaces. HE, obj.10x. (D) Severe infiltrate of neutrophils in the alveolar spaces. HE, obj.20x.

bronchioles (48/50) and alveolar spaces (40/50) characterized these lesions (Fig.1C,D), in addition to occasional coccoid bacterial myriad mainly in the bronchi (11/50) and a severe and diffuse alveolar edema (32/50), which rarely involved the lobular septum (7/50). The suppurative infiltrate was often accompanied by fibrin deposition in the alveolar spaces (32/50), occasionally by interstitial fibrosis (11/50) and rarely by areas of intra-alveolar hemorrhage (3/50).

Lung suppurative processes involved a bacterial cause in 26 cases (26/50). Strains showing beta-hemolytic mucoid colonies (1–3 mm in diameter), with gram-positive spherical or ovoid cocci in pairs or chains, that were catalase-negative, with no growth in 6.5% NaCl were identified as *S. equi*. According to the biochemical characteristics available in Table 1, 21 isolates were identified as *Streptococcus equi* subsp. *zooepidemicus*, followed by *S. equi* subsp. *equi* (2/26), and *S. equi* subsp. *ruminatorum* (1/26). The identification of two strains was not possible through cultural and biochemical methods, and, thus, these were identified as β -hemolytic *Streptococcus* spp.

Granulomatous eosinophilic pneumonia

Granulomatous eosinophilic pneumonia was grossly characterized in all cases by multifocal pinpoint firm to hard yellow areas (0.1–0.3cm in diameter) involving mainly the lung parenchyma and occasionally the pleura (Fig.2A).

Microscopically, these pinpoint areas had a central area of dystrophic mineralization surrounded by abundant collagen fibers, occasional epithelioid macrophages and foreign body-type multinucleated giant cells. At the periphery of these, there was a severe inflammatory infiltrate mainly composed of eosinophils, with a few plasma cells and lymphocytes (Fig.2B). The lumen of bronchi and bronchioli was filled by an amorphous amphophilic material intermixed by an intense inflammatory infiltrate of eosinophils. At the ZN and GMS, neither acid-fast bacilli nor fungi were identified. No bacterial agent was obtained through aerobic culture.

Recurrent airway obstruction

Recurrent airway obstruction (RAO) was characterized grossly by non-collapsed areas in the lung, which on the cut surface showed multifocal discrete pinpoint firm white areas with accumulation of mucopurulent content inside bronchi (7/74). Histologically, bronchi and bronchioles contained in the lumen large amounts of an amphophilic amorphous material (mucus) intermixed by a mild inflammatory infiltrate of eosinophils and the mucosa was often hyperplastic with large numbers of goblet cells (metaplasia) (Fig.2C). The smooth muscle of these bronchi and bronchioles was variably hypertrophic. Alcian blue histochemistry exam evidenced the mucus in the

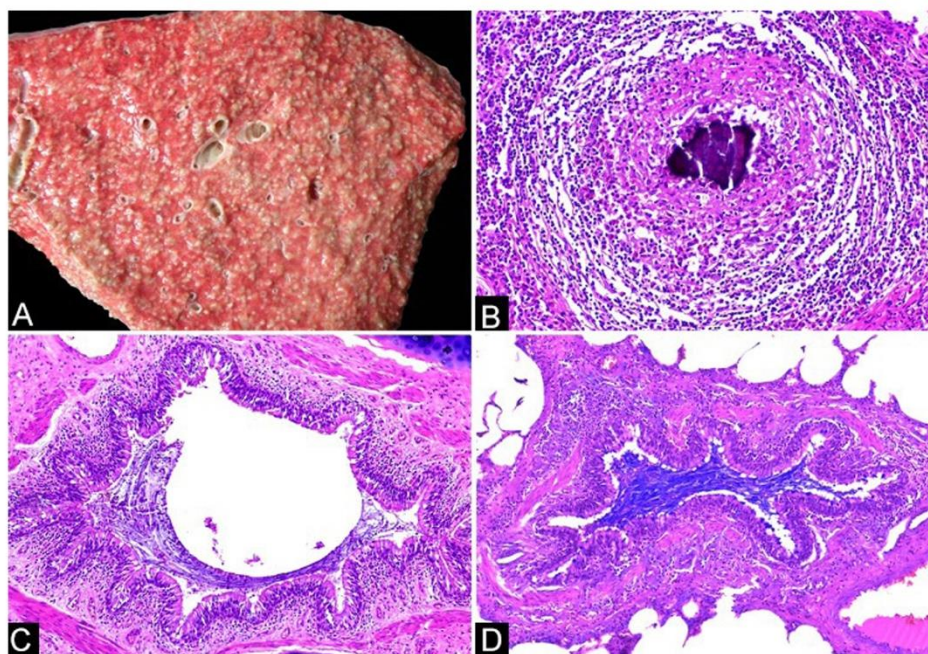


Fig.2. (A-B) Granulomatous eosinophilic pneumonia and (C-D) recurrent airway obstruction in slaughtered horses. (A) Multifocal pinpoint yellow areas (0.1–0.3cm in diameter) involving the lung parenchyma. (B) Microscopically these had a central area of mineralization surrounded by collagen fibers and occasional epithelioid macrophages, and at the periphery large numbers of eosinophils. HE, obj.20x. (C) Mucus filled the lumen of bronchi and the mucosa was hyperplastic. HE, obj.10x. (D) Mucus in the lumen and increased numbers of goblet cells in the mucosa of this bronchiole were evidenced. Alcian blue stain, obj.10x.

lumen of the bronchi and bronchioli, in addition to the large numbers of Goblet cells in the mucosa (Fig.2D).

Lung fibrosis

Lung fibrosis occurred in two distinct gross and microscopic patterns. The first one involved two cases and was grossly characterized by focally extensive whitish poorly limited areas of lung consolidation. Microscopically, these cases had interstitial to peribronchial fibrosis, in addition to moderate infiltrate of macrophages containing a brown granular pigment in the cytoplasm (hemosiderosis) in the alveolar septum. The second pattern occurred in two cases and was grossly characterized by a firm and whitish nodular area in each case, which measured 8x5x4cm and 4x3x2cm, and, on the cut surface, it was outlined by a red halo from the remaining of the lung parenchyma in one of the cases (Fig.3A). Microscopically, both cases had moderate to severe interstitial fibrosis and a severe type II pneumocyte hyperplasia in the alveolar spaces, which was associated to a moderate inflammatory infiltrate of degenerate neutrophils, macrophages and rare multinucleated giant cells. Multiple basophilic and round intranuclear inclusion bodies were observed in the macrophages in one of these cases (Fig.3B) and, thus, this was compatible with equine multinodular pulmonary fibrosis (EMPF) caused by equine

herpesvirus 5 (EHV-5). Masson's trichrome stain evidenced the interstitial fibrosis in all cases.

Lung hemorrhage

Grossly, lungs with hemorrhage were reddened on gross examination, while microscopically there was a moderate to severe diffuse hemorrhage in the alveolar spaces (3/3), occasionally associated to a multifocal infiltrate of macrophages containing granular brownish pigment (hemosiderosis) in the alveolar septum (1/3).

Pulmonary pythiosis

Pulmonary pythiosis corresponded to a focal nodular and firm area in the lung parenchyma, measuring 11x9x5cm. On the cut surface, this nodular area had multifocal to coalescing yellow, irregular, firm, but friable masses (compatible with "kunkers"), which were surrounded by a moderate red halo and outlined by a thick white tissue (capsule) (Fig.3C). Microscopically, these consisted of multifocal to coalescing nodular structures which contained a central amorphous eosinophilic necrotic material surrounded by a marked inflammatory infiltrate of neutrophils, eosinophils, epithelioid macrophages and Langhans-type multinucleated giant cells, in addition to severe fibrous tissue proliferation. At the periphery of the necrotic

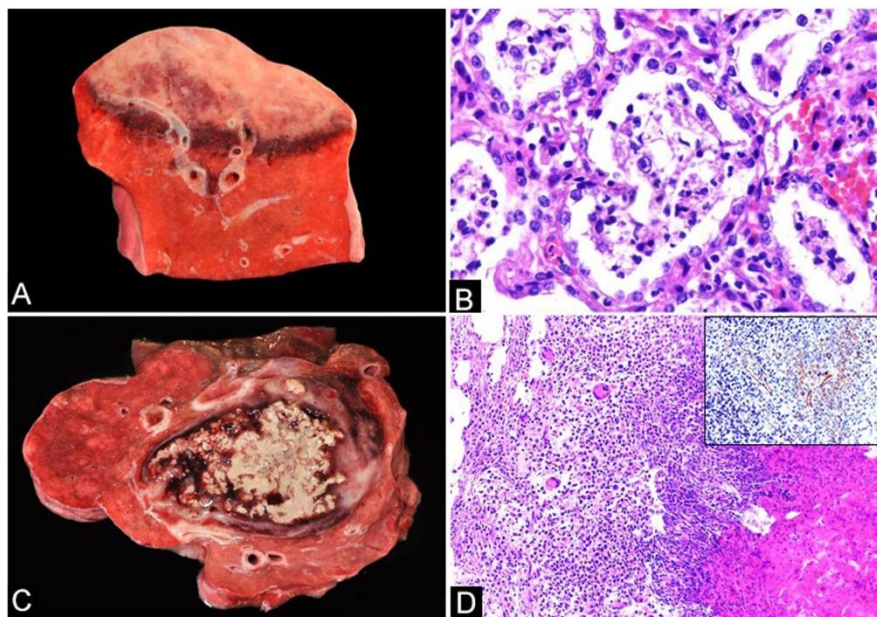


Fig.3. (A-B) Equine multinodular pulmonary fibrosis (EMPF) and (C-D) pulmonary pythiosis. (A) Focally extensive whitish nodule outlined by a red halo in the lung parenchyma. (B) Alveolar spaces had severe pneumocyte hyperplasia associated to infiltrate of degenerate neutrophils and macrophages, often containing intranuclear inclusion bodies (suggestive of EHV-5; arrow). HE, obj.40x. (C) Pythiosis corresponded to a focal nodule that on the cut surface had multifocal to coalescing "kunkers". (D) Microscopically, these had a central eosinophilic necrosis surrounded by a marked inflammatory infiltrate of neutrophils, eosinophils, epithelioid macrophages and Langhans-type multinucleated giant cells. Negatively stained sections of hyphae were observed at the periphery of the necrotic areas. HE, obj.10x. Inset: marked immunostaining of hyphae within the "kunkers". Immunohistochemistry anti-*Pythium insidiosum*. AEC, obj.40x.

areas, multiple negatively stained transverse and longitudinal sections of hyphae were observed (Fig.3D). Sections of lung stained by GMS evidenced abundant branching, rarely septate and 5-µm-in-diameter irregular hyphae, which were also stained by IHC for *P. insidiosum* (Fig.3D inset).

DISCUSSION

The diagnosis in all cases was obtained by the association of the gross, microscopical, immunohistochemical, histochemical and bacteriological features. Our findings indicated that suppurative bronchopneumonia was the main lung lesion in slaughtered horses. Primary pneumonia in adult horses may happen due to stressful events, such as transportation and long distance travel (Mair & Lane 1989), as we have observed in this study. Stress may predispose lower respiratory tract infection in horses by impairing the pulmonary defense mechanisms (Bayly et al. 1986), and, thereby, it facilitates colonization by *Streptococcus equi* subsp. *zooepidemicus*, which is considered an upper respiratory tract commensal bacterium. Still, it is one of the most frequently isolated bacterial pathogen of young (Lakritz et al. 1993) and adult horses (Bailey & Love 1991). Since horses of the present study were transported for long distances with variable duration of trips, we speculated that these were the main predisposing factors for the lesions observed. *S. equi* subsp. *zooepidemicus* was identified in 21 cases, followed by *S. equi* subsp. *equi* in two cases. When these pathogens are compared, *S. equi* subsp. *zooepidemicus* has a broader tissue tropism (Carvalho et al. 2017), being able to infect, in addition to the lungs, other reproductive organs (Erol et al. 2012). On the other hand, *S. equi* subsp. *equi*, the etiologic agent of strangles, predominantly involves the upper airways and associated lymph nodes with abscess formation, but spread to other organs may occur in "bastard strangles" through blood, lymphatic or aspiration of pus into the lungs (Timoney 2004, Caswell & Williams 2016), with the development of necrotizing pneumonia. In this study, however, both *S. equi* subsp. *zooepidemicus* and *S. equi* subsp. *equi* most likely spread through the airway system, affecting the lower respiratory tract with a suppurative bronchopneumonia. This lesion distribution is consistent with inhalation of aerolised bacteria and has been referred as acentric-acinar, corresponding, grossly, to well-defined areas of dark red consolidation (Oikawa et al. 1995, Racklyeft & Love 2000). Still, further evaluation of the pathogenesis of the condition was compromised by the lack of all lung lobes in the samples analyzed. A bacterial agent was not obtained in 24 cases, yet the gross and microscopical lesions were characteristic of a bacterial cause. However, culture may be unsuccessful during the incubation and early clinical phases of the condition (Timoney 2004).

Fibrosing granulomas involving the lungs were initially described as primary lesions of the liver and peritoneum of horses, and were grossly characterized by numerous firm and gritty nodules (Buergelt & Greiner 1995), similarly to the ones observed in the present study. Likewise, similar gross lesions in the lungs without systemic involvement have been described in horses, being referred as idiopathic eosinophilic pneumonia or disseminated eosinophilic pulmonary granulomas (Uhlhorn et al. 2006, Bell et al. 2008, Magi et al. 2016). Since lesions observed in the present study were both gross and microscopically similar to these studies,

the condition was referred as granulomatous eosinophilic pneumonia, which was the second most common condition involving the lungs of slaughtered horses. Microscopically, the granulomas of the present study had large amounts of dense fibrous tissue, epithelioid macrophages and multinucleated giant cells surrounding a necrotic or mineralized center, and at the periphery there was a severe inflammatory infiltrate of eosinophils, corroborating with previous findings (Uhlhorn et al. 2006), but it differed from another description of associated vascular lesions (Magi et al. 2016).

After extensive search for parasitary structures in the lesions, these were absent. Previous studies have also failed to identify the cause of these lesions in the lungs (Buergelt & Greiner 1995, Uhlhorn et al. 2006, Magi et al. 2016). Yet, we believe that these were initially caused by a parasite migration, considering that while the chronic reaction progresses, the dead eggs or larvae may disappear through degeneration and calcification in these lesions (Buergelt & Greiner 1995). Migrating helminths parasites, as *Parascaris equorum* or *Dictyocaulus arnfieldi*, are possible causes of these lesions (Nicholls et al. 1978, Mackay & Urquhart 1979). Yet, the larvae of *P. equorum* usually undergo pulmonary migration when horses are young (Bell et al. 2008), whilst the horses of the present study were all adults, and the lesions are usually transitory (Nicholls et al. 1978). On the other hand, *Dictyocaulus arnfieldi* causes eosinophilic bronchitis in adult horses (Mackay & Urquhart 1979), which was not observed in the present study. *Heterobilharzia americana*, a schistosome that causes canine schistosomiasis in the USA, is able to cause a similar granulomatous and eosinophilic pneumonia in llamas and horses, but is mostly an hepatic disease (Buergelt & Greiner 1995, Corapi et al. 2012, 2015). Still, this parasite has not been reported in Brazil, neither have the characteristic eggs and empty eggshells been observed in the center of the granulomas in the present study.

Granulomatous and eosinophilic lesions in the lungs of horses should be differentiated mainly from *Mycobacterium* spp. infections, fungi infections (Buergelt & Greiner 1995), multisystemic eosinophilic epitheliotropic disease (MEED), eosinophilic bronchiolitis and parasitic bronchopneumonia (Bell et al. 2008, Magi et al. 2016). *Mycobacterium* spp. and fungi infections were ruled out in these cases based on the absence of staining through ZN and GMS, as reported previously (Buergelt & Greiner 1995, Uhlhorn et al. 2006, Magi et al. 2016), as well as by the marked eosinophilic infiltrate, which are not typical of these conditions (Uhlhorn et al. 2006). MEED typically affects young horses (Uhlhorn et al. 2006), while the horses of the present study were all adults, and significant involvement of the respiratory tract is uncommon (Pucheu-Haston & Del Piero 2013).

RAO or "heaves" is an inflammatory airway disease of mature horses (Caswell & Williams 2016), formerly known as chronic obstructive pulmonary disease (Buergelt & Del Piero 2014). Similarly to the observed in the present study, the most characteristic gross lesions of this condition in horses are the lack of pulmonary collapse (Buergelt & Del Piero 2014) and the accumulation of mucopurulent secretions throughout the tracheobronchial tree (Pirie 2014). Lesions are usually more severe in the caudodorsal lung (Caswell & Williams 2016); however, since only fragments of lung lobes were evaluated in this study, this focal involvement was not analyzed, as well as

the clinical importance of this lesion may not be extrapolated from these cases. Microscopically, this condition presents the accumulation of mucus and neutrophils in bronchiolar lumens and hyperplasia of goblet cells (Caswell & Williams 2016), which were observed in all horses, in addition to a variable peribronchial and peribronchioli smooth muscle hypertrophy (Caswell & Williams 2016), which may explain the gross pinpoint white firm areas, as previously described (Buergelt & Del Piero 2014).

Interstitial lung fibrosis is a proliferative response to the damage of the alveolar walls and loss of functional alveolar-capillary in horses. A specific cause for this condition may not be recognized (Buergelt et al. 1986), as in three of the four cases in this study. Interstitial lung fibrosis was identified in six horses in a retrospective study in Brazil (Estima-Silva et al. 2017), which were microscopically similar to all the four cases described in the present study. In older horses, this condition may result in interalveolar septal thickening by fibrosis, in addition to fibrosis in the visceral pleura (Buergelt et al. 1986). This pattern of lesion was observed in two of the horses of the present study, while in the other two cases we observed a preservation of the "alveolar-like" architecture with the lumen of the alveoli delineated by type II pneumocyte hyperplasia, which have been described as characteristic of EMPF (Williams et al. 2007). This is a chronic infectious disease caused by EHV-5 in adult horses, which forms numerous coalescing nodules in the lungs (Williams et al. 2007). This condition has been previously described in Brazil in two horses (Panziera et al. 2014, Estima-Silva et al. 2017), and in this study a suggestive diagnosis of EMPF by EHV-5 in one of the cases was obtained by the association of the gross and the microscopic features. Similarly to the previously described changes in a race horse, the multiple intranuclear inclusion bodies associated to the interstitial fibrosis are characteristic of this condition (Williams et al. 2007, Carvallo et al. 2017). The histological features of EMPF are distinct from other causes of fibrosis (Williams et al. 2007), as it was observed in the remaining two cases of interstitial fibrosis, in which a specific cause could not be determined.

Pythiosis is a tropical to subtropical disease caused by *Pythium insidiosum* that mostly affects horses (Mendoza et al. 1996), and lesions are usually cutaneous to subcutaneous (Souto et al. 2016). This condition is considered endemic in the Brazilian Pantanal (Leal et al. 2001) and in the Rio Grande do Sul state (Weiblen et al. 2016). Pulmonary pythiosis has been described in a *Panthera onca* (Camus et al. 2004) and in horses (Goad 1984, Reis Junior et al. 2003, Souto et al. 2016), being mainly associated to the inhalation of the agent in water and/or ingestion with subsequent aspiration of infective material (Goad 1984). It is possible that the lesion observed was associated to a nasal infection, since it has been reported previously in horses (Reis Junior et al. 2003, Vaz et al. 2009, Galiza et al. 2014, Souto et al. 2016), but grossly the origin of this lesion was not determined. In addition to that, some authors have described previous cutaneous lesions that would allow the hematogenous dissemination of the pathogen to internal organs, such as the lungs, through detached hyphal segments of *P. insidiosum* (Reis Junior et al. 2003). This form of dissemination would lead to a multifocal distribution of these lesions (Goad 1984, Reis Junior et al. 2003), and seems

less likely considering that the focal nodular was isolated from the remaining parenchyma. Sequestra in the lungs, which commonly develops with masses of necrotic lung parenchyma (Caswell & Williams 2016), has been described in a case of lung pythiosis in a horse (Goad 1984), similarly to the present case, suggesting that the response of the organism may be effective in isolating the lesion. On the other hand, the presence of "kunkers" seems to be a constant finding in horses both in cutaneous, nasal, alimentary and pulmonary lesions (Goad 1984, Reis Junior et al. 2003, Martins et al. 2012, Souto et al. 2016). Microscopical lesions were similar to those observed in nasal and lung lesions (Goad 1984, Souto et al. 2016), but distinct from cutaneous or subcutaneous lesions, in which an eosinophilic dermatitis associated with the formation of granulation tissue predominates (Martins et al. 2012). The gross and microscopic lesions, in addition to the immunohistochemical features observed, were characteristic of the condition, and allowed us to differentiate it from *Conidiobolus* spp. and *Basidiobolus* spp. infections, as previously described (Reis Junior et al. 2003).

CONCLUSIONS

Bacterial bronchopneumonia caused by *Streptococcus equi* subsp. *zooepidemicus* is the main cause of lung lesions in slaughtered horses.

No etiological agent was detected in granulomatous eosinophilic pneumonia cases, although microscopically these were characteristic of parasite induced lesions with a mineralized center surrounded by collagen and large numbers of eosinophils at the periphery.

Recurrent airway obstruction had mild gross lesions, while microscopically it presented an accumulation of mucus and eosinophils in the airways associated to hyperplasia of the epithelium and goblet cells.

Lung interstitial fibrosis was mostly of unknown cause, while in one of the cases the lesion was characteristic of EMPF caused by EHV-5.

Lung pythiosis occurred in one horse and shared similar gross and microscopic features to cutaneous, alimentary and nasal pythiosis. Immunohistochemistry is essential for pythiosis diagnosis in the absence of culture.

Conflict of interest statement. - The authors have no competing interests.

Animal welfare/ethical statement. - We authors of the article entitled "Lung lesions of slaughtered horses in Southern Brazil" declared, for all due purposes, the project that gave rise to the present data of this manuscript has been submitted for evaluation to the Ethics Committee of the Universidade Federal do Rio Grande do Sul (UFRGS), and has been approved under the number 33501 at December 7th, 2017.

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3. ARTIGO 2

Nesse item é apresentado o artigo intitulado:

Etiological and pathological aspects of pneumonia and pleuritis in 50 horses

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Title page

Etiological and pathological aspects of pneumonia and pleuritis in 50 horses

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Abstract

Respiratory diseases are the fourth to fifth most common causes of death in horses, but the pathological aspects of these conditions are yet insufficiently described. This study described the gross, microscopic and etiologic findings related to pneumonias and pleuritis in 50 horses necropsied between 2005 and 2017 in Southern Brazil. Suppurative pneumonia was the main condition diagnosed (17/50), followed by pyogranulomatous pneumonia (14/50), aspiration pneumonia (5/50), mycotic pneumonia (4/50), bronchointerstitial pneumonia (3/50), pleuritis (3/50), and embolic pneumonia (3/50). Suppurative pneumonias were further divided into acute (8/17), subacute (6/17), and chronic (3/17) based on the morphological pattern of lesions. *Streptococcus* sp. was identified through bacterial culture or immunohistochemistry in eleven cases. Pyogranulomatous pneumonias were caused by *Rhodococcus equi*, and occurred mainly in young foals, with multifocal to coalescent cranioventral yellow nodules, which consisted microscopically of areas with caseous necrosis intermixed by a pyogranulomatous inflammatory infiltrate. Aspiration pneumonia occurred mainly in hospitalized horses, with cranioventral purple-red focally extensive unilateral or bilateral consolidated areas within the lungs, but foreign materials were rarely observed grossly. Microscopically, these were identified in all cases within the bronchi and the bronchioli, often extending into the adjacent alveolar spaces. Mycotic pneumonia predisposing factors included prolonged antimicrobial therapy and comorbidities (salmonellosis). Lesions were grossly characterized by randomly distributed nodular soft areas, which consisted microscopically of fibrinonecrotic lesions. *Aspergillus* spp. was identified as the cause based on the morphological features and immunohistochemistry. Bronchointerstitial pneumonia was caused by Influenza A, and occurred only in young foals. Grossly, the lungs were diffusely enlarged and with ribs impressions, while microscopically all cases had multifocal to diffuse fibrinous alveolitis and type II pneumocyte hyperplasia. Pleuritis occurred in adult horses, with long-distance transportation and hospitalization identified as predisposing factors. Grossly, lesions were restricted to the pleura, with superficial fibrin exudate intermixed by an inflammatory infiltrate of neutrophils, and supported by a marked fibrovascular tissue proliferation. Embolic pneumonias occurred in association with primary distant lesions in adult horses. Grossly, there were randomly distributed firm red to dark-red elevated areas within the lungs, while microscopically necrosuppurative

pneumonia was associated to thrombosis and large amounts of bacterial structures. Pneumonias are important causes of death in horses in Southern Brazil. Epidemiological, pathological, bacteriological and immunohistochemical features are essential to obtain a final diagnosis.

Keywords: suppurative, *Streptococcus* sp., *Rhodococcus equi*, aspiration, *Aspergillus* spp., Influenza A, embolic

Introduction

Respiratory diseases are the most common cause of morbidity and mortality in foals up to 12 months old (Cohen, 1994; Galvin and Corley, 2009), while in a general horse population these are the fourth to fifth most common cause of death according to retrospective studies (Baker & Ellis, 1981; Pereira et al., 2012; Pierezan et al., 2009). Still, the pathological findings of pneumonia in horses are insufficiently described (Pierezan et al., 2009), limited to sudden death unspecific lung lesions (Lyle et al., 2011) or restricted to the pathological findings for specific categories of horses, such as racehorses (Carvallo et al., 2017), slaughtered horses (Bianchi et al., 2018), young (Lakritz et al., 1993) and adult horses (Mair & Lane, 1989). Nevertheless, no previous study has investigated the pathological and microbiological findings of pneumonia in a broad population of horses, involving all age ranges.

The aim of this study was to describe the gross, microscopic, and etiologic findings related to naturally occurring pneumonia and pleuritis in 50 horses in Southern Brazil.

Materials and methods

A retrospective study of the necropsy database from the veterinary pathology laboratory was conducted from January 2005 to December 2017 in search of pneumonias affecting horses. The general population of horses and horses affected by pneumonias were classified with regards to the age range into five categories: younger than one-year-old (foals), between one-year-old and five-years-old (young adults), between five-years-old and ten-years-old (adults), older than ten-years-old (senile), and information not provided. Information regarding age, breed, clinical course, predisposing factors, and comorbidities were retrieved from the necropsy protocols. Gross lesions were reevaluated through photographs and later combined with the previous descriptions available on the necropsy

reports. Histological slides of the selected cases were promptly analyzed when available, or paraffin-embedded tissues were routinely processed for histological exams and stained by hematoxylin and eosin (H&E). Grocott's methenamine silver stain (GMS) was employed when a fungal etiology was suspected. The cases were then grouped into seven main categories: suppurative pneumonia, pyogranulomatous pneumonia, aspiration pneumonia, mycotic pneumonia, bronchointerstitial pneumonia, pleuritis, and embolic pneumonia. Cases that did not fit in any of the above categories were listed as others. Suppurative pneumonia cases had distinct gross and microscopical features which were mainly associated to the clinical course, and, thus, were classified into acute, subacute, and chronic, as described previously in other species (Dagleish et al., 2010; Hansen et al., 2010; Caswell & Williams, 2016).

Bacterial culture was carried out when a bacterial etiology was suspected, consisting in the inoculation of lung fragments and purulent contents in Blood agar plates (5% sheep blood, Mueller Hinton, Kasvi®, Brazil) and MacConkey agar (Kasvi®, Brazil) followed by aerobic incubation at 37°C for 72 h. *Rhodococcus equi*, *Streptococcus equi* subsp. *zooepidemicus*, *S. equi* subsp. *equi*, *S. pneumoniae*, *Escherichia coli*, and *Chromobacterium violaceum* were identified by Gram staining, microscopic appearance, and by epidemiology, cultural and biochemical characteristics (Table 1) (Fernández et al. 2004, Markey et al. 2013)

Immunohistochemistry (IHC) anti-*E. coli* and anti-*Streptococcus equi* (polyclonal) were employed, according to the protocol in Table 1, when bacterial culture was not available or when bacterial culture yielded no growth. The sections were counterstained with Harris hematoxylin. Two positive controls from lung lesions with a pure culture of *S. equi* subsp. *equi* or *S. equi* subsp. *zooepidemicus* were employed at *S. equi* IHC, and a case of colibacillosis in a pig was used as the positive control for the IHC anti-*E. coli*, as described (De Lorenzo et al., 2018).

IHC anti-*Aspergillus* spp. and anti-*Rhizopus arrhizus* were performed when fungal structures were evidenced through GMS. The IHC protocol employed is also available in Table 1. The sections were counterstained with Harris hematoxylin. Positive controls consisted of histological lesions with a previous fungal isolate of *Aspergillus* spp. and *Rhizopus arrhizus*, respectively.

Paraffin-embedded lung tissues were subjected to RNA extraction according to a previously described protocol established by CDC (Centers for Disease Control and Prevention) for *Influenza*. RNA obtained was submitted to a real-time PCR (RT-PCR), employing the following primers forward (5'-GACCRATCCTGTACCTCTGAC-3') reverse (5'-AGGGCATTYTGACAAAKCGTCTA-3') and TaqMan probe (5'-FAM-TGCAGTCCTCGCTCACTGGGCACG-BHQ1-3') for *Influenza A*. The RT-PCR was performed in one step: an initial transcriptase reverse phase at 50 °C for 30 min, followed of 40 cycles at 95 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s, and 7 min of final extension at 72 °C.

Results

From January 2005 to December 2017, 654 horses were necropsied in the veterinary pathology laboratory. Of these, 121 horses were foals (18.5%), 109 were young adults (16.7%), 156 were adults (23.9%), 122 were senile horses (18.6%), and this information was not provided in 146 horses (22.3%). A total of 50 cases had a diagnosis of pneumonia and pleuritis as the cause of death (7.64%; 50/654). The main cause for these were suppurative pneumonia (17/50), pyogranulomatous pneumonia (14/50), aspiration pneumonia (5/50), mycotic pneumonia (4/50), bronchointerstitial pneumonia (3/50), pleuritis (3/50), and embolic pneumonia (3/50). Other causes included one case of eosinophilic granulomatous pneumonia. With regards to the age range, 31 of these horses were foals (62%), nine were young adults (18%), four were adults (8%), and six were senile horses (12%).

Suppurative pneumonia accounted for 34% of the cases (17/50), of which eight were acute, six were subacute, and three were chronic. Horses had an age range of one-day-old to 19 years-old, and a median of seven months-old. Regarding the breeds, mixed breeds were the most affected (6/17), followed by Criollo (4/17), Thoroughbred (4/17), Brazilian Sport Horse (1/17), Lusitano (1/17) and Quarter horse (1/17) breeds. The age, clinical course, predisposing factors, bacterial isolates, immunohistochemistry findings and the morphological pattern of the lesions are described in Table 2.

Acute cases (8/17) had a shorter clinical course (one to three days), with poor colostrum intake identified as the most common predisposing factor (4/8). Most of the horses (7/8) were neonates to young foals (less than seven months old). Grossly, two patterns of lung lesions were observed. The

first pattern was characterized by cranioventral to diffuse dark-red firm areas (3/8) with the thoracic cavity filled by moderate amounts of dark red serous liquid in two of the cases (Figure 1A). The second pattern had focally extensive to multifocal cranioventral purple-red areas of consolidation (5/8), with a mild amount of a reddish serous liquid in the thoracic cavity. On the cut surface, both had multifocal to coalescent mild areas of lung parenchyma consolidation (Figure 1B). Microscopically, the first pattern had mostly necrotic lesions, characterized by a severe hemorrhage, edema, fibrin exudation, vascular fibrinoid necrosis and thrombosis in association to neutrophilic infiltrate, and a myriad of bacterial clusters mainly involving the alveolar spaces (Figure 2A). The second pattern had microscopical airway centered neutrophilic inflammatory infiltrate extending to the alveolar spaces. The alveolar spaces were also filled by moderate to severe edema and occasionally had multifocal fibrin exudate associated to mild moderate amounts of basophilic bacterial clusters (Figure 2B). *S. pneumoniae*, *Klebsiella pneumoniae*, and *E. coli* were cultured in one case each, while in other four cases a positive stain for *S. equi* was obtained through IHC. In two cases, the etiology could not be determined.

Subacute cases (6/17) had a clinical course of two to seven days, with multiple predisposing factors, but most of them were related to previous airway diseases (strangles, guttural pouch empyema, recurrent airway obstruction and strangles), and most of these horses (4/6) were adults older than three years-old. Grossly, the lungs had focally extensive cranioventral purple-red consolidated areas intermixed by pinpoint (2/6) to nodular (4/6) soft yellowish lesions (abscesses; Figure 1C). On the cut surface, multifocal to coalescent irregular to nodular demarcated areas with a friable center were observed in all cases (Figure 1D). Microscopically, all cases had in the lung parenchyma multifocal to coalescent areas of marked necrosis intermixed by large amounts of bacterial colonies, which were outlined by a rim of neutrophilic inflammatory infiltrate (Figure 2C). In addition to that, all cases had an airway centered neutrophilic inflammation, alveolar edema and fibrin exudate, and most of the cases (5/6) had moderate to severe fibrin thrombi within the lung parenchyma (Figure 2D). A bacterial etiology was identified in five cases, as described in Table 2, and in the remaining case a positive immunostaining for *S. equi* was observed (Figure 2F).

Chronic cases (3/17) had a longer clinical course of six to 15 days, only adult horses over 2.5 years-old were affected, and predisposing factors included hospitalized animals (2/3) and previous strenuous exercise (1/3). Grossly, all cases had moderate to severe unilateral pleural fibrinous thickening, in addition to red to dark-red cranioventral consolidated areas and large amounts of dark-red to brown turbid liquid (Figure 1E). On the cut surface, there were extensive friable devitalized greenish to brownish areas within the lung parenchyma, which were demarcated by a thick whitish tissue (compatible with sequestration of necrotic tissue; Figure 1F). Microscopically, the pleura was effaced by a severe fibrinous and suppurative inflammatory infiltrate intermixed by a myriad of bacterial clusters, in addition to moderate to marked fibrovascular tissue proliferation, and moderate neutrophilic inflammation within the alveolar spaces (Figure 2E). The areas of devitalized tissue (necrosis) were surrounded by a rim of macrophages infiltrate and fibroblastic proliferation. Thrombosis and fibrinoid degeneration of blood vessels were also constant findings. In addition, two cases had severe atelectasis. Bacterial isolates were obtained in all cases, with the identification of *S. pneumoniae*, *S. equi* subsp. *zooepidemicus* and *Enterococcus* sp.

Pyogranulomatous pneumonia accounted for 28% of the cases (14/50), and *R. equi* was isolated through bacterial culture in all cases. Age ranged from one to three months-old, with a median of 2 months-old. Regarding the breeds, Holsteiner was the most affected (11/14), followed by Quarter horse, Brazilian Sport Horse, and Mangalarga Marchador breed with one case each (1/14). All Holsteiner foals were from the same farm. Information regarding clinical course was available in ten cases, varying from sudden death to 30 days of clinical evolution, and respiratory clinical signs were reported in seven cases. Grossly, the lungs had multifocal to coalescent predominantly yellow, but occasionally red, cranioventral nodules measuring from 1 to 10 cm in diameter (Figure 3A). On the cut surface, these nodules extended into the parenchyma, often exuded yellowish liquid (7/14) to friable (6/14) contents, and rarely were solid (1/14) (Figure 3B). Besides these lesions, other organs affected included: lymph nodes in nine cases, caecum/large colon in seven cases, joints in seven cases, eyes in two cases, and bones in two cases. Microscopically, within the alveolar spaces there were multifocal to coalescent poorly limited nodular areas with caseous necrosis intermixed by an inflammatory infiltrate of degenerate neutrophils, foamy macrophages, and few Langhans and foreign-

type multinucleate giant cells. Occasionally, within the cytoplasm of these cells there was a myriad of basophilic coccoid bacteria, and rare multinucleate giant cells contained neutrophils within the cytoplasm (Figure 3C-D). In one of the cases, the alveolar septum had a severe and diffuse mineralization.

Aspiration pneumonia accounted for 10% of the cases (5/50), and predisposing factors included hospitalized horses (3/5), cleft palate (1/5) and unknown history (1/5). Age of affected horses ranged from two-days-old to eight years-old, with a median of 24 months-old and a mean age of 42 months-old. Most of the horses were mixed breed (4/5), followed by Argentino breed. Grossly, lesions were unilateral (2/5), involving only the apical right lobe, or bilateral (3/5), involving both apical lobes. In both cases, the lung had cranioventral purple-red focally extensive consolidated areas (Figure 4A). Occasionally, there were also nodular whitish areas (2/5) and focally extensive greenish areas (1/5). Alimentary vegetal materials were observed within the trachea and bronchi only in one case. Microscopically, all cases had foreign materials (alimentary content primarily within the bronchi and the bronchioli, but often extending into the adjacent alveolar spaces. These were surrounded by an inflammatory infiltrate of degenerate neutrophils intermixed by fibrin and a myriad of bacterial aggregates. A moderate alveolar edema was also observed (Figure 4B). In two cases, foreign type multinucleate giant cells were observed around the foreign materials.

Mycotic pneumonia accounted for 8% of the cases (4/50), with a clinical course of 9 to 20 days, and predisposing factors included prolonged antimicrobial therapy (3/4), comorbidities (salmonellosis in three cases), and hospitalized horses (1/4). Most of the horses were younger than four months-old (3/4), and the other was a 13 years-old mare. Affected breeds included Mangalarga Marchador (2/4), Criollo and Brazilian Sport Horse with one case each. Grossly, there were multifocal randomly distributed nodular soft areas ranging from 0.5 to 5 cm in diameter that varied in color (yellow, white, dark-red to black; Figure 4C). Two patterns of gross lesions were observed: predominantly subpleural (2/4) and distributed throughout the parenchyma (2/4). Microscopically, the lesions had a fibrinonecrotic pattern, with multifocal areas of coagulative necrosis within the alveolar spaces, which were associated to numerous fungal structures, inflammatory infiltrate of neutrophils, fibrin exudate, thrombosis and, occasionally, fibrinoid degeneration of blood vessels. These lesions

often extended into the pleura in focally extensive areas (2/4) and into the bronchioli causing necrosis of the epithelium (2/4) (Figure 4D). GMS stain evidenced these fungal structures, which were narrow (4-6 μm width), septate, had dichotomous branching oriented in the same direction and occasional vesicular swellings (Figure 4E). A positive immunostaining for *Aspergillus* spp. was obtained in all cases (Figure 4F), while these were negative for *R. arrhizus*. Similar lesions were not observed in other organs.

Bronchointerstitial pneumonia occurred in 6% of the cases (3/50), and in all cases Influenza A was identified as the cause through RT-PCR. All horses were younger than 2 months-old, with a median of 1 month-old and a mean age of 1.17 months-old. Breeds affected included Criollo (2/3) and Mangalarga breeds. Grossly, the lungs were diffusely enlarged, reddish to whitish, not collapsed and with ribs impressions (Figure 5A). On the cut surface, these lungs had a meaty appearance (Figure 5B). Another gross finding included multifocal areas of cutaneous alopecia in all horses. Microscopically, all cases had multifocal to diffuse necrosis of the alveolar surfaces associated to moderate fibrin exudate (alveolitis), severe type II pneumocyte hyperplasia, and occasional mineralization of the alveolar septum (Figures 5C-D). Two cases also had necrosis of the bronchioli epithelium.

Pleuritis cases occurred in 6% of the cases (3/50), only in adult mixed breed horses (median age of five years-old and mean age of 6.6 years-old) in a clinical course of 10 to 13 days, and predisposing factors included long-distance transportation (1/3) and hospitalized horses (1/3). Two of these horses were submitted to antimicrobial therapy. Grossly, lesions were restricted to the pleura, which was diffusely and bilaterally expanded by large amounts of fibrin intermixed by purulent content (Figure 6A). The thoracic cavity also had large volumes of turbid brown-yellow fluid and multifocal areas of pleural adhesions. Additionally, there was lung atelectasis in two cases. Microscopically, there was a severe and diffuse pleural thickening, characterized by a marked fibrovascular tissue proliferation covered by a severe superficial fibrin exudate and inflammatory infiltrate of neutrophils intermixed by rare bacterial structures. The lung parenchyma had areas of atelectasis intermixed by areas of alveolar edema, but no inflammation was present (Figure 6B). In one of the cases, *E. coli* and *Pseudomonas aeruginosa* were identified through bacteriological culture,

but immunostaining for *E. coli* was absent at IHC, suggesting that *E. coli* was a contaminant. In the remaining two cases, immunostaining for *S. equi* and *E. coli* was absent.

Embolic pneumonias accounted for 6% of the cases (3/50), and only adult horses were affected (age ranged from 4.5 to 26 years-old, with a median age of 15 years-old and a mean age of 15.17 years-old). Predisposing factors included primary distant lesions, such as purulent endometritis and meningitis in one case each. Two horses were mixed breed, and the other was a Criollo. Grossly, there were multifocal to coalescent randomly distributed firm red to dark-red elevated areas (Figure 6C). On the cut surface, these had a friable content. Microscopically, there was a necrosuppurative pneumonia with multifocal coagulative necrosis occurring in association to thrombosis, large amounts of bacterial structures, marked inflammatory infiltrate of neutrophils, and moderate fibrin deposition (Figure 6D). *E. coli* was identified through bacteriological culture in the case related to purulent endometritis, while in another case a positive immunostaining for *E. coli* was also observed. The case related to the purulent meningitis did not stain either for *E. coli* and *S. equi*.

Discussion

The diagnosis of the present cases was based on the gross, microscopical, immunohistochemical and bacteriological findings. Unlike previous studies, which reported pleuropneumonia as the most common presentation of pneumonia in racehorses (Carvallo et al., 2017), suppurative pneumonia was, indeed, the most common presentation, with the involvement of the pleura, or pleuropneumonia, only in chronic cases. Prior predisposing factors were identified in 16 of 17 cases of suppurative pneumonia, as it has been described in a previous study which identified transport, strenuous exercise, and upper airway respiratory infections as the most common stressful events associated to the onset of pneumonia in horses (Racklyeft & Love, 2000).

S. equi subsp. *zooepidemicus* has been reported as the most commonly isolated bacteria in cases of pleuropneumonia and bronchopneumonia in horses (Bianchi et al., 2018; Carvallo et al., 2017; Oikawa et al., 1995; Racklyeft & Love, 2000). In the present study, however, this bacterium was identified only in two cases of suppurative pneumonia through bacterial culture. Probably other factors, such as hospitalization and poor colostrum intake, played major roles when compared to

previous transportation as the most important factor related to *S. equi* subsp. *zooeconomicus* pneumonic lesions (Oikawa et al., 1995). Although *S. equi* was identified in other six cases through immunohistochemistry, the antibody employed had a positive stain for both *S. equi* subsp. *zooeconomicus* and *S. equi* subsp. *equi*, and we were unable to infer which of the agents was involved. Yet, most of these horses were younger than five months-old (5/6), with strangles identified as a predisposing factor in two foals, which makes *S. equi* subsp. *equi* as the most likely etiologic agent for these cases. This is not considered a normal commensal agent in the equine respiratory tract, and bronchopneumonia is a possible form of secondary disease (Mallicote, 2015), as observed in the present study. In addition, *K. pneumoniae*, *E. coli* and *Enterococcus* sp. were identified in four cases, and these have been also described as common aerobic bacterial isolates from adult horses with pneumonia and pleuropneumonia along with β -hemolytic *Streptococcus* (Sweeney et al., 1991). Moreover, *Streptococcus pneumoniae* was identified in two cases both in peracute and chronic lesions. This agent is a common pathogen of humans; in horses it has been associated to disease in foals (Meyer et al., 1992), while it has been isolated from the respiratory tract of adult horses without clinical disease (Burrell et al., 1986). Thus, the predisposing factors identified in the two adult horses of the present study (long-distance transportation and hospitalization) may have facilitated the infection, since the natural introduction of small numbers of these bacteria into stressed animals may have similar effects to the observed in ponies (Blunden et al., 1993; Chanter, 1994).

Grossly and microscopically, the lung suppurative lesions in the present study varied from acute to chronic. Independently of the morphological pattern of the lesions, all cases had a cranioventral distribution of the lesions, which suggests that the infection gained entry to the lungs through the airways, and the aspiration of these bacteria may be favored during transport and hard exercise (Racklyeft & Love, 2000). The first pattern of acute lesions with a predominance of hemorrhage, edema, fibrin exudation, vascular fibrinoid necrosis and thrombosis may possibly reflect a difference in pathogen invasiveness or a temporal difference in the process of lesion formation, similarly to what has been observed in three horses transported for varying time and distance in a similar clinical course (0.3 to two days) (Oikawa et al., 1994) to the horses of the present study (one to three days). The preponderance of circulatory changes in these lesions may be related to the associated

vascular changes, which were also demonstrated within three to four hours after horses were inoculated endobronchially with *S. equi* subsp. *zooepidemicus* suspensions (Yoshikawa et al., 2003). The second pattern of acute lesions had mostly an airway centered neutrophilic inflammatory infiltrate, similarly to those caused by *S. equi* subsp. *zooepidemicus* in adult horses previously transported (Oikawa et al., 1995) and similar to those observed in the lungs of adult horses inoculated with *S. equi* subsp. *zooepidemicus* suspensions (Yoshikawa et al., 2003). Similarly, *S. equi* was involved in four horses with acute suppurative pneumonia; suggesting that this is a common agent mainly in neonatal to young foals, and poor colostrum intake was an important predisposing factor related to these cases. Although neonatal foals are more likely to develop bacterial pneumonia secondary to sepsis (Reuss & Cohen, 2015), primary suppurative pneumonia was most common in the present study.

Subacute lesions of cranioventral purple-red consolidated areas from the present study were grossly similar to those observed in four horses that died of *S. equi* subsp. *zooepidemicus* pneumonia associated with transport (Oikawa et al., 1994). In addition, the abscess formation, which was characteristic of this phase, has also been described at seven days post-infection (dpi) in calves inoculated with *Pasteurella multocida* (Dagleish et al., 2010), in a similar clinical course to the horses of the present study (two to seven days). The suggested pathogenesis for these lesions, as well as for the multifocal to coalescent areas of lung necrosis, is related to the formation of fibrin thrombi within the lung parenchyma, as described in calves (Dagleish et al., 2010).

Although the gross subacute lesions from the present study were similar to those described for other horses (Oikawa et al., 1994), the clinical course (two to seven days) is distinct from that previously identified (9-30 days). This is, probably, a reason for the microscopical differences between both studies, such as fibrinopurulent pleurisy and organized granulation tissue (Oikawa et al., 1994). These were mostly related to chronic cases in our study with a clinical course of 6-15 days, and a constant finding was unilateral pleural thickening (pleuritis), as observed after two weeks in horses inoculated with *S. equi* subsp. *zooepidemicus* (Yoshikawa et al., 2003). Similarly, subpleural lesions were observed ten dpi in calves inoculated with *P. multocida* (Dagleish et al., 2010). Pleuritis secondary to pneumonia may develop by direct spread from the infected lung tissue or as a result of

blockage of the pulmonary lymphatics by inflammatory debris, with subsequent leakage into the pleural cavity (Mair & Lane, 1989). In the present study, the involvement of the pleura was most likely a result of direct spread from lung lesions, as has been observed previously (Carvallo et al., 2017), since the etiological agents identified (*S. pneumoniae*, *S. equi* subsp. *zooepidemicus*, and *Enterococcus* sp.) are common lung pathogens (Erol et al., 2012; Tomlinson et al., 2015).

Furthermore, pleural effusions were exclusively unilateral, differing from previous studies (Arroyo et al., 2017; Carvallo et al., 2017). This may have occurred due to the fact that inflammation and fibrin deposits related to pleuritis may close the normally fenestrated equine mediastinum, dividing the pleural cavity effectively into two hemithoraces (Schott & Mansmann, 1990). Moreover, sequestration of necrotic tissue was observed at the chronic lesions in a similar manner to that observed in calves at seven to ten dpi (Dagleish et al., 2010). Unlike previous studies which identified recent travel as an important predisposing factor to pleuropneumonia development (Arroyo et al., 2017), the cases herein were mostly related to horses hospitalized or submitted to previous strenuous exercises.

R. equi infection, as seen here, may present as an acute respiratory distress, with foals dying within a few hours or days (Muscatello, 2012). Moreover, some foals may succumb to severe infection without presenting acute fever and respiratory distress (Cohen, 2014; Heidmann et al., 2006), similarly to this study in which some foals died suddenly without any clinical signs, as well as the involvement of other organs may influence the predominant clinical signs (Dedar et al., 2017; Reuss et al., 2009). The disease is restricted to foals (Cohen, 2014; Muscatello, 2012), as observed in this study, and this is most likely related to the immaturity of the immune system of the foal at this age range (Dawson et al., 2010). Holsteiner foals were mostly affected in this study, since all these foals were originated from the same property where pneumonia caused by *R. equi* was endemic (Oliveira et al., in press), and the disease may occur recurrently at some farms (Cohen, 2014; Muscatello et al., 2006).

R. equi is a facultative intracellular pathogen that leads to necrosis of alveolar macrophages, which are often followed by a pyogranulomatous infiltrate (Muscatello, 2012), as observed in the present study. Still, previous studies reported that not all foals with clinical *R. equi* infection have pulmonary abscesses/pyogranulomas (Leclere et al., 2011). In the present study, however, we demonstrated that at necropsy all horses affected by *R. equi* have characteristic gross lung lesions

(pyogranulomas), which are a hallmark of advanced *R. equi* pneumonia (Heidmann et al., 2006). In all cases, the final diagnosis of *R. equi* pneumonia was based on the bacteriological culture, which is relied as the gold standard for the diagnosis (Cohen, 2014), since other organisms, such as *Streptococcus* spp. and anaerobes may also cause pulmonary abscesses (Heidmann et al., 2006). Other organs are commonly affected by *R. equi*, causing septic uveitis, ulcerative enterocolitis, colonic and/or mesenteric lymphadenitis, osteomyelitis and septic arthritis (Heidmann et al., 2006; Reuss et al., 2009), similarly to the present study in which other organs were unevenly affected, such as the lymph nodes and the alimentary tract in nine and seven cases, respectively.

Previous studies reported pulmonary infections secondary to the inhalation of food and/or saliva as an important cause of pneumonia in horses mainly related to dysphagia and grass sickness (Mair & Lane, 1989). In the present study, however, aspiration pneumonia was the third major cause of pneumonia in horses, and this difference may have occurred due to the predominance of hospitalization and cleft palate as predisposing factors. Similarly, anesthesia and hospitalization were identified as important predisposing factors related to aspiration pneumonia in an adult horse (Anderson et al., 2017), while another study reported that aspiration of feed material through the lungs was present in seven out of nine foals with cleft palate (Shaw et al., 2015). Aspiration pneumonia is usually localized and unilateral rather than bilateral and symmetrical (Caswell & Williams, 2016). Nevertheless, lesions were mostly bilateral in the present study, while unilateral lesions involved only the apical right lobe. Grossly, the lung lesions were similar to those observed in acute to subacute suppurative pneumonias, as described (Caswell & Williams, 2016). Microscopically, all cases had alimentary content within the bronchi, bronchioli and/or alveolar spaces, although alimentary content was not observed grossly in most of the cases. Grossly visible plant material is often absent in cases of aspiration pneumonia, and when present it may be of little diagnostic significance, whereas microscopically it is usually a constant feature (Caswell & Williams, 2016).

Mycotic pneumonia caused by *Aspergillus* spp. is rare in horses, but it may cause a variety of clinical conditions from sudden death (Johnson et al., 1999) to chronic disease (Headley et al., 2014), and an age predilection has not been detected previously (Slocombe & Slauson, 1988). In this study, foals were mostly affected, and due to the shorter clinical course (less than 20 days), lesions were

acute with a predominance of necrosis intermixed by large amounts of fungal hyphae, similarly to the previously described (Slocombe & Slauson, 1988). A constant gross finding related to this condition was the presence of randomly distributed nodular lesions that varied in color, similarly to that described with variable tan white to yellow and dark-red to black nodules disseminated throughout the lungs mainly in the proximities of small airways (Carrasco et al., 1997; Johnson et al., 1999; Stefanetti et al., 2015).

Fungal respiratory diseases are most frequently acquired via inhalation of the causative agent (Stewart & Cuming, 2015). Still, the nodular lesions were observed predominantly in the subpleural spaces in two cases, which is possibly related to the clinical course of the condition, since a previous study had described fungal-related chronic nodules both at the pleural and sectioned surface, deep within the pulmonary parenchyma (Headley et al., 2014), and fungal lesions tend to be peripheral in distribution (Stewart & Cuming, 2015). Microscopically, all cases had fungal structures morphologically similar to those previously described for *Aspergillus* spp. (Headley et al., 2014; Johnson et al., 1999; Slocombe & Slauson, 1988; Thirion-Delalande et al., 2005), which was also supported by the positive immunostaining at IHC, a specific technique that should be considered in the absence of culture methods available (Thirion-Delalande et al., 2005). These fungi involved the alveolar spaces, in addition to the subpleural spaces and bronchioli lumen in two cases each, with necrotic lesions. Blood vessels often were involved, as described in similar cases (Slocombe & Slauson, 1988; Stefanetti et al., 2015), suggesting that other disseminated/secondary lesions were not observed due to the acute clinical course. These findings associated to the absence of mycotic lesions in other organs suggests that aspergillosis was, indeed, a primary lung lesion favored by the concomitant predisposing factors (antimicrobial therapy, salmonellosis and hospitalization), as suggested by other authors (Carrasco et al., 1997; Johnson et al., 1999; Stefanetti et al., 2015), in spite of previous descriptions of mycotic invasion from the gut (Slocombe & Slauson, 1988; Thirion-Delalande et al., 2005).

Bronchointerstitial pneumonias are characterized by four phases: parenchymal injury and alveolitis, proliferative phase, interstitial fibrosis and end-stage irreparable fibrosis of the lung (Wilkins & Lascola, 2015). In the present cases, microscopic lesions were consistent with the first

phase (parenchymal injury and alveolitis) with some proliferative changes as well (type II pneumocyte hyperplasia), and occasionally the bronchioli were also affected. Grossly, the lungs are usually diffusely consolidated and pale to dark-red (Patterson-Kane et al., 2008), similarly to the present study. Nevertheless, these features may be similar to the gross and microscopical findings observed in acute respiratory distress in foals (Lakritz et al., 1993). However, this condition usually occurs in older foals (Lakritz et al., 1993), while the foals of the present study had a median age of one month-old. Similarly, equine influenza infection is mostly described in neonatal to less than 6 months-old foals (Patterson-Kane et al., 2008; Peek et al., 2004; Wilkins & Lascola, 2015). Moreover, this condition should be differentiated from other viral, bacterial, parasitic, protozoal, and fungal diseases, mainly through the distribution of the lesions, since interstitial pneumonia has a diffuse pattern (Wilkins & Lascola, 2015). Still, RT-PCR may be employed for the identification of other agents involved in the condition (Wilkins & Lascola, 2015).

Pleuritis usually occurs as a complication of lung abscesses or pneumonia, and most horses will survive if properly treated in the acute phase (Arthur, 1983). In the present study the lesions were mainly chronic, since horses had a longer clinical course (10-13 days), and microscopically, in addition to the fibrinous exudate over the pleural surface, there was also marked fibrovascular tissue proliferation. This is a characteristic feature of the organization stage, when fibroblasts grow into the exudate from both pleural surfaces and produce an inelastic pleural layer that involves the lung, leaving it functionless (Reuss & Giguère, 2015). The chronicity of the condition possibly enabled that lesions were bilateral in all cases. In addition, a bacterial isolate was obtained in only one case, similarly to a previous study (Carvallo et al., 2017). The etiology and pathogenesis of pleuritis may be obscure since this is a chronic condition, and an early but resolved pulmonary infection cannot be discarded, as well as direct chest penetrations with no associated lung damage may have occurred (Mair & Lane 1989). Moreover, a failure in bacterial culture may also have been influenced by the intense antibiotic therapy employed in two of these horses before sample collection, as described previously (Lakritz et al., 1993).

Embolic pneumonia results from hematogenous distribution of infectious agents within the lungs (Caswell & Williams, 2016), as it was observed in two cases with purulent endometritis and

meningitis. In this study, embolic pneumonia was mainly induced by *E. coli*, differing from previous studies which revealed *Actinobacillus* spp. as the main cause (Carvalho et al., 2017).

Pneumonias are important causes of death in horses in Southern Brazil. Suppurative pneumonias were the main cause for these deaths, can be divided in acute, subacute and chronic forms based on the morphological pattern of lesions. Multiple predisposing factors were identified in these horses, and *Streptococcus* sp. was the main agent identified through IHC and bacterial culture. Pyogranulomatous pneumonia was represented by *R. equi* pneumonia in foals, and grossly multifocal to coalescent cranioventral yellowish nodules (pyogranulomas) were characteristic of the condition. Aspiration pneumonia had hospitalization and cleft palate as predisposing factors, with gross unilateral to bilateral consolidated lung lesions. Grossly visible vegetal material was uncommon, while microscopically it was a constant finding. Mycotic pneumonia affected mainly horses submitted to prolonged antimicrobial therapy and/or affected by comorbidities (salmonellosis). Gross lesions included multifocal random nodules (0.5-5 cm) in a variety of colors, involving both the lung parenchyma and pleural surface. IHC was an important tool to confirm the presence of *Aspergillus* spp. within these lesions. Bronchointerstitial pneumonia caused by Influenza A occurred only in young foals, with diffusely enlarged and reddish to whitish lungs. Microscopically, alveolitis and regenerative lesions were observed. Pleuritis affected only adult horses, with long-distance transportation and hospitalization as important predisposing factors. Lesions were bilateral, involving only the pleura with microscopic marked fibrovascular tissue proliferation covered by fibrin and neutrophilic exudate. Embolic pneumonias were less common, and had primary distant lesions (endometritis and meningitis) as predisposing factors.

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Table 1. Antibodies and immunohistochemical protocols employed in lung tissues of horses with pneumonia.

Antibody (code)	Clone	Antigen retrieval	Dilution	Amplification signal	Chromogen
<i>Escherichia coli</i> ^a	Polyclonal	5 min/microwave oven, citrate buffer, pH 6.0	1:200	MACH 4 ^d	AEC
<i>Streptococcus equi</i> ^b	Polyclonal	40 min/96°C, digital pressure cooker, Tris EDTA buffer	1:500	MACH 4	AEC
<i>Aspergillus</i> spp. ^c	Monoclonal (WF-AF-1)	40 min/96°C, digital pressure cooker, Tris EDTA buffer	1:200	MACH 4	DAB
<i>Rhizopus arrhizus</i> ^c	Monoclonal (WSSA-RA-1)	40 min/96°C, digital pressure cooker, Tris EDTA buffer	1:50	MACH 4	DAB

^a Virostat; ^b non-commercial antibody obtained in rabbits; ^c Bio-Rad; ^d Universal HRP-Polymer, Biocare Medical; AEC = 3-amino-9-ethylcarbazole; DAB = 3,3'-diaminobenzidine.

Table 2. Suppurative pneumonias in horses of the Southern Brazil.

Horse	Age	Clinical course (days)	Predisposing factor	Bacterial isolate	Immunohistochemistry	Morphological pattern of lesion
1	5 months-old	2	None	NA	Positive for <i>Streptococcus equi</i>	Acute
2	2 days-old	2	Poor colostrum intake	NA	Positive for <i>S. equi</i>	Acute
3	11 years-old	1	Long-distance transportation	<i>Streptococcus pneumoniae</i>	NA	Acute
4	7 months-old	2	Hospitalization and strangles	NA	Positive for <i>S. equi</i>	Acute
5	2 days-old	2	Poor colostrum intake	NA	Negative for <i>S. equi</i>	Acute
6	6 days-old	2	Intestinal intussusception	<i>Klebsiella pneumoniae</i>	NA	Acute
7	3 days-old	3	Poor colostrum intake	NA	Positive for <i>S. equi</i>	Acute
8	1 day-old	1	Poor colostrum intake	<i>Escherichia coli</i>	Positive for <i>E. coli</i>	Acute
9	5 months-old	7	Strangles	NA	Positive for <i>S. equi</i>	Subacute
10	5 years-old	NA	Guttural pouch empyema	<i>E. coli</i>	Positive for <i>E. coli</i> and <i>S. equi</i>	Subacute
11	19 years-old	4	Recurrent airway obstruction	<i>S. equi</i> subsp. <i>zooepidemicus</i>	Positive for <i>S. equi</i>	Subacute
12	3 years-old	2	Recurrent pneumonia	<i>Chromobacterium violaceum</i> ^a	NA	Subacute
13	4 years-old	2	Long-distance transportation	<i>S. equi</i> subsp. <i>equi</i>	Positive for <i>S. equi</i>	Subacute
14	7 days-old	7	Poor colostrum intake	<i>Staphylococcus</i> sp.	NA	Subacute
15	2.5 years-old	15	Hospitalization	<i>S. pneumoniae</i>	NA	Chronic
16	7 years-old	12	Hospitalization	<i>S. equi</i> subsp. <i>zooepidemicus</i>	NA	Chronic
17	4 years-old	6	Strenuous exercise	<i>Enterococcus</i> sp.	Negative for <i>S. equi</i>	Chronic

NA: not available; ^a Hammerschmitt *et al.* 2017

Figures and figures legends

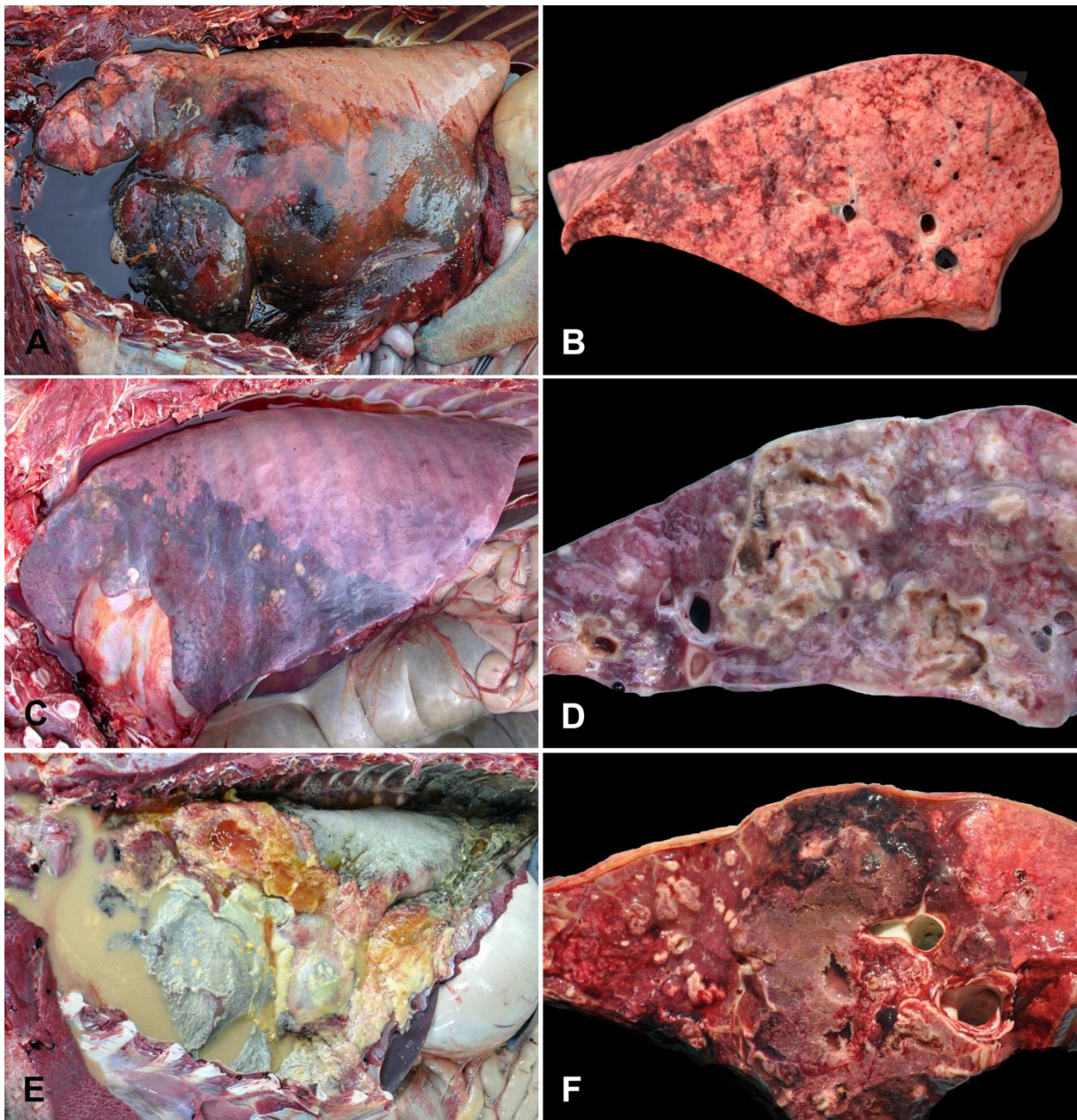


Figure 1. Gross findings of suppurative pneumonia in horses. **A-B.** Acute cases. Cranioventral dark-red areas within the lung parenchyma, in addition to variable amounts of dark-red serous liquid. The cut surface revealed multifocal to coalescent dark-red areas intermixed by normal lung parenchyma. **C-D.** Subacute cases. Focally extensive purple-red cranioventral areas intermixed by multifocal abscess formation. The cut surface revealed multifocal to coalescent irregular to nodular demarcated areas with a friable center. **E-F.** Chronic cases. Severe unilateral pleural fibrinous thickening, in addition to red to dark-red cranioventral areas and large amounts of yellowish turbid liquid. The cut surface revealed extensive friable devitalized greenish to brownish areas within the lung parenchyma, which were demarcated by a thick whitish tissue (compatible with sequestration of necrotic tissue).

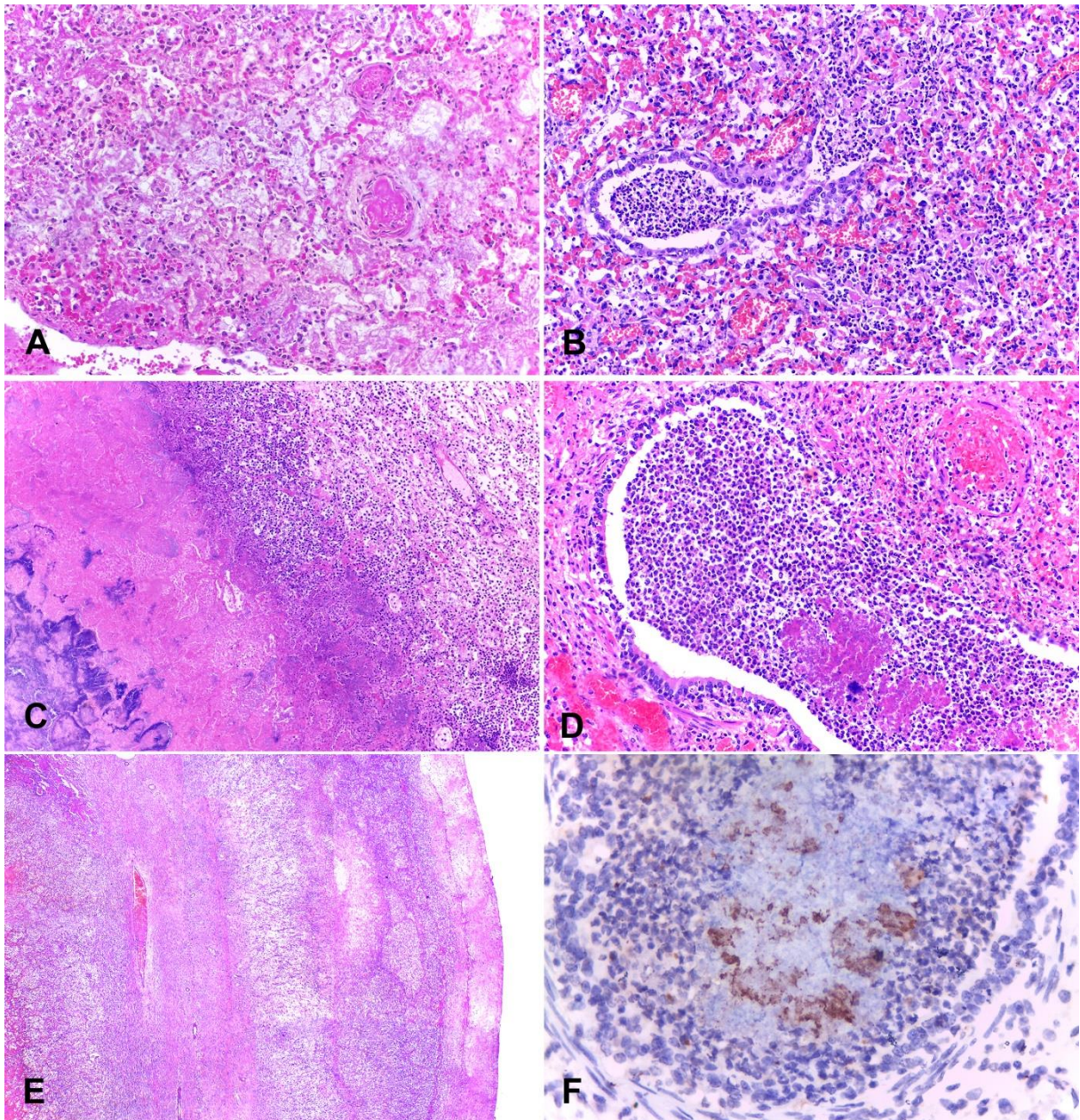


Figure 2. Microscopic findings of suppurative pneumonia in horses. **A.** Acute suppurative pneumonia. First pattern of lesion characterized by hemorrhage, edema, fibrin exudation, vascular fibrinoid necrosis and thrombosis. Hematoxylin and eosin (HE), 20x. **B.** Acute suppurative pneumonia. Second pattern of lesion characterized by airway centered neutrophilic inflammatory infiltrate extending to the alveolar spaces, in addition to multifocal fibrin exudate. HE, 20x. **C.** Subacute suppurative pneumonia. Focally extensive area of marked necrosis intermixed by large amounts of basophilic bacterial colonies, which was outlined by a rim of neutrophilic inflammatory infiltrate. HE, 10x. **D.** Subacute suppurative pneumonia. The bronchioli were also filled by a neutrophilic inflammation, which was intermixed by fibrin exudate and a myriad of basophilic bacterial colonies. HE, 20x. **E.** Chronic suppurative pneumonia. The pleura has a severe fibrinous and suppurative inflammatory infiltrate (right), in addition to marked fibrovascular tissue proliferation, and moderate neutrophilic inflammation within the alveolar spaces (left). HE, 2.5x. **F.** A positive granular immunostaining was observed within the necrotic areas. Immunohistochemistry anti-*Streptococcus equi*, 40x.

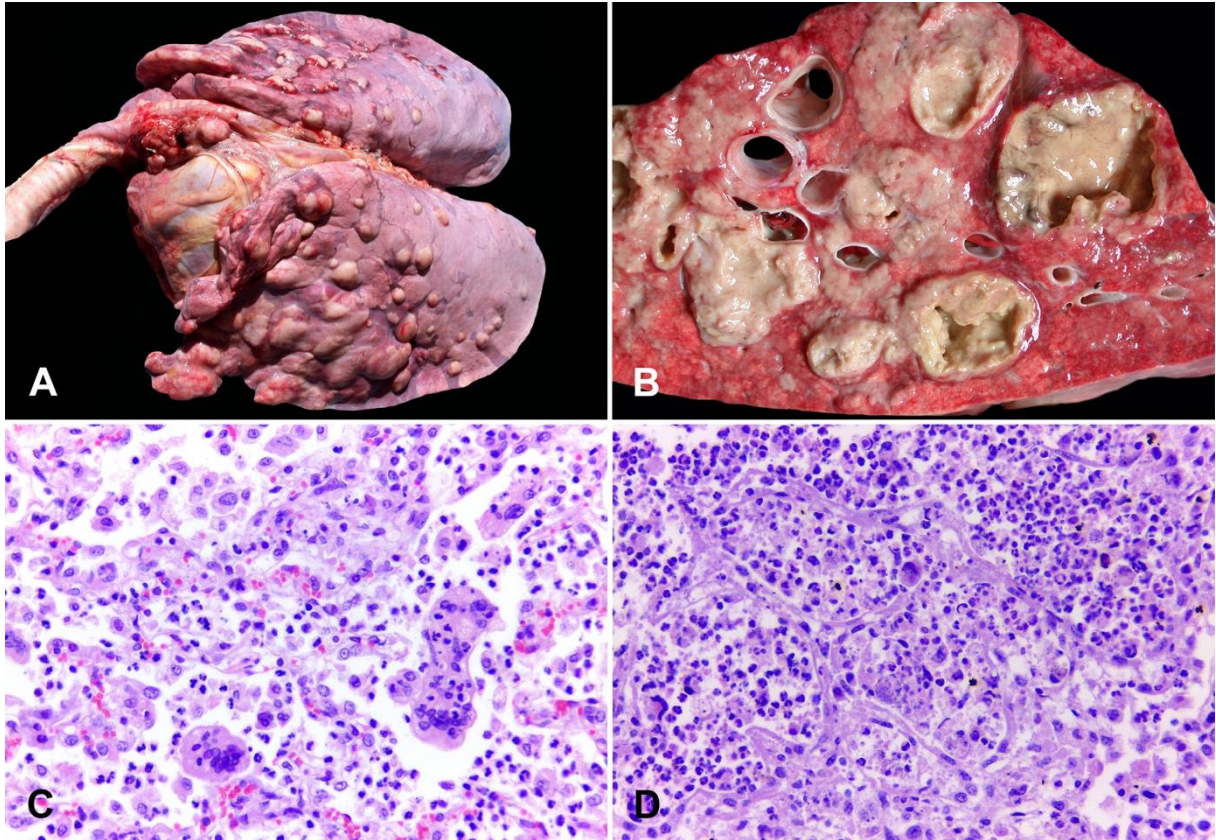


Figure 3. Pyogranulomatous pneumonia in horses caused by *Rhodococcus equi*. **A.** Multifocal to coalescent predominantly yellow, but occasionally red, cranioventral nodules. **B.** The cut surface revealed that these nodules extended into the parenchyma, and exuded yellowish liquid to friable contents. **C-D.** Within the alveolar spaces there was caseous necrosis intermixed by an inflammatory infiltrate of degenerate neutrophils, foamy macrophages, and foreign-type multinucleate giant cells. Occasionally, within the cytoplasm of these cells there was a myriad of basophilic coccoid bacteria, and rare multinucleate giant cells contained neutrophils within the cytoplasm. Hematoxylin and eosin, 20x.

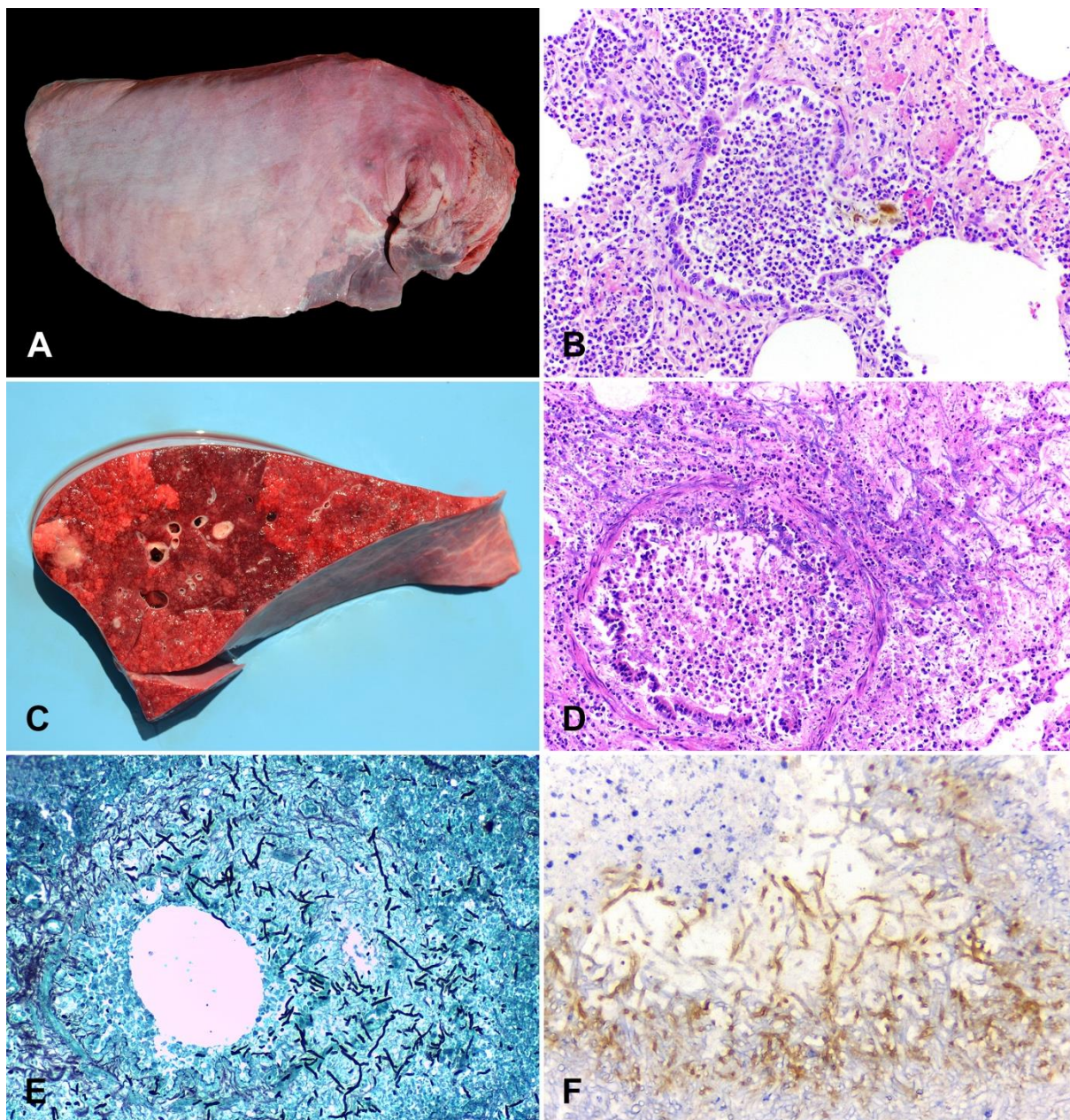


Figure 4. A-B. Gross and microscopic findings of aspiration pneumonia in horses. **A.** Cranioventral purple-red focally extensive area within the apical right lobe. **B.** The bronchioli contained foreign material surrounded by an inflammatory infiltrate of degenerate neutrophils intermixed by fibrin. The adjacent alveolar space had similar inflammatory infiltrate, and moderate edema. Hematoxylin and eosin (HE), 20x. **C-F.** Gross, microscopic and immunohistochemical findings of mycotic pneumonia in horses. **C.** Multifocal nodular yellowish to whitish areas randomly distributed throughout the parenchyma. **D.** Focally extensive area of necrosis of the bronchioli epithelium associated to numerous amphophilic fungal structures, inflammatory infiltrate of neutrophils, and fibrin exudate, which often extended into the alveolar spaces. HE, 20x. **E.** These fungal structures were narrow (4-6 μm width), septate, and had dichotomous branching. Grocott's methenamine silver stain, 20x. **F.** A positive immunostaining for *Aspergillus* spp. was observed within the necrotic lesions. Immunohistochemistry anti-*Aspergillus* spp., 40x.

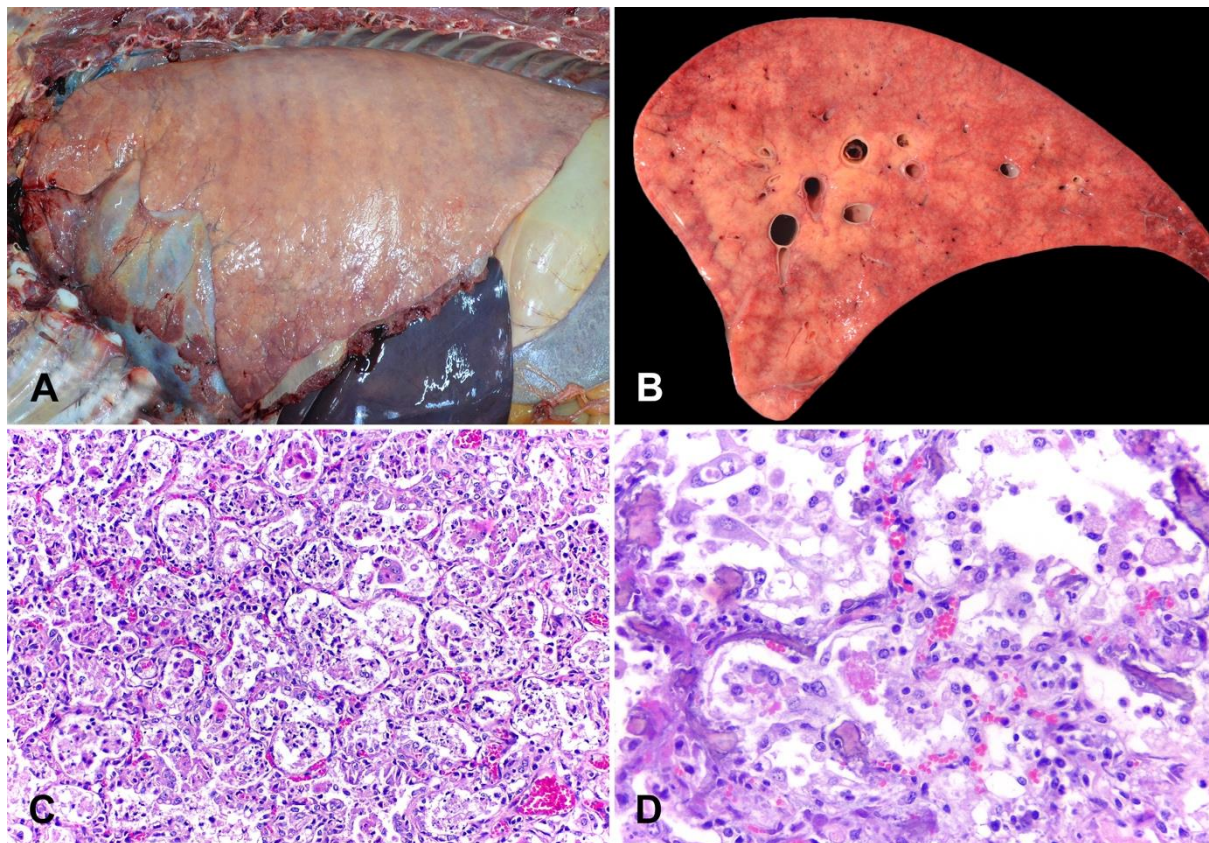


Figure 5. Gross and microscopical findings of bronchointerstitial pneumonia in horses caused by Influenza A. **A.** Lungs were diffusely enlarged, whitish, not collapsed and with ribs impressions. **B.** The cut surface of the lungs revealed a meaty appearance. **C-D.** Multifocal to diffuse necrosis of the alveolar surfaces associated to moderate fibrin exudate (alveolitis), severe type II pneumocyte hyperplasia, and mineralization of the alveolar septum. Hematoxylin and eosin, 20x and 40x.

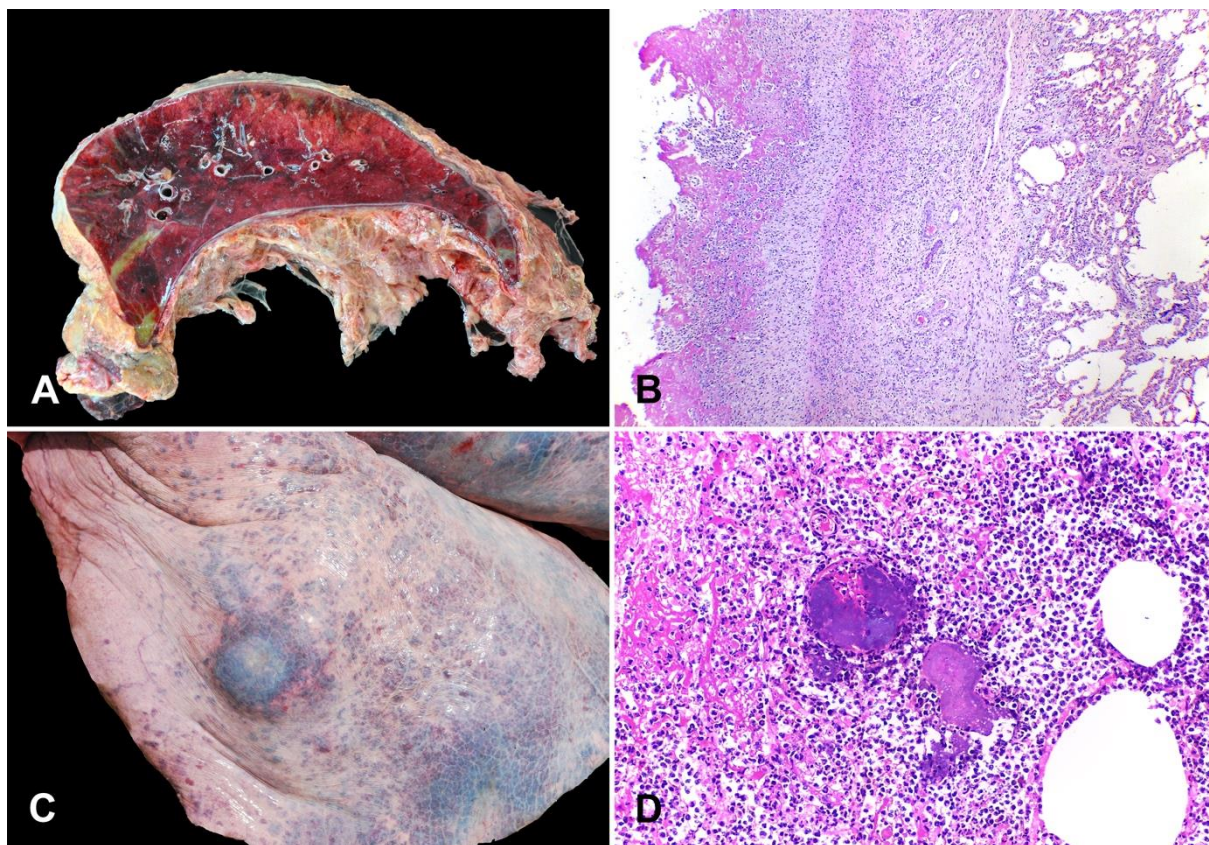


Figure 6. Gross and microscopical findings of pleuritis and embolic pneumonia in horses. **A.** The pleura was diffusely expanded by large amounts of fibrin, while the lung parenchyma had no abnormality. **B.** Severe and diffuse pleural thickening, characterized by a marked fibrovascular tissue proliferation covered by a superficial fibrin exudate and inflammatory infiltrate of neutrophils. The lung parenchyma had areas of atelectasis, but no inflammation. Hematoxylin and eosin (HE), 4x. **C.** Embolic pneumonia had multifocal to coalescent randomly distributed firm red to dark-red elevated areas. **D.** Multifocal areas of thrombosis associated to large amounts of bacterial structures, which extended into the adjacent lung parenchyma causing a marked inflammatory infiltrate of neutrophils intermixed by marked fibrin deposition. HE, 20x.

4. ARTIGO 3

Nesse item é apresentado o artigo intitulado:

Fatal parasite-induced enteritis and typhlocolitis in horses in Southern Brazil

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Fatal parasite-induced enteritis and typhlocolitis in horses in Southern Brazil

Enterites e tif ocolites parasitárias fatais em equinos no Sul do Brasil

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Abstract

Diseases related to the alimentary system are the main cause of death in horses. This retrospective study aimed to describe the pathological findings of fatal parasite-induced enteritis and typhlocolitis caused by cyathostominae, *Eimeria leuckarti*, *Balantidium coli*, and *Strongylides westeri* in horses. The records of parasite-induced intestinal lesions in horses necropsied in Southern Brazil between 2005 and 2017 were reviewed. Ten horses had fatal parasitic enteritis and/or typhlocolitis, and the main causes were: cyathostominae typhlocolitis (6/10), *E. leuckarti* enteritis (1/10), *S. westeri* enteritis (1/10), *B. coli* colitis related to cyathostominae (1/10), and infection by multiple agents (1/10). Cyathostominae typhlocolitis showed marked mucosal thickening, with multifocal elevated nodules containing tangled filiform parasites. Microscopic examination revealed that the mucosa and submucosa had encysted parasitic structures surrounded by eosinophilic and granulomatous inflammation. *E. leuckarti* enteritis was microscopically characterized by macrogamonts, microgamonts, and oocysts inside the host cells. *S. westeri* enteritis showed microscopic atrophy of the villi with numerous mucosal encysted parasitic structures. *B. coli* typhlocolitis showed severe diffuse mucosal reddening, with microscopic superficial mucosal necrosis associated with multiple protozoan trophozoites. Fatal parasite-induced enteritis and typhlocolitis are important causes of death in horses in Southern Brazil.

Keywords: Parasitic diseases, colic, cyathostominae, *Eimeria leuckarti*, *Strongylides westeri*, *Balantidium coli*.

Resumo

Doenças relacionadas ao sistema alimentar são as principais causas de morte em equinos. Esse estudo teve o objetivo de descrever aspectos patológicos de enterites e tif ocolites parasitárias fatais por ciatostomíneos, *Eimeria leuckarti*, *Balantidium coli* e *Strongylides westeri*, em equinos. Foi revisado o banco de dados de lesões intestinais parasitárias em equinos necropsiados de 2005 a 2017 no Sul do Brasil. Dez equinos apresentaram enterite e/ou tif ocolite parasitária fatal, e as principais foram: tif ocolite por ciatostomíneos (6/10), enterite por *E. leuckarti* (1/10), enterite por *S. westeri* (1/10), colite por *B. coli* com ciatostomíneos (1/10), e infecção por múltiplos agentes (1/10). A tif ocolite por ciatostomíneos exibiu acentuado espessamento da mucosa, com nódulos multifocais elevados contendo parasitas filiformes. Microscopicamente, a mucosa e submucosa apresentavam estruturas parasitárias encistadas envoltas por inflamação eosinofílica e granulomatosa. A enterite por *E. leuckarti* era caracterizada microscopicamente por macrogamontes, microgamontes e oocistos no interior de células do hospedeiro. Microscopicamente, a enterite por *S. westeri* apresentava atrofia de vilosidades com numerosas estruturas parasitárias encistadas na mucosa. A tif ocolite por *B. coli* exibiu avermelhamento acentuado difuso da mucosa, e microscopicamente necrose superficial associada a múltiplos trofozoítos protozoários. Enterites e tif ocolites fatais parasitárias são importantes causas de morte em equinos no Sul do Brasil.

Palavras-chave: Doenças parasitárias, cólica, ciatostomíneos, *Eimeria leuckarti*, *Strongylides westeri*, *Balantidium coli*.

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Introduction

The horse industry is an important part of the Brazilian economy, with an effective herd size of 5.5 million animals, and the second largest population of horses in Brazil (553,191 horses) is located in Rio Grande do Sul state (IBGE, 2017). The industry is estimated to generate an annual economic flow of R\$ 16.15 billion and to generate approximately 3.000.000 direct and indirect jobs (BRASIL, 2016). Diseases related to the alimentary system are the main cause of death in horses in this state (PIEREZAN et al., 2009b), as in other countries (BAKER & ELLIS, 1981). Among these diseases, displacements of the intestines, obstructions, impactions, perforations, and ruptures are the main cause of death in horses (BAKER & ELLIS, 1981; PIEREZAN et al., 2009b).

Gastrointestinal parasites are also one of the most important problems in developing countries such as Brazil (PEREIRA & VIANNA, 2006). However, most studies have only addressed the general occurrence of gastrointestinal parasites in equines through fecal examination (MARCOLONGO-PEREIRA et al., 2014; PEREIRA & VIANNA, 2006; SHEFERAW & ALEMU, 2015). Moreover, a few studies have described the clinical and pathological findings related to fatal larval cyathostomiasis (BODECEK et al., 2010; PIEREZAN et al., 2009a), *Strongyloides westeri* enteritis (LUCENA et al., 2012) and *Balantidium coli* colitis (HEADLEY et al., 2008). Nevertheless, recent studies have expressed concerns regarding the increasing prevalence of cyathostomins, their potential pathogenicity, and anthelmintic drug resistance (CORNING, 2009; LOVE et al., 1999). Thus, these parasites continue to pose a potential threat to equine health and are becoming increasingly difficult to control (NIELSEN & LYONS, 2017).

Therefore, the aim of this study was to describe the gross and microscopic findings related to fatal parasite-induced enteritis and typhlocolitis caused by cyathostominae, *Eimeria leuckarti*, *B. coli* and *S. westeri* in horses.

Materials and Methods

A retrospective study of the necropsy database of horses was conducted to search for records of parasite-induced lesions involving the small and large intestines detected between January 2005 and December 2017 at the veterinary pathology laboratory. These cases were grouped into non-fatal (incidental) and fatal conditions, and only cases of fatal parasite-induced enteritis

and typhlocolitis were selected. All horses studied were from the metropolitan area of Porto Alegre, Rio Grande do Sul state. Information regarding the clinical signs, seasonal occurrence, age, sex, breed, and gross lesions were retrieved from the necropsy database. Paraffin-embedded tissues of the selected cases were routinely processed for histological examination, and stained using hematoxylin and eosin. The Gordon & Whitlock (1939) technique was performed post-mortem in two cases (horses #4 and #5) with three replicates for each horse. Histological classification of cyathostominae larval stages was based on previous studies (NIELSEN et al., 2015; PIEREZAN et al., 2009a), with larger larvae classified as late L3/L4 and small larvae as early L3.

Results

Between January 2005 and December 2017, 654 horses were necropsied at the veterinary pathology laboratory. Of these, 10 horses had fatal parasitic enteritis and/or typhlocolitis. The main cause for these were cyathostominae typhlocolitis in six cases, and *E. leuckarti* enteritis, *S. westeri* enteritis and *B. coli* colitis associated with cyathostominae in one case each. One of the horses was infected by multiple agents, including large amounts of *S. westeri* and *E. leuckarti* in the small intestine and few cyathostominae in the large intestine. Age, sex, breed and seasonal occurrence of fatal parasite-induced enteritis and typhlocolitis in each horse are described in Table 1. Clinical signs associated with the conditions diagnosed are provided in Table 2.

Cyathostominae typhlocolitis was grossly characterized in all six cases by abundant greenish liquid and fetid content in both the cecum and large colon, with large amounts of filiform reddish free parasites measuring 1 cm in length in three cases (Figure 1A), and marked thickening of the mucosa in all cases. The mucosal surface had multifocal to coalescent red pinpoint areas (petechial hemorrhages) in addition to multifocal elevated nodules measuring up to 3 mm in diameter (Figure 1B), which occasionally had erosions on the surface (3/6 cases) and commonly contained tangled filiform parasites on the cut surface, measuring 0.5 to 1 cm in length (Figure 1C). In addition, moderate to severe colonic and cecal lymphadenomegaly was observed in all six cases. Microscopic examination in all cases revealed that the cecum and large colon had their mucosa and submucosa disrupted by moderate to large amounts of encysted parasitic structures surrounded by an eosinophilic and granulomatous inflammatory response (Figure 1D). These parasitic structures were

Table 1. Age, breed, sex and seasonal occurrence of fatal parasite-induced enteritis and typhlocolitis in horses.

Horse	Age	Breed	Sex	Seasonal occurrence	Condition diagnosed
1	1.4 years old	Criollo	M	Autumn	CT
2	1.5 years old	Criollo	NI	Spring	CT
3	10 months old	Criollo	F	Spring	CT
4	1 year old	Mixed breed	M	Spring	CT
5	1 year old	Mixed breed	M	Spring	CT
6	7 months old	Criollo	F	Autumn	CT
7	20 years old	Mixed breed	M	Winter	EE
8	1 month old	Criollo	M	Spring	SwE
9	2 months old	Quarter horse	F	Spring	BcT and CT
10	4 months old	Mangalarga marchador	M	Autumn	SwE, EE and CT

M = male; F = female; NI = not informed; CT = Cyathostominae typhlocolitis; EE = *Eimeria leuckarti* enteritis; SwE = *Strongyloides westeri* enteritis; BcT = *Balantidium coli* typhlocolitis.

Table 2. Clinical signs associated to fatal parasite-induced enteritis and typhlocolitis in horses.

Horse	Condition diagnosed	Clinical signs
1	CT	Chronic liquid diarrhea
2	CT	Profuse diarrhea
3	CT	Diarrhea, pale mucous membranes, weight loss and weakness
4	CT	Liquid diarrhea, abdominal increase, inappetence, polydipsia and weakness
5	CT	Liquid diarrhea, abdominal increase, inappetence, polydipsia and weakness
6	CT	Diarrhea, pale mucous membranes, bilateral ocular opacity and blindness
7	EE	Severe weight loss (cachexia)
8	SwE	Chronic liquid diarrhea, fever, apathy and dehydration
9	BcT and CT	Inappetence, apathy and diarrhea
10	SwE, EE and CT	Sudden death

CT = Cyathostominae typhlocolitis; EE = *Eimeria leuckarti* enteritis; SwE = *Strongyloides westeri* enteritis; BcT = *Balantidium coli* typhlocolitis.

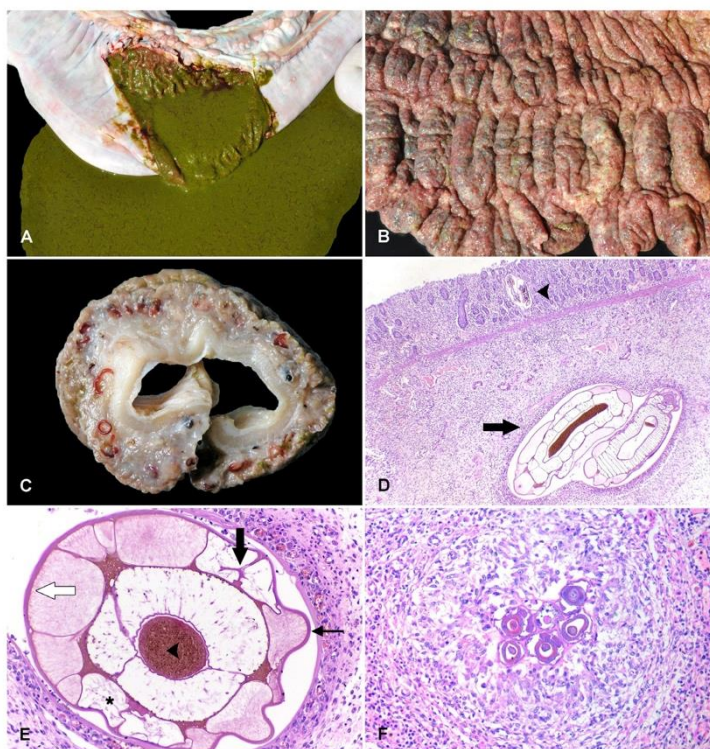


Figure 1. Gross and microscopic findings of cyathostominae typhlocolitis. (A) The large colon has an abundant greenish liquid content, with uncountable filiform reddish free parasites measuring 1 cm in length; (B) The mucosa is markedly thickened with multifocal to coalescent red pinpoint areas (petechial hemorrhages) in addition to multifocal elevated nodules; (C) On the cut surface, these nodules have tangled filiform reddish parasites inside; (D) The mucosa and submucosa of the large colon are disrupted by encysted parasitic structures: late L3 and L4 parasites (arrow) and early L3 parasites (arrowhead). Hematoxylin and eosin (H&E), 40× magnification; (E) Late L3 and L4 parasites have a thick eosinophilic cuticle (thin arrow), thin platymyarian musculature (empty arrow), finely vacuolated lateral cords (asterisk), prominent internal cuticular crests (thick arrow), and multinucleate intestines with a brush border in the apex and brown granular content (arrowhead). H&E, 200× magnification; (F) The submucosa is expanded by an eosinophilic and granulomatous inflammatory response that surrounds the parasitic structures. H&E, 200× magnification.

characterized by: multiple late L3 and L4 parasites (300–400 µm in diameter), which were composed of a thick eosinophilic cuticle, thin platymyarian musculature, finely vacuolated lateral cords, prominent internal cuticular crests, and multinucleate intestines with a brush border in the apex and brown granular content (Figure 1E), as well

as few early L3 parasites (80–100 µm in diameter), which were only located in the mucosa, were composed of a thick eosinophilic cuticle, and had a digestive tract with granular basophilic cells. The submucosa was expanded because of a similar inflammatory response that surrounded the parasitic structures, and showed

severe edema, marked muscle layer hypertrophy of the arterioles, moderate lymphoid hyperplasia with random necrosis, and mild to moderate thrombosis (Figure 1F). The superficial erosions on gross observation (3/6 cases) corresponded to focal areas of mucosal ulceration with associated hemorrhage and fibrin deposition. Frequently, the muscular layer of the mucosa was ruptured by the emergence of the encysted larvae, and the mucosa was eroded. Marked lymphoid hyperplasia and erythrophagocytosis were also observed on the mesenteric lymph nodes. Surprisingly, the eggs per gram of feces (EPG) of horses #4 and #5 were negative on all three replicates. Moreover, a direct analysis of the larvae observed in the

luminal content of these two horses revealed that only L4 parasites were present, and the adult forms were absent.

Gross abnormalities of *E. leuckarti* enteritis were only evidenced by the abundant liquid content in the small intestine, in addition to severe cachexia, pale mucous membranes, dehydration, and serous fat atrophy in the pericardial adipose tissue. Microscopic examination revealed that the villi of the jejunum and ileum had mild atrophy and multiple parasitic structures at distinct stages (macrogamonts, microgamonts, and oocysts) inside the hypertrophied host cells, which were flattened (signet-ring appearance) at the apex (Figure 2A). Macrogamonts were round, measured up to 80 µm in diameter, and contained multiple peripheral eosinophilic round granules

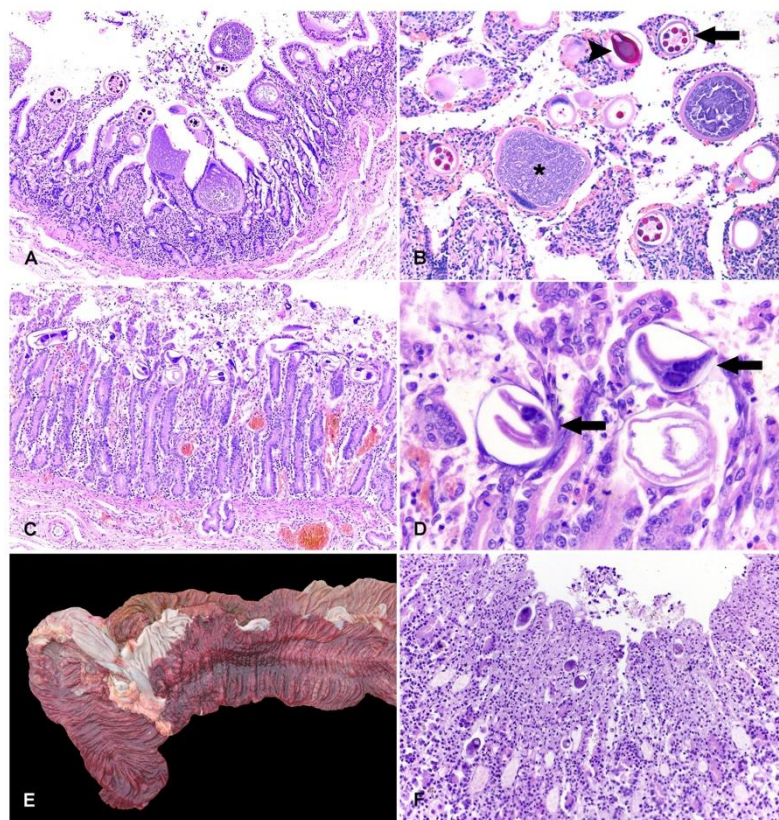


Figure 2. Microscopic findings of *Eimeria leuckarti* and *Strongyloides westeri* enteritis (A-D), and gross and microscopic findings of *Balantidium coli* typhlocolitis (E, F). (A) The villi of the jejunum and ileum are mildly atrophied and have multiple *Eimeria* parasitic structures at distinct stages (macrogamonts, microgamonts, and oocysts) inside hypertrophied host cells at the apex. Hematoxylin and eosin (H&E), 100× magnification; (B) Macrogamonts are round and contain multiple peripheral eosinophilic round granules (arrow). Microgamonts are round and contain multiple swirling microgametes inside, which are characterized by pinpoint basophilic granules (asterisk). Unsporulated oocysts are oval, with a micropyle at one end, an enlarged region at the opposite side, a thick eosinophilic capsule, and a sporoblast with granular eosinophilic material (arrowhead). H&E, 200× magnification; (C) *S. westeri* enteritis shows severe atrophy of the villi with numerous encysted parasitic structures at the base of the villi. H&E, 100× magnification; (D) These have external unevenly spaced cuticular ridges, a thin eosinophilic cuticle, with platymyarian musculature, a uninucleate large intestine with granular dark pigment in the cytoplasm, and a paired genital tract often containing embryonated eggs (arrow). H&E, 400× magnification; (E) *B. coli* typhlocolitis is grossly characterized by diffusely reddened mucosa of the cecum and ventral large colon; (F) The mucosa of the large colon and cecum present superficial necrosis associated with multiple oval parasitic structures covered by cilia on the surface (trophozoites of *B. coli*). H&E, 200× magnification.

(4–20 μm). Microgamonts were round, measured up to 190 μm in diameter, and contained multiple swirling masses of microgametes characterized by pinpoint basophilic granules. Unsporulated oocysts were oval (70 \times 50 μm), with a micropyle at one end, an enlarged region at the opposite side, a thick eosinophilic capsule and a sporoblast with granular eosinophilic material (Figure 2B). Surrounding these structures there was moderate fibrous connective tissue proliferation and mild inflammatory infiltration of eosinophils in the lamina propria.

S. westeri enteritis was grossly characterized by abundant liquid and fetid content in the small intestine, which had on the mucosa multiple irregular red areas. In addition, mesenteric lymphatic and blood vessels were evident. Microscopic examination revealed that the segments of the duodenum to ileum had severe atrophy of the villi with numerous encysted parasitic structures at the base of the villus, measuring from 45 \times 60 μm (transverse cut surface) to 190 \times 30 μm (longitudinal cut surface) (Figure 2C). These had a characteristic morphology of *S. westeri* (LUCENA et al., 2012), with external unevenly spaced cuticular ridges, a thin eosinophilic cuticle, with platymyarian musculature, a uninucleate large intestine with granular dark pigment in the cytoplasm, and a paired genital tract often containing embryonated eggs (Figure 2D). Numerous embryonated eggs were also observed retained in the epithelial tunnels at the base of the villi. The mucosa also showed marked hyperemia, hemorrhage, and moderate inflammatory infiltration of lymphocytes, neutrophils, and eosinophils.

B. coli typhlocolitis associated with cyathostominae in horse #10 was grossly characterized by a diffusely reddened serosa of the cecum and ventral large colon. Both had the mucosa diffusely and markedly reddened and intermixed with multifocal whitish pinpoint areas (Figure 2E). In addition, cyanotic mucous membranes and moderate mesenteric lymphadenomegaly were observed. Microscopic examination revealed that the mucosa of the large colon and cecum had superficial necrosis associated with multiple oval parasitic structures covered by cilia on the surface (trophozoites of *B. coli*; Figure 2F). These trophozoites measured 25 \times 40 μm and contained a prominent basophilic macronucleus, multiple micronuclei, occasional clear contractile vacuole, and prominent cytostome. In addition, there was severe inflammatory infiltration of eosinophils, lymphocytes and plasma cells in the lamina propria and submucosa; mild crypt-cell hyperplasia; decreased goblet cells; severe lymphoid hyperplasia; and moderate edema in the submucosa. Rare cut sections of the large intestine had in addition to protozoans, occasional encysted cyathostominae in the mucosa. Mesenteric lymph nodes showed diffuse hyperplasia.

Discussion

The diagnose in the present cases were obtained by associating the gross and microscopic findings with the observation of parasites inside the mucosa and associated surrounding lesions. Parasitological examinations were performed for identifying cyathostominae species in two cases (horses #4 and #5), but no adult parasites were identified, and only L4 parasites were detected. Most pathogenic effects related to cyathostomins arise when the developed L4 parasite emerges from the cysts and into the intestinal

lumen (CORNING, 2009), as was observed in three of the cases wherein large numbers of filiform reddish parasites were freely intermixed in abundant liquid intestinal content. Interestingly, these three cases also showed associated mucosal ulceration similar to that reported in a previous study (NIELSEN et al., 2015), suggesting that the mucosal lesions are important in the pathogenesis of this condition, and differing from a previous study which stated that ulceration of the mucosa in these cases is mostly related to the alimentary behavior of adult cyathostomin (REINEMEYER, 1986). Nevertheless, the remaining three cases may have showed a distinct pathogenesis mostly related to the invasion of the mucosa by the parasites, since L3 enter the glands of Lieberkuhn in the large intestine and penetrate the cell at the base (REINEMEYER, 1986), with subsequent development of encysted larvae, and later reduced nutritional metabolism in the intestine (COLLOBERT-LAUGIER et al., 2002).

Clinically, cyathostominae typhlocolitis had diarrhea as a constant finding in all horses, and this was different from the findings of a previous study wherein it was considered variable (PIEREZAN et al., 2009a). Several clinical syndromes may occur with cyathostome infection, including acute protein-losing diarrhea, recurrent diarrhea, rapid weight loss, subcutaneous edema, lethargy, inappetence and variable fecal consistency (MAIR, 2002). In a previous study, cyathostomiasis caused acute diarrhea in three horses, of which only one had a clinical recovery (BODECEK et al., 2010). This study, in which all the horses were younger than 1.5 years old, theorized that foals are more susceptible to severe acute protein-losing diarrhea, while aged horses may present mostly recurrent diarrhea (BODECEK et al., 2010). Previous studies have also shown that foals have less resistance to helminths, with higher mean EPG (SHEFERAW & ALEMU, 2015), and are thus more susceptible to this condition (PIEREZAN et al., 2009a) because of their as-yet undeveloped immune system (NIELSEN & LYONS, 2017); these findings are similar to the present findings.

On gross examination, the mucosa of the colon and cecum was markedly thickened with numerous elevated nodules containing tangled filiform parasites, similar to those previously described (PIEREZAN et al., 2009a). On histological examination, these nodular lesions had both early and late stages of L3 and L4 parasites surrounded by an eosinophilic and granulomatous inflammatory infiltrate. Both larval stages were expected to be observed, since are part of the cyathostomins life cycle progression, as has been previously described in juvenile horses infected by a fenbendazole-resistant cyathostominae population (NIELSEN et al., 2015) and in 5 horses described in other study (PIEREZAN et al., 2009a). However, early L3 parasites were observed only in the mucosa, and this can may be related to the acute clinical course of the present cases with a concomitant mucosal invasion by these stages (CORNING, 2009), followed by the emergence of immature parasites to the intestinal lumen (REINEMEYER, 1986). Corroborating these data, previous studies have shown that early stage L3 parasites may be observed exclusively in the lamina propria if horses are treated with a less efficient anthelmintic (fenbendazole), while late stage L3 and L4 parasites may be observed in both the mucosa and submucosa (NIELSEN et al., 2015), similarly to the observations in the present study. Moreover, previous studies showed that L3 and L4

parasites are more likely to be encysted in the mucosa than in the submucosa (BODECEK et al., 2010; STEUER et al., 2018), but these works may be related to the same species of cyathostomes, since some species may prefer to occupy a particular tissue level (REINEMEYER, 1986). Indeed, our cases may be related to similar cyathostome species, as the distribution of late stage L3s and L4 parasites was predominantly in the mucosa/submucosa, which is similar to the distribution described in other foals (NIELSEN & LYONS, 2017). Still, prolonged persistence of larvae in the third stage may occur, since these stages molts to the L4 within the tissue cyst (REINEMEYER, 1986).

The presence of larvae on gross and histopathological examinations is essential to obtain a final diagnosis of cyathostomiasis, since fecal egg counts are often zero or very low during larval cyathostomiasis, with the passage of many L4 with the feces (LOVE et al., 1999; LYONS et al., 2000; REINEMEYER, 1986). However, identification at the species level was not possible in the present study, since foals less than 1 year old mostly present juvenile nematodes (KUZMINA et al., 2011), with no adult parasites present, as in the present study. This probably occurred due to the fact that cyathostomiasis is related to the emergence of immature parasites that do not reproduce yet (REINEMEYER, 1986), and species identification relies mainly on adult parasites rather than larval stages (CORNING, 2009), because the larval stages possess fewer morphological characters than do adult worms (KHARCHENKO et al., 2009). Additionally, the size of cyathostome populations, mostly adult stages, may vary with the age of the horse, exposure history, management, use of anthelmintics, and the level of nutrition (REINEMEYER, 1986). Another potential differential pathological diagnosis would be typhlocolitis and lymphadenitis caused by *Rhodococcus equi*; however, unlike in the present cases, the mucosal lesions would have been observed in both the small and large intestines with purulent elevated ulcers (pyogranulomas), and the mesenteric or colonic lymph nodes would have been obliterated by edema and caseous/purulent foci (UZAL et al., 2016).

Cyathostominae typhlocolitis was observed mainly in the spring (4/6 cases) and autumn (2/6 cases) in the present study, unlike in a previous study in which most of the cases occurred in the winter and autumn (PIEREZAN et al., 2009a). This seasonal difference may be explained by the subtropical climate of the region, with marked differences in temperature and rainfall between the seasons (COPAERGS, 2018), since the development of larvae is directly related to the environmental temperature, with lower temperatures being very lethal to preinfective larval stages (REINEMEYER, 1986). Thus, in the warmer summer months, the larvae are inhibited and may reemerge in the autumn, while the opposite may occur in the cooler months (winter), with the larvae reemerging as the weather warms up in the spring (BAUDENA et al., 2000; REINEMEYER, 1986).

Strongyloides spp. are small nematodes that infect all species of domestic animals, and their larvae typically establish and persist within tunnels in the small intestine (GARDINER & POYNTON, 2006; UZAL et al., 2016). The pattern of lesions, which is related to the morphological features of the parasite and the involvement of the duodenum/ileum, was characteristic of *S. westeri* infection in this case. This species mostly infects foals (LUCENA et al.,

2012) and may be fatal in heavily infected animals (UZAL et al., 2016), as was observed in horse #8. Outbreaks usually occur in the summer (LUCENA et al., 2012); however, since lactation is an important pathway for *S. westeri* infection (LYONS et al., 1973) and since foals are usually born in the spring in the study area, these factors were probably related to the fatal enteritis observed in horse #9. Nevertheless, the occurrence of *S. westeri* enteritis in horses seems to be low so far, most likely because of the use of highly effective drugs, such as benzimidazoles and ivermectin (LYONS et al., 1993). Despite that, a recent study showed an overall elevated prevalence of patent *S. westeri* infections in foals in the USA, probably resulting from the diminished use of ivermectin owing to recent resistance in *Parascaris equorum* (LYONS & TOLLIVER, 2014), which may increase the overall importance of that parasite in horses in the near future.

The clinical findings observed in our study in *S. westeri* parasitism (chronic diarrhea, apathy, fever and dehydration) are similar to those previously described in an outbreak in Brazil (LUCENA et al., 2012), and are related to the gross and microscopic lesions caused by this parasite, wherein heavy parasite loads may persist for at least 10 weeks in foals (BOWMAN, 2014). The gross findings were mainly characterized by multifocal red areas on the mucosa and the accumulation of fluids in the intestinal lumen. This loss of fluids most likely occurred because of numerous encysted parasitic structures occupying the base of the villi; these may also be related to the atrophy of the villi, as described in previous studies (LUCENA et al., 2012).

E. leuckarti is the only valid species of *Eimeria* in equids (DUBEY & BAUER, 2018), and oocyst detection has been widely reported through fecal examinations, mainly in foals (SOUZA et al., 2009; DUBEY & BAUER, 2018; FIGUEIREDO et al., 1993). However, as shown in the present study, it may also occur in older horses (HIRAYAMA et al., 2002). Nevertheless, the present study reinforces the fact that post-mortem examinations may provide useful diagnostic insights in these cases, since the morphological features of unsporulated oocysts are similar to those described through coproscopic techniques (SOUZA et al., 2009). Clinically, this infection may present with or without clinical signs of lower alimentary tract disorders (HIRAYAMA et al., 2002), as in the present study wherein horse #7 had few clinical signs other than severe cachexia. A minor proportion of horses excreting *E. leuckarti* organisms may present with temporary diarrhea (STUDZIŃSKA et al., 2008). This probably led to the accumulation of abundant liquid content in the small intestine in the present case, similarly to previous descriptions (REPPAS & COLLINS, 1995), and most likely resulted in severe dehydration, serous fat atrophy, and death.

On microscopic examination, *Eimeria* organisms (macrogamonts, microgamonts, and oocysts) are frequently found in the cytoplasm of hypertrophied host cells in the lamina propria at the tips of the villi of the jejunum and ileum (HIRAYAMA et al., 2002). However, microgamonts may be mistaken for schizonts because of their size; even though these have not yet been identified in naturally infected equids (DUBEY & BAUER, 2018). The parasites may also be found in the extracellular spaces surrounded by macrophages and lymphocytes (HIRAYAMA et al., 2002), but this was not observed in the present study, and only minor changes were evident.

In this study, *B. coli* typhlocolitis was diagnosed mainly on the basis of the association between the gross and microscopic findings. Morphology has been the basis for species identification, and, thus, *B. coli* was identified by its large size, ovoid shape, dense curved macronucleus, adjacent small micronucleus, contractile vacuoles, and the presence of cilia on the surface (HEADLEY et al., 2008; SCHUSTER & RAMIREZ-AVILA, 2008; UZAL et al., 2016). In horses, it has previously been associated with an abrupt onset of colic and severe hemorrhagic and eosinophilic colitis without any obvious simultaneous primary infection (HEADLEY et al., 2008). This differs from the present case wherein rare cut sections of cyathostominae were also observed in the mucosa of the large intestine, suggesting that this infection probably favored *B. coli* invasion and proliferation in the large intestine of horse #9. A similar pathogenesis has been proposed for abomasal and lymphatic balantidiasis in the Barbary sheep (*Ammotragus lervia*), which was associated with a previous coccidial development of *Eimeria* spp. in the epithelial lining of the intestine (CHO et al., 2006).

B. coli infection may have three clinical manifestations: asymptomatic, chronic infection with non-bloody diarrhea, and fulminant balantidiasis with mucoid and bloody stools (SCHUSTER & RAMIREZ-AVILA, 2008). The latter seems to be the case in horse #9, which showed gross lesions mainly characterized by hemorrhagic typhlocolitis. In support of this theory, the parasite was also considered pathogenic since it was observed invading the mucosa and elicited a predominantly necrotic and eosinophilic inflammatory response, as reported in previous studies (HEADLEY et al., 2008).

Horse #10 was infected by multiple parasites, including helminths (*S. westeri* and cyathostomins) and protozoans. Mixed infection by helminths could be attributed to the fact that these roundworms have direct life cycles, with their free-living larvae having similar bionomics (MATTO et al., 2015).

Conclusions

Fatal parasite-induced enteritis and typhlocolitis are important causes of death in horses, especially in foals aged less than 1.5 years old in Southern Brazil. Cyathostominae typhlocolitis was the main condition diagnosed, followed by *E. leuckarti* enteritis, *S. westeri* enteritis and *B. coli* colitis.

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5. ARTIGO 4

Nesse item é apresentado o artigo intitulado:

**Epidemiological and pathological aspects of noninfectious diseases of the
gastrointestinal tract in horses in Southern Brazil**

Matheus V. Bianchi, Paula R. Ribeiro, Alanna S. Stolf, Marianna Bertolini, Cláudio J.

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

Epidemiological and pathological aspects of noninfectious diseases of the gastrointestinal tract in horses in Southern Brazil ¹

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Equine colic has been regarded as the most common cause of death in horses, but few studies have investigated specifically the conditions at the post-mortem exam (necropsy). This study aimed to describe the epidemiological and pathological features of noninfectious diseases of the gastrointestinal tract in horses. A retrospective study was conducted in search of cases of these diseases affecting horses between 2005 and 2017. During this period, 114 horses died due to noninfectious diseases of the gastrointestinal tract, and the main causes were: primary gastric dilation (27/114), volvulus (27/114), enterolithiasis (20/114), rectal (colonic) perforation (15/114), gastric or cecocolonic impaction (10/114), incarcerations (6/114), intussusception (4/114), and others (5/114). Mixed breeds horses (56/114) and males (69/114) were mostly affected. The horses had a median and mean age of 10 and 10.9 years-old, respectively. Primary gastric dilation was characterized by distension of the stomach by moderate to large amounts of content, which in 21 cases caused tearing of the stomach wall at the greater curvature (peritonitis), and the main predisposing factor was alimentary overload (17/27). Intestinal volvulus occurred within the small intestine and within the large intestines (14 and 13 cases). Grossly, there was intestinal ischemia with reddened to deep-black serosa, and diffusely red mucosae. Enterolithiasis caused partial or complete obstruction of the right dorsal colon (8/20), transverse colon (4/20), small colon and right dorsal colon (3/20), rectum and right dorsal colon (2/20), small colon (2/20), and large colon (1/20). Viscera perforation and peritonitis occurred in 11 cases. Rectal (colonic) perforation involved the rectum (10/15), rectum/small colon (4/15), and the small colon (1/15). It was characterized by a focally extensive transmural tearing, associated to reddened borders, and retroperitoneal to diffuse peritonitis. Palpation related iatrogenic injuries (11/15) were the main cause. Impactions affected the large colon (7/10), the cecum (2/10), and the stomach (1/10). Grossly, cecocolonic impactions had large amounts of impacted and dry digesta (6/9), variable amounts of sand (2/9) or plastic material within the lumen (1/9), while gastric impaction had impacted and dry ingesta. Predisposing factors (often multiple per case) included previous colic surgery (4), poor dentition (3), water deprivation (3), and dehydration (2). Incarcerations consisted of inguinoscrotal hernias (2/6), small intestine entrapment by a mesenteric failure (2/6), diaphragmatic hernia, and umbilical eventration. Grossly, the organs were constricted by an hernial ring, with intestinal ischemia and reddened to dark-red serosa. Predisposing factors included previous surgeries (2/6), and patent inguinal ring (1/6). Intussusception involved the small intestine (3/4) and ileocecum (1/4). Foals, lack of colostrum intake and concomitant pneumonia was a characteristic presentation (3/4). Grossly, the intussusceptum slipped into intussusciens, with a diffuse deep black-red discoloration. Other causes included large colon displacement (2/5), extrinsic and intrinsic obstruction of the small intestine (2/5), and an intestinal adenocarcinoma. Noninfectious gastrointestinal diseases are

important causes of death in horses. Epidemiological and gross features of the conditions should be accounted to obtain a final diagnosis of the cause of the colic.

INDEX TERMS: colic, gastric dilation, volvulus, enterolithiasis, rectal perforation, impaction, incarceration, intussusception.

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RESUMO.- [Aspectos epidemiológicos e patológicos de doenças não infecciosas do trato gastrointestinal de equinos no sul do Brasil.] A cólica em equinos é considerada como a principal causa de morte de cavalos, porém poucos estudos têm investigado especificamente as condições envolvidas através de exames post-mortem (necropsia). O objetivo desse estudo foi descrever os aspectos epidemiológicos e patológicos de doenças não infecciosas do trato gastrointestinal de equinos. Foi conduzido um estudo retrospectivo em busca de casos dessas doenças envolvendo cavalos de 2005 a 2017. Durante esse período, 114 equinos morreram devido a doenças não infecciosas do trato gastrointestinal, e as principais causas foram: dilatação gástrica (27/114), vólvulos (27/114), enterolitíase (20/114), ruptura (colônica) retal (15/114), compactação gástrica ou cecocolônica (10/114), encarceramentos (6/114), intussuscepções (4/114), e outros (5/114). Animais sem raça definida (56/114) e machos (69/114) foram mais afetados. Os equinos apresentavam uma média e mediana de idade de 10 e 10,9 anos, respectivamente. A dilatação gástrica primária era caracterizada por distensão do estômago por moderada a grande quantidade de conteúdo, que em 21 casos provocava ruptura da parede gástrica na curvatura maior (peritonite), e o principal fator predisponente foi sobrecarga alimentar (17/27). Vólvulo intestinal ocorreu no intestino delgado e no intestino grosso (14 e 13 casos). Macroscopicamente, havia isquemia intestinal com serosa avermelhada a enegrecida e mucosa difusamente avermelhada. A enterolitíase causou obstrução parcial ou completa do cólon dorsal direito (8/20), cólon transversal (4/20), cólon menor e cólon dorsal direito (3/20), cólon menor (2/20), reto e cólon maior direito (2/20) e cólon maior (1/20). Perfuração de vísceras e peritonite foram observadas em 11 casos. A ruptura (colônica) retal envolveu o reto (10/15), reto/cólon menor (4/15) e cólon menor (1/15). Era caracterizada por ruptura transmural focalmente extensa, com bordos avermelhados e peritonite retroperitoneal a difusa. Traumas relacionados à palpação (11/15) foram a principal causa. Compactações afetaram o cólon maior (7/10), ceco (2/10) e estômago (1/10). Macroscopicamente, as compactações cecocolônicas apresentavam grande quantidade de digesta compactada e seca (6/9), quantidades variáveis de areia (2/9) ou material plástico no interior do lúmen (1/9), enquanto a compactação gástrica exibia ingesta compactada e seca. Os fatores predisponentes (frequentemente múltiplos por caso) incluíram cirurgia de cólica anterior (4), problemas dentários (3), privação de água (3) e desidratação (2). Encarceramentos consistiram em hérnias inguinoescrotais (2/6), encarceramento de alças intestinais por falha no mesentério (2/6), hérnia diafragmática e eventração umbilical. Macroscopicamente, os órgãos estavam constrictos por um anel hernial, com isquemia intestinal e serosa avermelhada a vermelho-escura. Fatores predisponentes incluíram cirurgias prévias (2/6) e anel inguinal patente (1/6). Intussuscepções envolveram o intestino

delgado (3/4) e íleoceco (1/4). A apresentação característica foi em potros com falta de colostro e concomitante pneumonia. Macroscopicamente, o intussuscepto deslizava em direção ao intussusceptente, exibindo coloração vermelho-enebecida difusa. Outras causas incluíram deslocamento de cólon maior (2/5), obstrução extrínseca e intrínseca do intestino delgado (2/5), e um caso de adenocarcinoma intestinal. Doenças não infecciosas são importantes causas de morte em equinos. Os aspectos epidemiológicos e macroscópicas das condições devem ser considerados para o que o diagnóstico final da causa da cólica seja obtido.

TERMOS DE INDEXAÇÃO: cólica, dilatação gástrica, vólvulo, enterolitíase, ruptura retal, compactação, encarceramento, intussuscepção

INTRODUCTION

Horses are monogastric grazers, with a gastrointestinal tract that may be divided into three segments: foregut (stomach and esophagus), midgut (small intestine), and hindgut (large colon and cecum). The foregut and midgut receive continuous amounts of food that reach finally the hindgut, which is essentially a fermentation chamber. However, modern management practices have substituted/replaced the horse's natural grazing alimentary habits to a diet with high contents of grains and fats, which may not be properly digested by the horse (Bland 2016). Colic is an acute gastrointestinal disease characterized by a clinical syndrome of abdominal pain in horses, which can be infectious or noninfectious (Tinker et al. 1997). Almost 10-11% of the horses will present clinical signs of colic at some point in their lives, and mortality rates are usually high (Bland 2016). Surgical intervention is often necessary to treat colic in horses when a noninfectious cause is defined, but these are expensive, extremely invasive, and with a relatively high postoperative mortality (Immonen et al. 2017, Mair & Smith 2005a, Proudman et al. 2002).

Colic has been considered the most common cause of death in horses, accounting for at least 28% of the deaths (Tinker et al. 1997). With this perspective, numerous studies have described the alimentary system as the most commonly involved in the cause of death of horses, followed by the locomotor, nervous, cardiovascular and respiratory systems (Baker & Ellis 1981, Pierezan et al. 2009, USDA, 2017). Most of these have listed the noninfectious diseases of the alimentary tract in horses (Baker & Ellis 1981, Marcolongo-Pereira et al. 2014, Pierezan et al. 2009), but few of them have investigated specifically its conditions, focusing on the epidemiological and pathological features. Still, often nonspecific colic cases (unknown causes) are clinically referred as spasmodic, flatulent, or mild colic, specially due to the lack of proper surgery or necropsy (Tinker et al. 1997).

The knowledge of common causes and outcomes of equine colic may aid veterinarians to consider appropriate diagnostic methods, therapeutics, and determine the prognosis for the individual horse (Voigt et al., 2009). Furthermore, equine colic might have a substantial economic impact since sports or reproductive horses are becoming more valuable, and there has been an increase in insurance expenses. In 2001, by instance, the United States horse insurance industry has paid over US\$ 115 million in claims due to horse's deaths (Bland 2016). This information is also important to be considered in Brazil due to current trends of insured horses, which may require, if these animals are submitted to necropsy, more detailed descriptions into the cause of death, in a way that insurance companies may reimburse the owners (Immonen et al. 2017). Therefore, this study aimed to describe the epidemiological

and pathological features of noninfectious diseases of the gastrointestinal tract in horses necropsied in Southern Brazil.

MATERIALS AND METHODS

This work was conducted through a retrospective study of the necropsy database of the veterinary pathology laboratory from January 2005 to December 2017 in search of cases of noninfectious diseases affecting the gastrointestinal tract of horses. All horses were from properties located at the Rio Grande do Sul state, especially at the metropolitan region of Porto Alegre. The necropsy protocols were reviewed, and information regarding the cause of death, predisposing factors (one or multiple per horse), location of lesions, breed, age (age range, mean and median age), and sex were compiled in a spreadsheet. Gross lesions were reevaluated through photographs and combined with the previous descriptions available on the necropsy reports. The cases were grouped into categories: primary gastric dilation, volvulus, enterolithiasis, rectal (colonic) perforation, gastric or cecocolonic impaction, incarceration, and intussusception. Displacements, extrinsic obstruction, intrinsic obstruction and neoplasm did not fit into any of the above categories, and, thus, were listed as others causes. Each category was later evaluated in terms of secondary gastrointestinal abnormalities, such as ruptures, peritonitis, gastric dilation and impactions. Additionally, enteroliths were classified according to the location, aspect, distribution (single or multiple), and composition of the central *nidus* (foreign body that originated the enterolith). Furthermore, the causes of rectal (colonic) perforation were characterized as iatrogenic (reproductive tract or colic related palpation) or natural mating. Since gross lesions were characteristic of many categories, histological features were reevaluated only for the neoplasm.

RESULTS

From January 2005 to December 2017, 654 horses were necropsied in the veterinary pathology laboratory. The alimentary system was related to the cause of death in 201 horses, of which 114 (56.7%) were due to noninfectious diseases of the gastrointestinal tract. These were mainly comprised of gastric dilation (27 cases), volvulus of the small or large intestines (27 cases), enterolithiasis (20 cases), rectal (colonic) perforation (15 cases), gastric or cecocolonic impaction (10 cases), incarcerations or hernias (six cases), intussusception (four cases), and others (five cases). Mixed breed horses were the most affected (56/114) breed, followed by Criollo (32/114), Ponies (6/114), Quarter Horse (5/114), Brazilian Sport Horse or “Brasileiro de Hipismo” (5/114), Thoroughbred (5/114), American Trotter (2/114), and Appaloosa, Dutch Warmblood, and Brazilian Saddle Horse or “Mangalarga Marchador” with one case each. Males were more affected (69/114) than females (45/114). The horses had a median age of 10 years-old and a mean age of 10.9 years-old, with an age range of 3 days-old to 35 years-old. The frequency of diagnosis, as well as information regarding the sex, age range, mean and median age of the affected animals, according to each category is presented in Table 1.

Gastric dilation

Gastric dilation was the most frequent noninfectious disease of the gastrointestinal tract (27 cases), along with intestinal volvulus. Grossly, the stomach was distended by

moderate to large amounts of liquid and, often, fetid content, which in 21 cases caused a tearing of the stomach wall at the greater curvature. This led to the stomach rupture, which was characterized by mild to large amounts of feed content released into the abdominal cavity causing peritonitis (Fig. 1A-B). In addition, the edges of the stomach rupture were reddened, while frequently the serosa was lacerated, with adjacent hemorrhages, which dissected the muscular layer (Fig. 1C). The main predisposing factor related to this condition was alimentary overload (17/27) by corn (10/17), grains (3/17), or both (4/17). Other lesions frequently observed in these horses were gastric ulcers (8/27), poor dentition (5/27), recent surgery (1/27), and postpartum period (1/27).

Intestinal volvulus

Intestinal volvulus was diagnosed in 27 horses, with the small intestine (mainly jejunum and ileum) and large intestine involved in 14 and 13 of the cases, respectively. Of these 13 cases, the entire large colon and cecum were rotated in five cases, the large colon in three cases, the left dorsal and ventral colon in three cases, and only the cecum in two cases. Grossly, independently of the tissue affected, there was intestinal ischemia with reddened to deep black-red serosa of the compromised portion (Fig. 1D-E), as well as moderate to severe luminal distension and diffusely red mucosae (Fig. 1F). The small intestinal contents were reddish and liquid (14/14), while the large intestinal contents were frequently dry and impacted (5/13). Other secondary gastrointestinal changes included secondary gastric impaction in three cases, and ruptures with peritonitis in two cases. Predisposing factors were detected in 11 cases (single or multiple per horse), with poor dentition identified in four cases, previous hospitalization/surgery in three, alimentary overload in three, feed changes in two, and recurrent colic in one case.

Enterolithiasis

Enterolithiasis was the cause of death in 20 horses causing partial or complete intestinal obstruction in all cases. Enterolithiasis was followed by viscera perforation and peritonitis (11/20), as well as cecocolic impaction (6/20), and secondary gastric dilation (5/20). The right dorsal colon was identified as the main location in eight cases (Fig. 2A), followed by the transverse colon in four cases (Fig. 2B), the small colon and the right dorsal colon simultaneously in three cases (perforation of the small colon was the cause of death, the small colon in two cases (Fig. 2C), the rectum and the right dorsal colon simultaneously in two cases (rectal perforation was the cause of death; Fig 2D), and the large colon (unspecified site) in one case. Enteroliths varied in size, being larger than 10.1 cm in diameter (9/20) or smaller than 10 cm in diameter (11/20). These concretions were mostly single (11/20) but also multiple (9/20) per horse, with varying shapes (Fig. 2E): rounded (13), irregular (7), pyramidal to flattened (4), and elongated (1). Besides that, on the cut surface, some exhibited concentric layers centered on a *nidus*: rope in seven cases, stone in two cases, and wire in one case (Fig. 2F). Another change commonly observed in these horses was poor dentition (six cases).

Rectal (colonic) perforation

Rectal (colonic) perforation was diagnosed in 15 cases, with involvement of the rectum in ten cases, rectum/small colon in four cases, and small colon in one case. Grossly, these organs had focally extensive transmural tearing, associated to reddened borders, in addition to retroperitoneal to diffuse peritonitis (Fig. 3A-B). Iatrogenic injuries were the most common (11/15) causes, with colic related palpation in six cases (males and females) and reproductive

tract palpation in five cases (only females), followed by one case of perforation occurring after natural mating in a mare. In three cases, the clinical history was insufficient to establish a possible cause.

Impactions

Impactions were diagnosed in ten cases, of which seven affected the large colon, two affected the cecum, and one affected the stomach. Grossly, cecocolonic impactions were caused by large amounts of impacted and dry content (6/9; Fig. 3C), variable amounts of sand (2/9; Fig. 3D) or plastic material within the lumen (1/9), while gastric impaction was caused solely by large amounts of impacted and dry ingesta, with a concomitant severe distal esophageal muscle hypertrophy. Locations affected within the large colon varied greatly from flexures (Fig. 3E) to the entire organ. Secondary gastrointestinal lesions associated to cecocolonic impactions included intestinal rupture and peritonitis (3/9), as well as secondary gastric impaction (3/9). Predisposing factors included previous colic surgery (four cases), poor dentition (three cases; Fig. 3F), water deprivation (three cases), dehydration (two cases), and anti-inflammatory treatment in one case. The horse affected by gastric impaction was found in a hole without any food or water supply.

Incarcerations

Incarcerations or hernias were detected in six cases, of which two consisted of inguinoscrotal hernias (Fig. 4A), other two of small intestine entrapment by a mesenteric failure, while the remaining two were composed by a diaphragmatic hernia and an umbilical eventration (Fig. 4B). Grossly, the small intestine was involved in five cases, and the large colon was involved in one case (diaphragmatic hernia). In all cases, these organs were constricted by a hernial ring, which led to intestinal ischemia with reddened to dark-red serosa. The intestinal contents were liquid and red. In the case of diaphragmatic hernia, there was also rupture of the large colon within the thoracic cavity. Predisposing factors included previous surgeries (two cases of small intestine entrapment within a mesenteric failure), and patent inguinal ring in one case.

Intussusception

Intussusception was diagnosed in four cases, of which three involved the small intestine (two in the jejunojejunum and one in the jejunoileum), and the other involved the ileocecum (Fig. 4C-D). Three cases occurred in foals and were associated to the lack of colostrum intake and to concomitant pneumonia, while the other case occurred in an eight-year-old horse in association to poor dentition. In all cases, grossly, a segment of the small intestine slipped (intussusceptum) into another portion of the small/large intestine (intussusciens), causing a focal area of luminal obstruction and ischemia, with the intussusceptum presenting a diffuse deep black-red discoloration.

Others

Other causes of noninfectious diseases of the gastrointestinal included two cases of left dorsal displacement of the colon, one case of an extrinsic obstruction of the small intestine, one case of intrinsic obstruction at the jejunum, and another case of a primary intestinal adenocarcinoma. The extrinsic obstruction of the small intestine was caused by foreign material (surgical dressing that led to fibrous adhesions between intestinal loops), and the intrinsic obstruction was caused by a linear foreign body (rope) with complete obstruction

of the intestinal lumen. The neoplasm was composed by a multifocal firm whitish mass (6 x 4 x 3 cm), which occluded the intestinal lumen partially, and was also associated to multifocal areas of intestinal stenosis. On the cut surface, it had a friable aspect (Fig. 4E). In addition, the mesenteric lymph node was enlarged and firm. Histologically, there was an unencapsulated neoplastic proliferation of epithelial cuboidal cells arranged in tubules and papillae, occasionally filled by a faint basophilic content, extending from the mucosa into the internal muscular layer. Additionally, the neoplastic cells were intermixed by a severe desmoplastic reaction (Fig. 4F), inflammatory infiltrate of lymphocytes and multifocal areas of necrosis. These cells were also observed within lymphatic vessels and within the mesenteric lymph node.

DISCUSSION

The diagnosis of the cases of the present study was obtained mainly by the association of the epidemiological and gross findings. Histological findings were restricted to the neoplasm case due to differential diagnosis to other tumoral conditions, such as pythiosis (Uzal et al. 2016). Noninfectious gastrointestinal diseases were related to the cause of death mostly in adult horses with a median age of 10 years-old. This is similar to the median age of horses submitted to colic surgeries (8.5 years-old) in a retrospective study conducted in a teaching hospital in Finland (Immonen et al. 2017), in an equine referral hospital in South Africa (Voigt et al., 2009), and it is equal to another study that evaluated the survival and complication rates of 300 horses submitted to surgical treatment of colic (Mair & Smith 2005a). Regarding to the breeds affected, there was an overrepresentation of mixed breeds and Criollo breed, that accounted for 76.8% of the cases, which in the opinion of the authors is mainly a reflection of the population of horses from the Rio Grande do Sul state, as well as from the population examined at the hospital. Males were more affected than females in this study (59.8%), similarly to a study conducted in South Africa, in which 58% of the horses were males (Voigt et al. 2009), and to another conducted in Canada wherein geldings accounted for 50% of the cases (Abutarbush et al. 2006).

Along with volvulus of the small and large intestines, primary gastric dilation was the most common diagnosis in this study. This is distinct from that observed in a previous study, in which gastric dilation was the fourth most common cause of death related to the alimentary system in the same state (Pierezan et al. 2009), but similar to another study conducted in horses previously submitted to celiotomy, of which 11% of the deaths were due to gastric rupture (Todhunter et al. 1986). Gastric dilation is often considered secondary due to obstruction of the stomach, small bowel, or of colic with adynamic (paralytic) ileus, which frequently follows abdominal surgery, especially when the intestines are roughly handled or traumatized (Uzal et al. 2016). Nonetheless, all cases of the present study were characterized as primary due to the absence of other gastrointestinal disturbances and due to the identification of predisposing factors. Alimentary overload was the most common of these and occurred mainly due to excess of corn, grain or both in the diet of these horses. In addition, other associated findings included gastric ulcers, which in horses are often incidental findings at the necropsy, but may also be rarely related to perforation (Uzal et al. 2016, Winfield & Dechant 2015). In the present study, it was not possible to associate the ulcers as the cause of the rupture, but in most of the cases in which ulcers were observed, no other predisposing factor was present. Nevertheless, gastric rupture and subsequent peritonitis was the most common pathological picture observed in association with primary gastric dilation, unlike previous studies (Pierezan et al. 2009). Diffuse peritonitis in horses is often acute and fatal, as observed, due to the small omentum of this species, which is probably related to the poor capacity of healing contaminated areas (Uzal et al. 2016). Grossly, all cases were differentiated from postmortem lesions due to the reddened edges at the ruptured sites at the greater curvature, which is the most common gastric rupture location in horses (Winfield & Dechant 2015). Moreover, the lesions seemed to begin at the serosa, which was frequently stretched, as has been described previously in gastric ruptures when generally, the gastric seromuscular tears are larger than the mucosal tears, indicating that the seromuscular layer weakened first (Todhunter et al. 1986).

Intestinal volvulus was, equally as gastric dilation, the most common cause of death related to noninfectious diseases of the gastrointestinal tract in horses. Mesenteric volvulus of the small intestines, also referred to mesenteric torsion in other studies (Pierezan et al. 2009), is a common condition in suckling ruminants and swine, and it frequently occurs in species with a long small intestinal tract, such as the horse (Buergelt & Del Piero 2014, Pierezan et al. 2009), in which strangulating obstructions of the small bowel are associated to reduced short-term survival rates after surgery (Mair & Smith 2005a). Similarly, in the present study small

intestine volvulus was the most common pathological presentation of volvulus and affected mainly adults older than six-years-old, as previously observed in a population of 115 horses, of which small intestine volvulus occurred in animals with a median age of five-years-old (Stephen et al. 2004). Furthermore, in horses the large colon and cecum are predisposed to volvulus due to the lack of mesenteric anchorage and potential mobility of the large intestines within the abdominal cavity (Uzal et al. 2016), as it was frequently observed in the present study, in which the large intestine was almost equally affected as the small intestine by volvulus. Colon volvulus has been considered one of the most painful and fatal cause of colic in the horse (Suthers et al. 2013), in addition to a relatively common complication of previous colic surgeries leading to relaparotomy (Mair & Smith 2005c), but in the present study this predisposing factor was not identified. It is estimated that large colon volvulus represents between 10 and 20% of horses with colic that undergo exploratory celiotomy (Suthers et al. 2013), similarly to the present study in which large intestine volvulus represented 11.4% of the total of causes of death (13/114). Grossly, small and large intestinal volvulus shared similar gross aspects as well as similar secondary gastrointestinal changes, similarly to that previously observed (Uzal et al. 2016).

Enterolithiasis has been previously identified in 15.1% of horses presenting for colic and in 27.5% of patients undergoing celiotomy for colic treatment in the California state, USA (Hassel et al. 1999), while in São Paulo state, Brazil, this condition accounted for approximately 15.4% of the horses submitted to surgical abdominal procedures (Corrêa et al. 2006), which is very similar to the frequency of causes of death observed in the present study (17.5%), reinforcing that in Southern Brazil this may be an important condition in horses with clinical signs of colic. Enteroliths are mineral concretions (calculi) usually formed in the ampulla coli of the right dorsal colon (Hassel 2002), and composed by distinct amounts of magnesium, ammonium and phosphate, which are often arranged in concentric lamellae around a central *nidus* (Uzal et al. 2016), as in the present study. These concretions most commonly are solitary (Hassel et al. 1999), as in 55% (11/20) of the cases in this study, but may also be multiple within the guts. Enterolithiasis is a historical common and worldwide distributed cause of obstructive colic with records dating up to 140 years (Hassel 2002). It occurs when the concretion is passed through the intestinal tract until it causes an intestinal obstruction with subsequent luminal distention, mucosal necrosis and, possibly, perforation of the guts (Uzal et al., 2016). This was often observed in the present study since all enteroliths were partially or completely obstructing the intestinal lumen, whereas in 55% (11/20) of the cases there was also intestinal perforation and peritonitis, which is distinct from a previous study that reported only 15% of intestinal rupture in association to enterolithiasis (Hassel et al. 1999). This difference probably occurred due to delay on the decision by the clinicians to perform a celiotomy in the present cases.

Common locations for the enteroliths to become entrapped are the transition into the transverse colon (Buergelt & Del Piero 2014), the right dorsal colon and the descending colon (small colon) (Hassel et al. 1999, Hassel 2002). Similarly to that, the right dorsal large colon was the most common location in the present study, while the transverse colon was affected in four cases. Moreover, an uncommon location (rectum) was also affected, but no enterolith was located in the pelvic flexure, suggesting that this is not a common location, unlike previously described (Buergelt & Del Piero 2014). Similarly to previous studies (Hassel et al., 1999; Pierezan et al., 2009), adult horses with a mean and median age of 10.3 and 10 years-old were most commonly affected. This was expected since the pathogenesis of enterolith formation is longstanding due to the fact that it depends on many factors: salts, a nidus for precipitation of triple phosphates, and some degree of intestinal hypomotility (Buergelt & Del Piero 2014). In vivo diagnosis of enterolithiasis relies mainly on abdominal radiography, rectum palpation, and exploratory laparotomy. Still, abdominal radiographs may fail if the enterolith is located in the small colon, as well as palpation per rectum may provide limited information mainly due to the size of the examined horse, with enteroliths located at the terminal right dorsal colon being rarely detected (Hassel 2002). This reinforces that concretions located at the right dorsal colon, transverse colon and small colon, such as in most of the cases of this study, may be related to poor prognosis if not properly detected early. Furthermore, smaller enteroliths, such as the ones involving the small colon and rectum in this study, are potentially more pathogenic than similar concretions present in the large dorsal colon, since are able to easily obstruct the lumen of these organs and become irreversibly lodged (Hassel et al. 2008), while larger enteroliths are always contained within the right dorsal colon (Hassel et al. 1999). Several different shapes of enteroliths may occur in horses, but the most common are spherical or tetrahedral, with the last being mostly multiple (Hassel 2002). In this study, most of these were rounded to spherical and often irregularly shaped. In six cases, poor dentition was observed, and interpreted as a possible consequence of imbalanced diets, similarly to a previous study in Criollo horses which reported 56.25% of dentition problems occurring in association with enterolithiasis (Santos et al. 2017).

Rectal (colonic) perforation in horses are job-related risks for equine veterinarians due to accidental iatrogenic events, such as obstetric interventions and/or rectal palpation (Buergelt & Del Piero 2014, McMaster et al. 2015, Uzal et al. 2016), as it was observed in the present study wherein iatrogenic injuries were reported in 73.3% of the cases (11/15). Most tears related to perforation occur 25 to 30 cm away from the anus, involve

the rectum mainly at the dorsal aspect (Uzal et al. 2016), and are, thus, difficult to be surgically accessed (McMaster et al. 2015). However, in this study, four cases were located at the limit between the rectum and small colon, while the perforation was located at the small colon in another horse. Therefore, a better nomenclature for these would be rectal-colonic perforation in cases where the boundaries between these two organs are not clear. Rectal tears may be classified into grades 1 to 4 based on the extent of the disruption, with full-thickness rupture observed only in grade 4 (Watkins et al. 1989), as all the cases of the present study would be classified. Moreover, only 6% of the horses with grade 4 lesions survive (McMaster et al. 2015), which is mainly due to the associated secondary peritonitis. This was a constant finding associated with these lesions in this study, occurring either as a located or disseminated lesion. As expected based on previous studies (Claes et al. 2008), females were mostly affected by injuries related to reproductive tract palpation. Horses older than nine-years-old, similarly to the ones of the present study, are more predisposed to these perforations (Claes et al. 2008), since the reproductive tract becomes more pendulous at these ages and the frequency of palpation in subfertile older mares is increased (LeBlanc et al. 1998). Moreover, colic related palpation affected both males and females, but specially males, which probably occurred due to the fact that stallions or geldings are less accustomed to palpation per rectum than mares, and other factors, such as restless behavior, small rectum, inadequate restraint, application of inadequate amounts of lubricants and absence of spasmolytic drugs, may have played a role in these cases (Stauffer 1981). Interestingly, a case of rectal perforation following natural mating in a mare was detected, which appears to be an unusual presentation, that was not observed in a previous study of 99 cases of rectal perforation in the USA (Claes et al. 2008), but was previously reported in a mare following accidental penetration of a stallion's penis into the rectum (Arnold et al. 1978).

Large intestine and stomach impaction was the fifth most common category of gastrointestinal noninfectious disease detected as the cause of death in horses of the present study, which is distinct from previous clinical studies that identified impactions as the most common causes of colic (Abutarbush et al. 2006, Voigt et al. 2009). Indeed, this difference may have occurred due to secondary impactions detected clinically without proper identification of the primary cause. Predisposing factors to this condition in horses are water deprivation, poor dentition, and dietary changes (Uzal et al. 2016), as was observed in the present study. Dental problems were evidenced in three cases, but, still, a previous study was not able to establish an association between dental pathology, fecal particle size, and impaction colic in horses (Gunnarsdottir et al. 2014). Furthermore, another important predisposing factor identified was previous colic surgery in four cases. Postoperative colic during the first year after surgery may reach an incidence of 20% in horses (Immonen et al. 2017), and these surgeries may act by modifying the colonic or cecal peristalsis, causing abnormal motility (Uzal et al. 2016). In horses, this pathogenesis is usually related to adynamic or paralytic ileus when intestines are not properly handled at surgery (Uzal et al. 2016), but may also apply to the present cecocolonic impaction cases. Unlike previous studies, impactions affected not only locations where the lumen was narrowed, such as the pelvic flexure and the transverse/small colon (Uzal et al. 2016), but also the entire large colon and cecum. In these cases, probably the association of predisposing factors, which were frequently multiple per horse, worsened the condition.

Sand impaction was detected in two cases, which are commonly related to horses grazing in poorly covered sandy soils (Uzal et al. 2016). The ingestion of this material culminates with a tendency for the sand to settle down within the cecum and the ventral large colon, leaving these organs extremely heavy with a predisposition to rupture (Buergelt & Del Piero 2014), as occurred in one of the cases of the present study. Nonetheless, the mortality rate for horses with sand impaction is reported to be low, as observed in this study in which a low frequency was detected (two cases), and early surgical intervention provides a good prognosis (Granot et al. 2008). Furthermore, the stomach was involved in one case as a primary impaction, which is a rare cause of colic in horses (Bird et al. 2012), and in this situation it was caused by the inadequate water supply, since this horse was found in a hole without any food or water available for three days. Primary gastric impactions are usually characterized by large quantities of ingesta (15-35 kg) associated to gross thickening of the muscle layers of the stomach wall, in addition to variable thickening of the pylorus and hypertrophy of the distal esophageal muscles (Bird et al. 2012), which was observed grossly in this case.

Incarcerations or hernias occurred mainly as internal and external hernias, such as inguinoscrotal and diaphragmatic, but also as internal failures of the mesentery and a case of eventration. Grossly, these lesions were typical of intestinal ischemia and infarction (Uzal et al. 2016), with reddened to dark-red serosa. The pathogenesis of this condition may be related to fixation of the hernial/entrapped contents within a location, which may be followed by distention of the viscera with accumulated gas or ingesta, and later the lesion becomes nonreducible (Uzal et al. 2016). Inguinal hernias may evolve to scrotal hernia when the herniated pass down the inguinal canal. These are usually lesions observed in foals since the inguinal ring tend to close as the animal grows up (Uzal et al. 2016). Unlike that, two cases of inguinoscrotal hernias were detected in adult horses in the present

study, and a patent inguinal ring-opening was detected in one of these. Moreover an umbilical eventration was observed in one adult horse, but it could be a result of a rare enterocutaneous fistula related to an umbilical hernia (Uzal et al. 2016). Another interesting finding were two cases of small intestine entrapment by a mesenteric failure, which were most likely related to previous surgeries that caused tissue gaps within the mesentery, allowing the small intestinal loops to slip into this failure and become entrapped (Buergelt & Del Piero 2014).

Intussusceptions were not frequently detected in this study, with only 3,5% of the diagnosis, as it was observed in another study, which had a prevalence of 8,7% cases of intussusceptions in horses undergoing laparotomy due to abdominal pain (Edwards 1986). This condition is usually related to preceding intestinal hypermotility or endoparasitism (Buergelt & Del Piero 2014), as well as it may be associated to previous intestinal surgery, enteritis and tumors (Uzal et al. 2016). In two of the present cases, decreased intake of colostrum and concomitant pneumonia were identified as predisposing factors, and probably resulted in hypermotility of the guts. Still, in horses ileocecal or cecocolic intussusceptions are most commonly detected (Buergelt & Del Piero 2014, Bell & Textor 2010), while in this study jejunojejunal and jejunoileal intussusceptions were more frequent. This difference probably occurred due to the lowest age of the animals affected in the present study, with a median age of 0.04 years (which corresponded to almost 15 days-old), when compared to another study that investigated cecal intussusceptions in which horses had a median age of 14.5 months-old (Bell & Textor 2010). Grossly, intussusception may present as a simple obstruction if mural blood flow is preserved, or as a necrotic lesion due to occlusion of the blood flow (Nelson & Brounts 2012). This second pattern was mostly observed in the present study, with ischemic lesions affecting the intussusceptum, which probably occurs in most severe cases when this condition is related to the cause of death of horses.

Intestinal neoplasms are rare in horses (Munday et al. 2017), and are usually mesenchymal, mainly represented by fibrosarcomas, leiomyosarcomas, and gastrointestinal stromal tumors (GIST) (Buergelt & Del Piero 2014), additionally to lymphoma and adenocarcinoma (Taylor et al. 2006). However, in this study, among the other lesions, a primary intestinal adenocarcinoma was detected, and, similarly to the previously described (Uzal et al. 2016), it represented less than 1% of the cases. These neoplasms usually involve the small intestines of adult horses with most of them invading the intestine wall and, rarely, causing a complete obstruction of the lumen (Taylor et al. 2006), similarly to the observed in the present study. Histologically, the neoplastic cells tend to be well differentiated and intermixed by marked fibrous metaplasia (desmoplastic reaction) (Munday et al. 2017), as in this case. Another finding of this study was one case of extrinsic obstruction of the small intestine caused by a foreign material that led to a focal adhesion in a similar manner to the previously reported in long-term complications of previous colic surgeries in horses (Mair & Smith 2005b). Adhesions become a clinical problem only when they mature into fibrous adhesions, which may compress or distort the intestine anatomically, causing an obstruction (Mair & Smith 2005b), which occurred in this case.

CONCLUSIONS

Noninfectious diseases of the gastrointestinal tract are among the leading causes of death of horses in general, and are the main diseases affecting the alimentary system in this species. Gastric dilation and volvulus are the most common conditions with gross characteristic lesions. Enterolithiasis was the third most common diagnosis, and viscera perforation with peritonitis frequently occurs. Rectal (colonic) perforation is most commonly related to iatrogenic injuries associated with colic and reproductive tract palpation, affecting more males and females, respectively. Impactions affected mostly the large colon and cecum, with previous colic surgery, poor dentition, water deprivation, and dehydration identified as important predisposing factors. Incarcerations were mostly related to hernias, mesenteric failures most likely induced by previous surgery, and eventration. Intussusception was observed mainly in foals at the small intestine, and it was often related to other concomitant diseases. Other causes were less common, and included a multifocal primary intestinal adenocarcinoma causing a partial occlusion of the intestinal lumen, as well as multifocal areas of stenosis. Epidemiological and gross lesions are essential to obtain a final diagnosis of these conditions.

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Conflict of interest statement.- The authors have no competing interests.

Animal welfare / ethical statement.- We authors of the article entitled “Epidemiological and pathological aspects of noninfectious diseases of the gastrointestinal tract in horses in the Southern Brazil” declared, for all due purposes, the project that gave rise to the present data of this manuscript has been submitted for evaluation

to the Ethics Committee of the Universidade Federal do Rio Grande do Sul (UFRGS), and has been approved under the number 33501 at December 7th, 2017.

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Table 1. Frequency of diagnosis of noninfectious diseases of the gastrointestinal tract in horses, as well as information regarding the sex, age range, mean and median age of the affected animals.

Categories	Frequency	Sex (M:F)	Age range	Mean age (y)	Median age (y)
Gastric dilation	23.7%	20:7	3 m - 26 y	10.6	11
Volvulus	23.7%	13:14	1.5 m - 35 y	9	8
Enterolithiasis	17.5%	13:7	5 y - 18 y	10.3	10
Rectal (colonic) perforation	13.2%	6:9	2 m - 20 y	10.5	12
Impactions	8.8%	6:4	2 y - 25 y	11.1	10
Incarcerations or hernias	5.3%	5:1	5 y - 15 y	11	12
Intussusception	3.5%	4:0	3 d - 8 y	2	0.04
Others	4.4%	2:3	1 y - 20 y	10.2	8

M: male; F: female; d: days-old; y: years-old; m: months-old.

Figures and figures legends

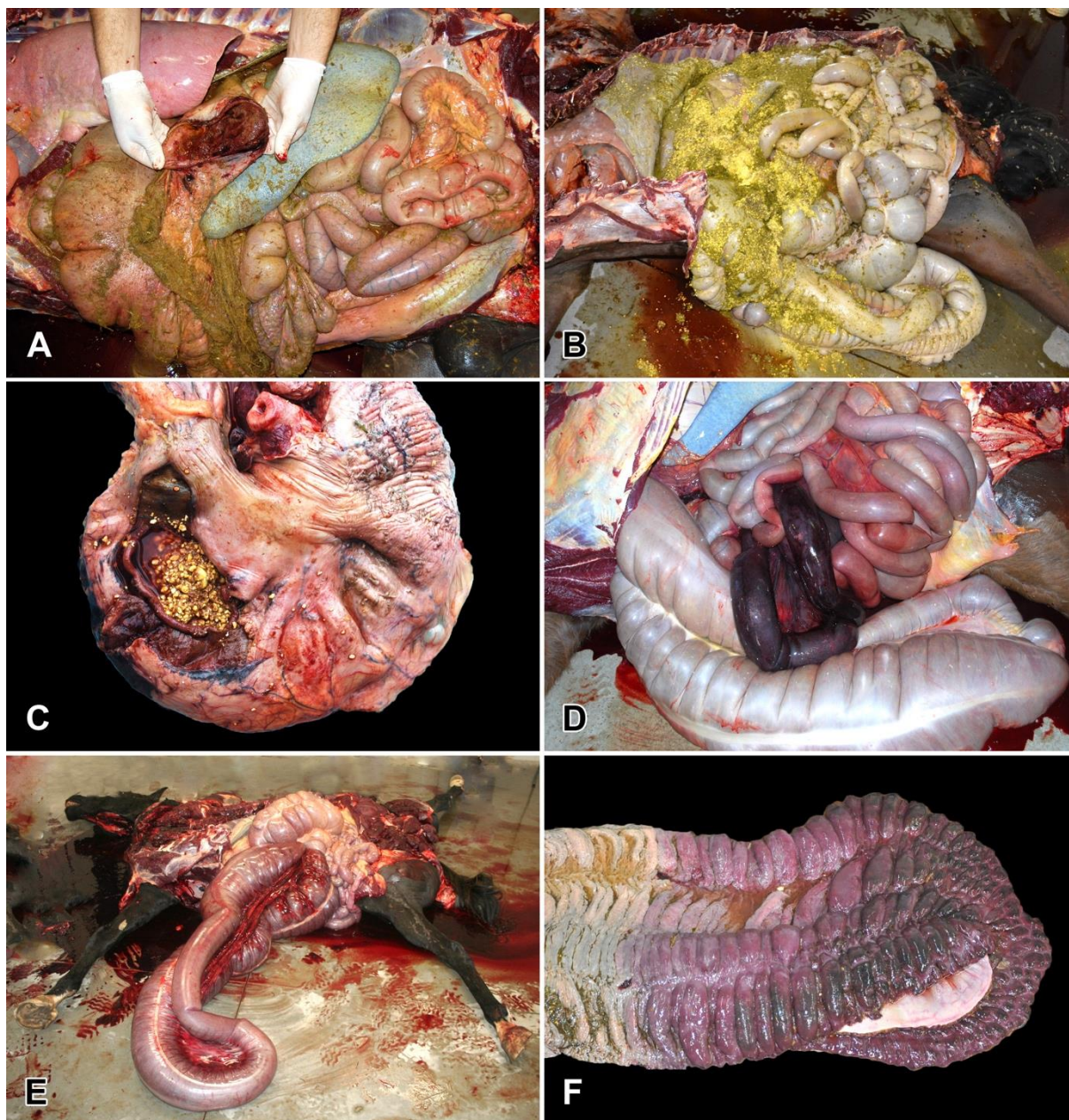


Fig.1. Gastric dilation and intestinal volvulus in horses in the Southern Brazil. **(A-B)** Rupture of stomach wall at the greater curvature with reddened edges, and a release of mild to large amounts of feed content into the abdominal cavity causing peritonitis. **(C)** The serosa of the stomach at the greater curvature was lacerated, with adjacent hemorrhages, and dissected the muscular layer. **(D)** A segment of the small intestine had a twist around its mesenteric axis, which caused intestinal ischemia, with deep black-red serosa of this segment. **(E)** The large colon had a 360° twist (volvulus) which caused a complete intestinal ischemia and diffuse dark-red serosa. **(F)** The left large colon (both ventral and dorsal) mucosa was diffusely reddish due to left large colon volvulus.

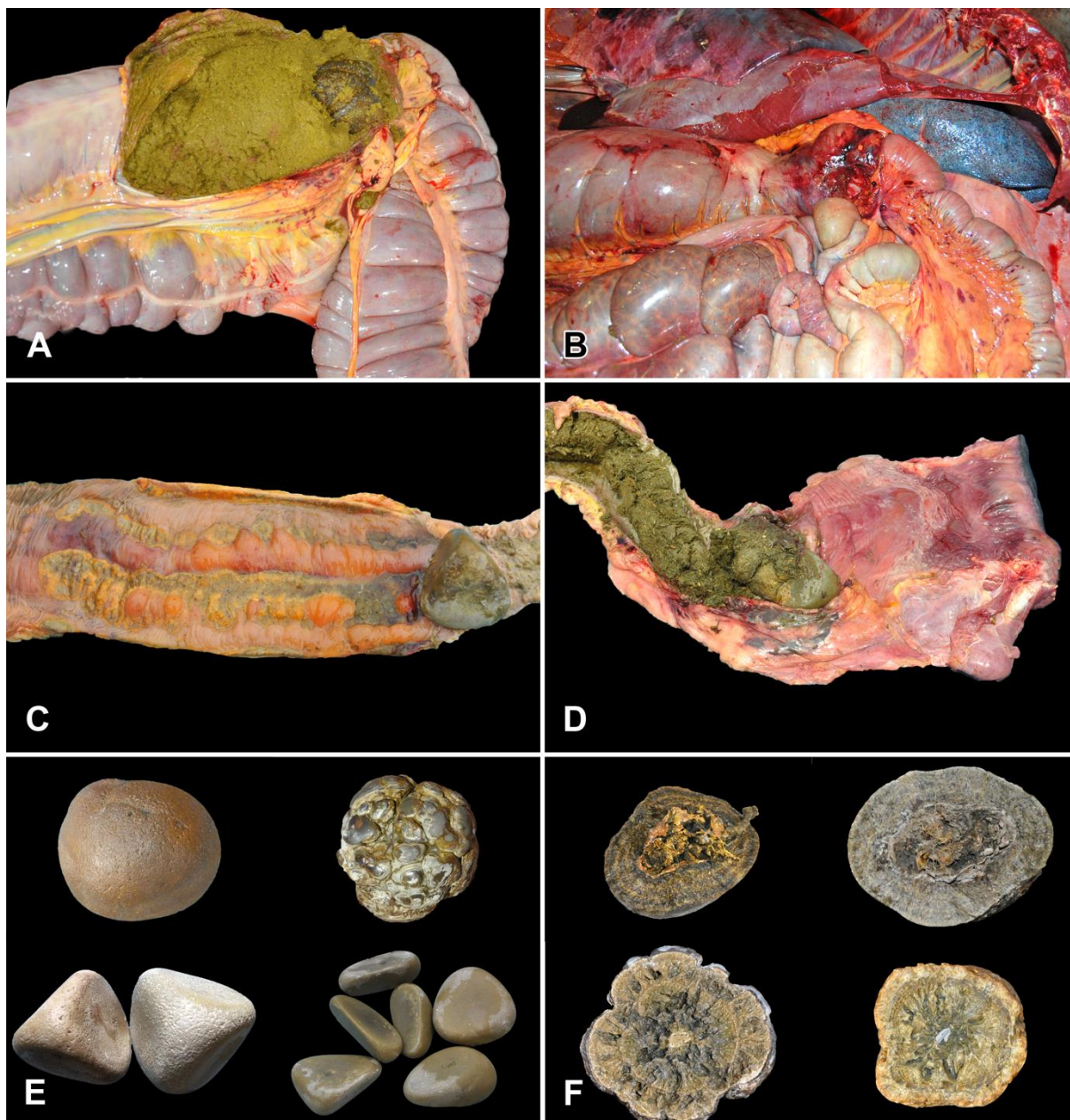


Fig. 2. Enterolithiasis in horses in the Southern Brazil. **(A)** A rounded enterolith has obstructed partially the lumen of the right dorsal large colon, and caused a secondary anterior large colon impaction. **(B)** The transverse colon was another common location of the enterolith, as in this case, where it completely obstructed the lumen of the organ. **(C)** This pyramidal to flattened enterolith was obstructing the final segment of the small colon, as well as it caused a severe focally extensive ulcer at the mucosa as it was moved posteriorly by the guts. **(D)** This flattened enterolith was located at the rectum, where it caused a complete obstruction, in addition to secondary anterior impaction. **(E)** Clockwise, concretions were rounded, irregular, and pyramidal to flattened. **(F)** Clockwise, the enteroliths had concentric layers centered on rope (two cases), wire, and stone.

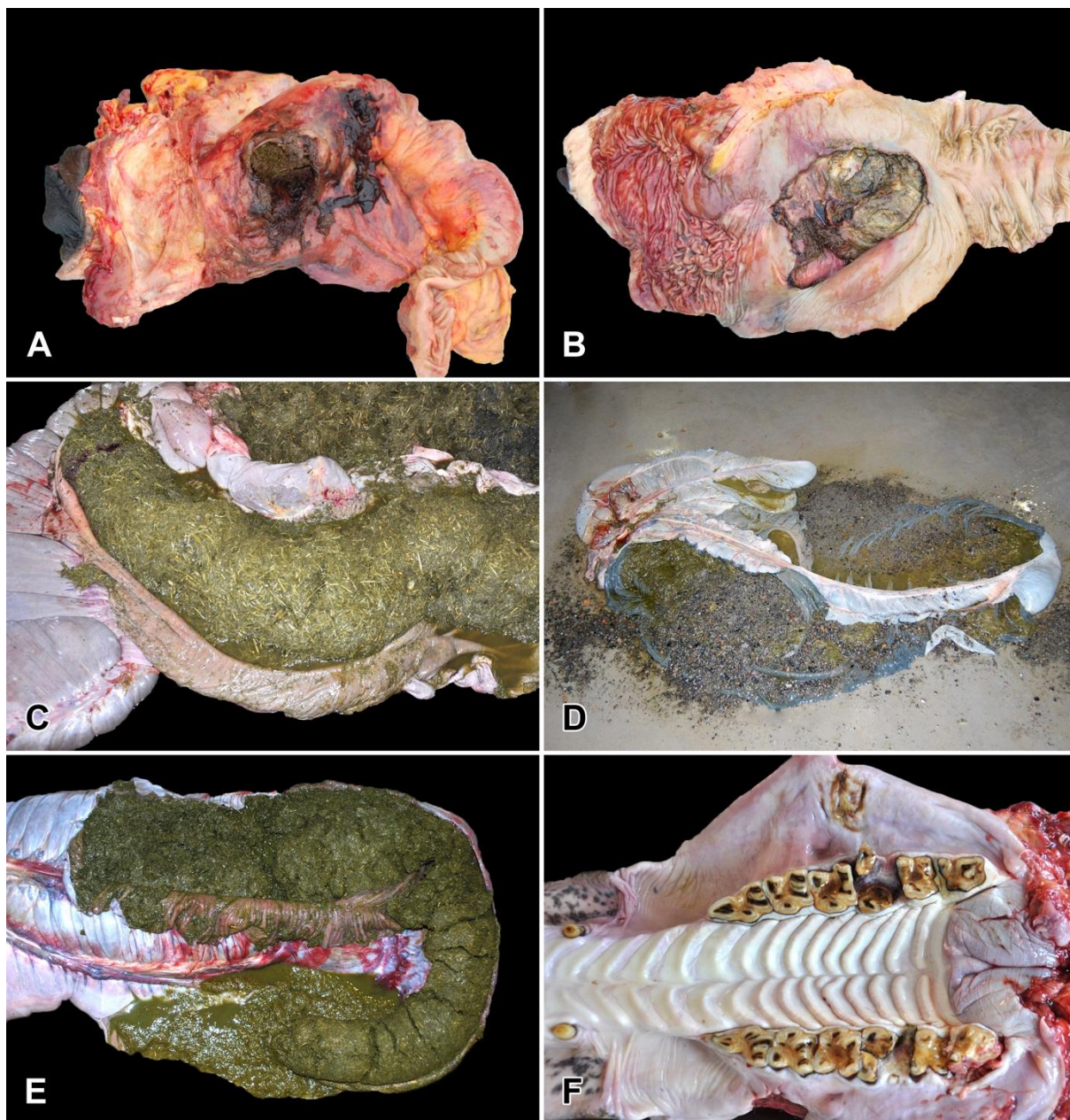


Fig. 3. Rectal (colonic) perforation and cecocolonic impaction in horses in the Southern Brazil. **(A)** The rectum displays a focally extensive area of rupture characterized by a transmural tearing, associated to reddened borders. **(B)** The mucosa of this ruptured area was severely ulcerated and covered by large amounts of fibrin and feces. **(C)** Impaction of the right ventral large colon by large amounts of dry digesta. **(D)** Impaction was also caused by variable amounts of sand material within the lumen of the large colon. **(E)** Locations affected within the large colon were variable, as in this case, where the pelvic flexure was involved. **(F)** Poor dentition was a common predisposing factor to the development of impactions in horses, with shallow or absent maxillary teeth.

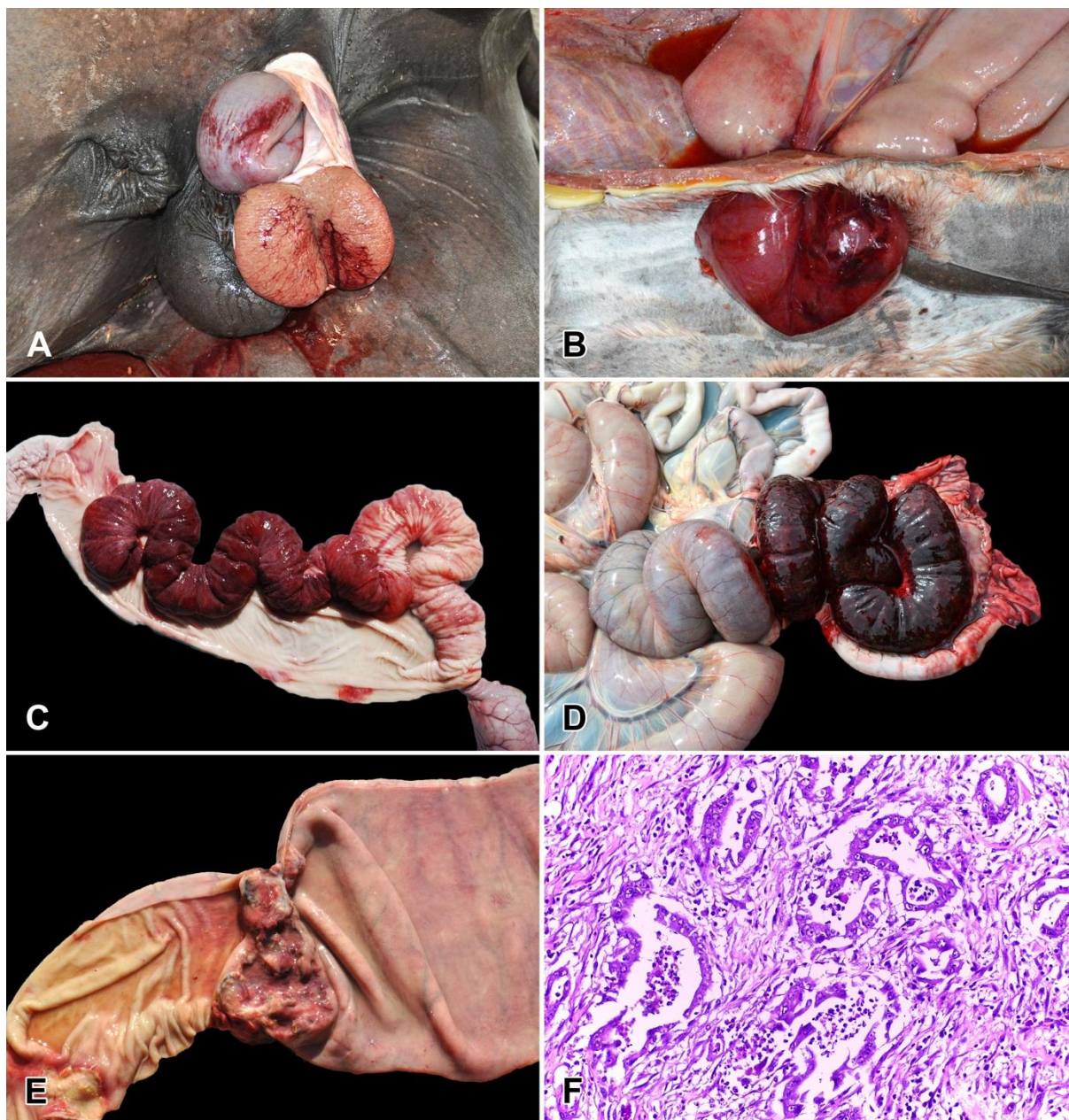


Fig. 4. Incarcerations or hernias, intussusceptions, and neoplasms in horses in the Southern Brazil. **(A)** A segment of the small intestine has passed through the inguinal canal and reached the scrotum, where these intestinal loops were constricted and presented a reddened serosa. **(B)** Umbilical eventration of a small bowel segment caused intestinal ischemia with dark-red serosa. **(C-D)** Small intestine intussusception characterized by the passage of an intussusceptum into an intussuscipiens, with the intussusceptum presenting a diffuse deep black-red discoloration. **(E)** A focal whitish mass (6 x 4 x 3 cm), with a friable aspect, occluded partially the intestinal lumen. **(F)** Histologically, this mass was composed by a neoplastic proliferation of epithelial cuboidal cells arranged in tubules and papillae extending into the muscular layer, often intermixed by desmoplastic reaction. Hematoxylin and eosin, 20x.

6. CONSIDERAÇÕES FINAIS

- Doenças respiratórias, especialmente, pneumonias são condições importantes em equinos abatidos em matadouro-frigorífico, assim como em equinos necropsiados no Sul do Brasil;
- Broncopneumonias supurativas causadas por *Streptococcus equi* subsp. *zooepidemicus* foram a principal condição identificada em equinos abatidos em matadouro-frigorífico, e provavelmente estão relacionadas ao estresse causado por transportes a longas distâncias;
- Por sua vez, em equinos necropsiados no Sul do Brasil, pneumonias supurativas, subdivididas em agudas, subagudas e crônicas, e pneumonias piogranulomatosas por *Rhodococcus equi* foram as principais condições observadas relacionadas à causa de morte dos animais;
- Enterites e tiflocolites parasitárias são importantes causas de morte em equinos, especialmente naqueles com menos de 1,5 anos de idade no Sul do Brasil. Essas são principalmente causadas por ciatostomíneos, *Eimeria leuckarti*, *Strongyloides westeri* e *Balantidium coli*;
- Doenças não infecciosas do trato gastrointestinal foram identificadas como a principal causa de morte relacionada ao sistema alimentar em equinos, dentre as quais destacaram-se as seguintes condições: dilatação gástrica primária, vólvulos intestinais, enterolitíases, rupturas retais e compactações. Nessas, os aspectos epidemiológicos e as características macroscópicas das lesões são essenciais para que o diagnóstico conclusivo da condição seja obtido.

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