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DOENÇAS DE CAPRINOS NO CENTRO-OESTE DO BRASIL

Santa Maria, RS, Brasil

2017

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Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Medicina Veterinária, Área de Concentração em Patologia e Patologia Clínica Veterinária, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de **Doutor em Medicina Veterinária.**

Orientadora: Prof. Mara I. B. Rubin

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Santa Maria, RS
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RESUMO

DOENÇAS DE CAPRINOS NO CENTRO-OESTE DO BRASIL

AUTOR: Fabio Brum Rosa
ORIENTADORA: Mara I. B. Rubin
Santa Maria, 25 de Janeiro de 2017

Esta tese envolveu o estudo retrospectivo e prospectivo de doenças de caprinos diagnosticadas no Laboratório de Anatomia Patológica da Universidade Federal de Mato Grosso do Sul. Para isso, foram examinados os laudos de necropsias de caprinos realizadas de Janeiro de 2012 a Agosto de 2015. Durante o período estudado foram realizadas necropsias de 74 caprinos, que resultaram em 72 diagnósticos conclusivos (em 6 necropsias houve mais de um diagnóstico) e em 16 não foi possível concluir por um diagnóstico. Do total de 72 diagnósticos conclusivos, 65,3% eram de doenças infecciosas e parasitárias, 25,0% eram de intoxicações e toxinfecções e 8,3% eram de doenças metabólicas e nutricionais. Outros distúrbios foram diagnosticados em menor proporção (1,4%). Hemoncose foi a condição mais prevalente sobre o total de diagnósticos (27,8%), seguida de broncopneumonia bilateral (20,8%) e de intoxicacao por *Brachiaria decumbens* (18,1%). Este estudo resultou em quatro trabalhos científicos: (1) Spontaneous poisoning by *Brachiaria decumbens* in goats; (2) Renal encephalopathy due to acute renal failure in a goat; (3) Granulomatous leptomeningitis in a goat associated with infection by *Cryptococcus neoformans* e (4) Hepatogenous chronic copper toxicosis associated with grazing *Brachiaria decumbens* in a goat. Esses quatros trabalhos já estão publicados e estão anexados a esta tese.

Palavras-chave: Doenças de caprinos. Doenças infecciosas e parasitárias. Intoxicações e toxinfecções. Hemoncose. Intoxicacao por *Brachiaria decumbens*.

ABSTRACT

DISEASES IN GOATS IN THE MIDWESTERN BRAZIL

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The data herein described is a compilation of retrospective and prospective studies of diseases in goats diagnosed at the *Laboratório de Anatomia Patologica* at the *Universidade Federal de Mato Grosso do Sul*. This investigation examined 74 necropsy reports of goats obtained for the period between January 2012 and August 2015. The results yielded 72 conclusive diagnoses (in 6 cases there was more than one diagnosis by necropsy), and 16 it was not possible to conclude for a diagnosis.. Of the 72 conclusive diagnoses, 65.3% were infectious and parasitic diseases, 25.0% were intoxications and toxinfections, and 8.3% were metabolic and nutritional diseases. Other disorders were diagnosed on one occasion only (1.4%). Haemonchosis was the most prevalent condition in all diagnoses (27.8%), followed by bilateral bronchopneumonia (20.8%) and poisoning by *Brachiaria decumbens* (18.1%). Four scientific papers resulted from this study: (1) Spontaneous poisoning by *Brachiaria decumbens* in goats; (2) Renal encephalopathy due to acute renal failure in a goat; (3) Granulomatous leptomeningitis in a goat associated with infection by *Cryptococcus neoformans* and (4) Hepatogenous chronic copper toxicosis associated with grazing *Brachiaria decumbens* in a goat. These four papers are already published and attached to this thesis.

Keywords: Diseases of goats. Infectious and parasitic diseases. Intoxications and toxinfections. Haemonchosis. Poisoning by *Brachiaria decumbens*.

SUMÁRIO

1 INTRODUÇÃO

A caprinocultura tem se destacado no agronegócio brasileiro e a tendência é que se mantenha em expansão, seja por fatores como o crescimento natural da população e da renda, seja pela organização desses setores para expandir seus mercados, dado o seu potencial. (EMBRAPA, 2016). Vários fatores nos cenários nacional e internacional mostram esta vertente, como a mudança de atitude da população no que se refere à alimentação. Como a carne caprina é uma das mais magras, tem conquistado cada vez mais adeptos. Além disso, com o desenvolvimento da caprinocultura leiteira e, consequentemente, as mudanças no sistema de criação, de extensivo para semi-intensivo, aumentaram a incidência e a gravidade das doenças que afetam o rebanho caprino, tornando necessária a pesquisa de medidas de controle cada vez mais eficazes e que tornem a atividade economicamente viável (CAVALCANTE et al., 2010). Segundo dados do IBGE, o rebanho nacional de caprinos em 2014 alcançou 8.851.879 cabeças, sendo 91,6% na Região Nordeste. A região Centro-Oeste ainda possui um rebanho pouco numeroso, mas do efetivo de 101.889 cabeças de caprinos da região, o maior número (37.927) está no Estado de Mato Grosso do Sul (IBGE, 2014).

Não há estudos retrospectivos abrangentes das causas de morte de caprinos no MS, não havendo também como estimar as perdas econômicas a relacionadas a elas.. Vários estudos têm sido realizados sobre doenças ou grupos de doenças de caprinos no Brasil (BANDARRA et al., 2011; LIRA et al., 2013; RIET-CORREA et al., 2008; RODRIGUES et al., 2007; ROSA et al., 2013). Estudos retrospectivos são importantes, pois determinam a prevalência das doenças que ocorrem em determinada espécie e em determinada região. Esses estudos permitem que clínicos, patologistas e veterinários de campo tenham acesso a uma lista de diagnósticos diferenciais mais frequentes em determinada região geográfica ao considerar o diagnóstico de uma doença.

Neste trabalho, foram estudadas as causas de morte de 74 caprinos provenientes de um capril na área de abrangência do Laboratório de Anatomia Patológica da Universidade Federal de Mato Grosso do Sul (LAP-UFMS). Os objetivos deste estudo foram determinar a prevalência e as características epidemiológicas e clínico-patológicas das doenças mais prevalentes. Desse estudo resultaram quatro trabalhos científicos já publicados.

A Introdução, Material e Métodos, Resultados e Discussão obtidos desses quatro trabalhos são, na íntegra, apresentados a seguir.

2 MATERIAL E MÉTODOS

Todo os casos presentes neste estudo foram provenientes de uma propriedade rural com rebanho de aproximadamente mil caprinos de diferentes categorias. A propriedade é exclusivamente voltada à caprinocultura de corte e os animais são provenientes de várias regiões do Brasil, especialmente do Nordeste. A propriedade é dividida em 13 lotes e as cabras são criadas em um sistema semi-intensivo, ou seja, permanecem no pasto durante o dia, e recebem suplementação com ração quando são recolhidas no final da tarde. A pastagem de todos os lotes é exclusivamente constituída por *Brachiaria decumbens*. Dos laudos foram coletados os dados epidemiológicos e os achados clínico-patológicos dos caprinos necropsiados. Os diagnósticos foram classificados em conclusivos e inconclusivos. Os casos com diagnóstico conclusivo foram agrupados, de acordo com a etiologia, em: 1) doenças infecciosas e parasitárias, 2) intoxicações e toxinfecções, 3) doenças metabólicas e nutricionais, 4) distúrbios iatrogênicos e 5) outros distúrbios.

3 RESULTADOS E DISCUSSÃO

Nesta tese são incluídos quatro artigos referentes às necropsias de caprinos realizadas no LAP-UFMS no periodo de Janeiro de 2012 a Agosto de 2015. Esses artigos incluem casos de intoxicação por *Brachiaria decumbens*; encefalopatia renal; leptomeningite granulomatosa causada por infecção por *Cryptococcus neoformans* e intoxicação por cobre. As demais doenças que não foram abordadas nestes artigos, foram incluídas no Quadro 1.

Durante o período estudado (2012-2015) foram realizadas necropsias de 74 caprinos, que resultaram em 72 diagnósticos conclusivos (em 6 casos havia mais de um diagnóstico por necropsia) e 16 casos com diagnóstico inconclusivo. Do total de 72 diagnósticos conclusivos, 65,3% eram de doenças infecciosas e parasitárias, 25,0% eram de intoxicações e toxinfecções e seis eram de doenças metabólicas e nutricionais. Outros distúrbios foram diagnosticados em uma ocasião (1,4%).

Do total das 74 cabras necropsiadas, 50 (67,6%) não tinham raça definida, 23 (31,1%) eram da raça Boer e uma (1,4%) era da raça Saanen. O sexo foi informado em todos os 74 protocolos de necropsia e havia 60,8% de fêmeas e 39,2%, de machos. A idade variou entre neonato e cinco anos de idade. Serão discutidos aqui somente os diagnósticos mais prevalentes de cada categoria e que não foram incluídos nos artigos científicos.

Hemoncose

Hemoncose foi a condição mais prevalente sobre o total de diagnósticos (27,8%). A idade dos animais afetados variou de seis meses a quatro anos. Os principais sinais clínicos apresentados foram: palidez das mucosas oral e ocular, apatia, emagrecimento progressivo, decúbito e edema subcutâneo da região submandibular. Os achados de necropsia mais frequentes foram ascite, hidropericárdio, edema pulmonar, acentuação do padrão lobular hepático e grande quantidade de parasitas (morfologicamente compatíveis com *Haemonchus* sp.) no abomaso na maioria dos casos. Em cinco dos vinte casos não foram observados nematódeos no abomaso. Microscopicamente observou-se degeneração e/ou necrose hepatocelular centrolobular em cinco casos.

Hemoncose foi também a doença mais prevalente em estudos retrospectivos das espécies caprina (ROSA et al., 2013) e ovina (RISSI et al., 2010). Portanto, demonstra grande importância como causa de morte em pequenos ruminantes, pois causam mortalidade e, principalmente, comprometem o processo produtivo, com redução do ganho de peso e da

qualidade da carcaça (MEDEIROS et al., 2009). Esta parasitose ocorre em locais com verões chuvosos, particularmente em regiões tropicais e subtropicais (CAVALCANTE et al., 2010). O diagnóstico de hemoncose nos casos aqui estudados foi realizado pelos achados clínico-patológicos característicos (BROWN et al., 2007), o qual é facilitado quando se observa grande número de exemplares do nematódeo (*Haemonchus contortus*) no abomaso. Entretanto, ausência dos parasitas no abomaso, observado em alguns casos, pode ser devido à autólise ou tratamento recente com antiparasitários (RADOSTITS et al., 2007). O pronto diagnóstico durante a necropsia permite que o proprietário aja rapidamente e empregue medidas de controle no rebanho. Diagnósticos diferenciais para anemia em caprinos incluem: leptospirose, intoxicação por cobre, coccidiose, fasciolose e deficiência de cobalto (NAVARRE & PUGH, 2002). Hemoncose foi diagnosticada associada com pneumonia em dois casos.

Broncopneumonia fibrinopurulenta bilateral com pleurite

Broncopneumonia bilateral foi a segunda condição mais prevalente neste estudo perfazendo 20,8% sobre o total de diagnósticos conclusivos. A idade dos animais afetados variou de dois meses a dois anos; em nove dos quinze casos os caprinos tinham cinco meses. Os principais sinais clínicos foram apatia, tosse e dificuldade respiratória. O curso clínico variou de 1-5 dias. Macroscopicamente observou-se marcada consolidação cranioventral de ambos os pulmões, e a parte consolidada estava vermelha escura ou roxa e coberta por múltiplos filetes de fibrina. Microscopicamente a broncopneumonia era caracterizada por áreas multifocais e extensas de infiltrado inflamatório preenchendo e dilatando os espaços aéreos (brônquios, bronquíolos e alvéolos) composto por neutrófilos íntegros e degenerados e macrófagos. Havia também áreas de necrose, hemorragia e agregados bacterianos cocoides e basofílicos. A pleura estava espessada por tecido conjuntivo fibroso e infiltrada de neutrófilos e macrófagos. Foram enviados fragmentos de pulmão para isolamento bacteriano, mas em nenhum caso se obteve crescimento, provavelmente devido ao tratamento com antibiótico realizado pelo veterinário da propriedade.

Infecções respiratórias causam grandes perdas econômicas decorrentes de retardo no ganho de peso e custos com diagnóstico e tratamento e são causadas pela interação de uma variedade de fatores que culminam na colonização microbiana do pulmão. Muitas vezes a pneumonia é de origem multifatorial; dentre as causas, a falha na ingestão de colostro é fator importante implicado na ocorrência mais frequente de broncopneumonias nos animais jovens (CASWELL & WILLIAMS, 2007). Dos 15 casos de broncopneumonia deste estudo, 10 foram

em animais de até cinco meses de idade. Outro fator importante na epidemiologia desta doença são as condições ambientais desfavoráveis que incluem mistura de animais de diferentes origens e faixas etárias, alta densidade animal, poeira e ventilação inadequada (CASWELL & WILLIAMS, 2007). Todas essas características ambientais estavam presentes na propriedade estudada. Os principais agentes envolvidos com as broncopneumonias em pequenos ruminantes são *Pasteurella* spp., *Chlamydia* sp, *Mycoplasma* spp. e *Streptococcus* spp., que ocasionam as broncopneumonias do complexo doença respiratória que podem ocorrer de forma enzóotica principalmente nos sistemas intensivos de criação ou na forma de surtos, denominada pasteurelose pneumônica ou febre dos transportes que é uma doença respiratória grave associada com *Pasteurella multocida* ou *Mannhemia haemolytica* (CASWELL & WILLIAMS, 2007).

Pneumonia por aspiração

Pneumonia por aspiração foi observada em 5,6% do total de diagnósticos conclusivos e ocorreu forma de broncopneumonia supurativa unilateral direita caracterizada por consolidação e escurecimento do lobo cranial direito com adesão à pleura costal. Em todos os quatro casos, além da lesão pulmonar havia lesão hepática acentuada devida à intoxicação por *Brachiaria decumbens* e que também contribuiu para a morte dos animais. Microscopicamente observou-se acentuado infiltrado de neutrófilos e macrófagos dilatando e preenchendo o lúmen de brônquios, bronquíolos e alvéolos associado a extensas áreas de necrose, hemorragia, deposição de fibrina e ocasionais fibras vegetais.

Broncopneumonia unilateral do lado direito é característica de pneumonia por aspiração. O conteúdo do rúmen é altamente irritante e causa uma pneumonia necrosante e fibrinosa. O lobo pulmonar cranial direito tende a ser mais severamente afetado porque o brônquio direito cranial é o ramo mais cranial e entra no aspecto ventrolateral da traqueia (LÓPEZ, 2012). O péssimo estado nutricional e a marcada emaciação causada pelo dano hepático devido à ingestão de *Brachiaria decumbens* podem ter contribuído para pneumonia aspirativa observadas nessas cabras.

Encefalomalácia focal simétrica

Os dois caprinos que morreram devido à encefalomalácia focal simétrica tinham quatro e seis meses de idade e apresentavam cegueira, mucosas hiperêmicas, apatia e decúbito lateral por quatro dias. Não foram observadas lesões macroscopicamente. Microscopicamente, no

mesencéfalo havia duas áreas focais bilaterais simétricas de malácia, caracterizadas por palidez e rarefação do neurópilo, com infiltrado leve de neutrófilos, tumefação endotelial, edema perivascular, hemorragias e tumefação axonal (esferoides). No rúmen de um caprino havia pequenas áreas multifocais de necrose e restos celulares de neutrófilos na mucosa associada à degeneração epitelial e focos de hiperceratose (rumenite). Em ambos os casos os caprinos tinham sido vacinado contra enterotoxemia tipo D apenas uma vez. As lesões histológicas no mesencéfalo são características de encefalomalácia focal simétrica (OLIVEIRA et al., 2010; PIMENTEL et al., 2010). Esta doença é comum em ovinos e rara em caprinos e é uma manifestação neurológica crônica da enterotoxemia causada pela proliferação no intestino de *Clostridium perfringens* tipo D e liberação de toxina épsilon (PIMENTEL et al., 2010). Imunidade deficiente devido a protocolo de vacinação incorreto, baixa eficácia da vacina usada, e dieta com concentrado não controlado foram fatores predisponentes apontados para o surgimento da doença. Adicionalmente, a proliferação de toxina épsilon em animais jovens, recém-desmamados é devido à inibição de tripsina pelo colostro. Sabe-se que a tripsina controla a quantidade de toxina épsilon, então com a diminuição de tripsina há um aumento de toxina épsilon culminando em enterotoxemia (UZAL & SONGER, 2008). Encefalomalácia focal simétrica ocorre de forma esporádica e tem sido relatada em leitões desmamados e ovelhas adultas quando há uma mudança brusca na dieta (UZAL & SONGER, 2008). O diagnóstico de encefalomalácia focal simétrica nesses casos foi baseado nas lesões histológicas similares às observadas em outro relato desta doença em caprinos, consideradas patognomônicas da doença (OLIVEIRA et al., 2010).

Desnutrição

Todos os casos de desnutrição ocorreram entre junho e setembro, que correspondem à estação de seca na região. Dois caprinos tinham quatro meses de idade e um caprino tinha seis meses de idade. Os animais apresentavam marcada letargia e fraqueza. Macroscopicamente havia ausência de tecido adiposo associado a marcado consumo das reservas corporais, caracterizada por atrofia serosa da gordura. Não foram observadas lesões histológicas. Como observados nos casos aqui descritos, a desnutrição ocorre principalmente em caprinos jovens ou recém-nascidos, devido à ingestão insuficiente de colostro, pastagem escassa ou comportamento materno inadequado da cabra (RISSI et al., 2010).

Quadro 1. Doenças de caprinos necropsiados pelo Laboratório de Anatomia Patológica, Universidade Federal de Mato Grosso do Sul, de março de 2012 a agosto de 2015.*

Doenças infecciosas e parasitárias	**N**	**% do total**
Hemoncose	20	27,8
Broncopneumonia fibrinopurulenta bilateral com pleurite	15	20,8
Pneumonia por aspiração	4	5,6
Linfadenite caseosa	2	2,8
Ectima contagioso	1	1,4
Eimeriose	1	1,4
Meningite por *Cryptococcus neoformans*	1	1,4
Abscessos hepáticos secundários à rumenite	1	1,4
Aborto por *Sarcocystis* sp.	1	1,4
Endometrite	1	1,4
Total	**47**	**65,3**
Intoxicações e toxinfecções	**N**	**% do total**
Intoxicação por *Brachiaria decumbens*	13	18,1
Intoxicação crônica por cobre	2	2,8
Encefalomalácia focal simétrica	2	2,8
Enterotoxemia	1	1,4
Total	**18**	**25,0**
Doenças metabólicas e nutricionais	**N**	**% do total**
Desnutrição	3	4,2
Polioencefalomalácia	1	1,4
Urolitíase	1	1,4
Encefalopatia renal	1	1,4
Total	**6**	**8,3**
Distúrbios iatrogênicos	**N**	**% do total**
Edema pulmonar por falsa via de administração de soro	1	1,4
Total	**1**	**1,4**
Total geral	**72**	**100**

4 ARTIGO 1- Spontaneous poisoning by *Brachiaria decumbens* in goats

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Spontaneous poisoning by *Brachiaria decumbens* in goats[1]

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ABSTACT.- Rosa F.B., Martins T.B., Lemos R.A.A., Barros C.L. & Rubin M.I.B. 2014. **Spontaneous poisoning by *Brachiaria decumbens* in goats.** Pesquisa Veterinária Brasileira 00(00):000-000. Departamento de Patologia, Universidade Federal de Santa Maria, Av. Roraima 1000, Camobi, Santa Maria, RS 97105-900, Brazil. E-mail: claudioslbarros@uol.com.br

This paper describes an outbreak of *Brachiaria decumbens* poisoning in goats in the state of Mato Grosso do Sul, Brazil. Of a herd of about 1,000 goats, seven goats that showed clinical signs died and were necropsied. Clinical signs included lethargy, dehydration and weight loss in all animals. Three goats showed signs of photosensitivity and four goats had jaundice. In all the goats there were macroscopic lesions in the liver. All livers had change in size - five were increased and two decreased; six had change in color - two were orange, two yellow and two gray; and two had accentuation of lobular pattern and in another two had punctate depressions, linear or stellate, whitish and slightly depressed and randomly distributed by capsular surface. In addition were observed macroscopic changes attributable to liver failure as hydropericardium (2/7) and ascites (2/7). Histologically, the main changes observed which contributed to the diagnostic by *B. decumbens* poisoning in goats of this outbreak were accumulation of refringent crystals in the bile ducts and infiltration of foamy macrophages in the liver of all goats. These cells were also present in the hepatic and mesenteric lymph nodes in two cases. The diagnosis of *B. decumbens* poisoning was based on epidemiology, clinical, necropsy and histopathology.

INDEX TERMS: *Brachiara* spp., saponins, diseases of goats, pathology, liver, photosensitization, plant poisoning.

RESUMO.- [Intoxicação espontânea por *Brachiaria decumbens* em caprinos]Neste trabalho, relata-se um surto de intoxicação por *B. decumbens* em caprinos no estado do Mato Grosso do Sul, Brasil. De um rebanho de aproximadamente 1.000 caprinos, sete cabras apresentaram sinais clínicos, morreram e foram necropsiadas. Sinais clínicos incluíam apatia, desidratação e emagrecimento em todos os animais. Três cabras apresentaram sinais de fotossensibilização e quatro cabras apresentaram icterícia. Em todas as cabras havia lesões macroscópicas no fígado. Todos tinham alteração no tamanho – cinco eram aumentados e dois, diminuídos; seis tinham alteração na cor – dois eram alaranjados, dois amarelados e dois, acinzentados; e dois tinham acentuação do padrão lobular e outros dois depressões puntiformes, lineares ou estreladas, brancacentas e levemente deprimidas distribuídas aleatoriamente pela superfície capsular. Adicionalmente, foram observadas alterações macroscópicas atribuíveis à insuficiência hepática como hidropericárdio (2/7) e ascite (2/7). Histologicamente, as principais alterações observadas, que contribuíram para o diagnóstico de intoxicação por *B. decumbens* nas cabras deste surto foram acúmulo de cristais refringentes nos ductos biliares e infiltrado de macrófagos espumosos no fígado de todas as cabras.

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Essas células também foram vistas nos linfonodos mesentéricos e hepáticos em dois casos. O diagnóstico de intoxicação por braquiária foi baseado na epidemiologia, achados clínicos, de necropsia e histopatológicos.

TERMOS DE INDEXAÇÃO: *Brachiara* spp., saponinas, doença de caprinos, patologia, fígado, fotossensibilização, intoxicação por plantas.

INTRODUCTION

Brachiaria spp. (Poaceae) is an important forage plant in the cattle pasture in the Brazilian cerrado. The easy diffusion, high-capacity production of green mass, good adaptation to low fertility soils and growth capacity during most of the year makes this grass a good source of food for ruminants (Tokarnia et al. 2012). However *Brachiaria* spp. presents toxicity to ruminants and significant economic losses resulting from this intoxication. The main species of cultivated *Brachiaria* are *B. decumbens*, *B. brizantha* and *B. humidicola* (Andrade et al. 2004), of which *B. decumbens* is the most toxic (Riet-Correa et al. 2011).

Lithogenic steroidal saponins that induce crystal formation in the biliary system are responsible for hepatogenous photosensitization in the intoxication by *Brachiaria* spp. (Cross et al. 2000). These crystals have been reported as insoluble salts resulting from the metabolism of saponins, toxic principle of *Brachiaria*, in the digestive tract of ruminants (Driemeier et al. 2002). *Brachiaria* spp. it has been associated to photosensitivity and death in cattle (Lemos et al. 1997, Ecco et al. 2004), sheep (Lemos et al. 1996, Brum et al. 2004, Albernaz et al. 2008), goats (Lemos et al. 1998), buffalos (Rozza et al. 2004, De Oliveira et al. 2013) and horses (Barbosa et al. 2006), and the clinical and pathological aspects is similar in all species.Intoxication occurs at any time of year (Souza et al. 2010), but most outbreaks are observed at the beginning of the rainy season when the pasture is greener and vigorously and when the plants are young, with higher amounts of saponins (Riet-Correa et al. 2011). Sheep and cattle are the most affected species. Sheep are more susceptible than cattle to poisoning and young animals (calves and lambs) are more susceptible than adults (Riet-Correa & Méndez 2007). Goats seem to be more susceptible than sheep because in a study where the two species were kept in the same pasture, only the goats got sick (Lemos et al. 1998). Nonetheless, spontaneous poisoning of *Brachiaria* spp. it appears to be rare in goats (Johnson et al. 1999, Lira et al. 2013, Rosa et al. 2013). In Brazil it was found only one report, which were two outbreaks that occurred liver damage and photosensitivity (Lemos et al. 1998).

The onset of clinical signs after ingesting the toxin is variable and depends on the content of saponins present in the grass, but usually begin 7-10 days after the introduction of grazing animals (Riet-Correa & Méndez 2007). Clinically, affected animals show typical signs of photosensitivity including photophobia and photodermatitis characterized by erythema and edema followed by ulcerations and crusting; regions of the face, ears, vulva and depigmented areas are the most affected. Jaundice is not always present. Anorexia, depression, prolonged recumbency, weight loss, eye discharge/hyperemic mucosa, nasal discharge and progressive weight loss are also observed in cases of poisoning by *Brachiaria* spp. (Tokarnia et al. 2012).

At necropsy, the main changes are skin lesions, different degrees of jaundice, subcutaneous edema, liver usually increased in size and yellowish or orange with accentuated lobular pattern, distension of the gallbladder and urine stained by bilirubin (Brum et al. 2007, Castro et al. 2011, Lemos et al. 2011). Histologically there is swelling, vacuolization and individual necrosis of hepatocytes, periportal fibrosis, bile duct proliferation, pericholangitis, cholangitis, and the presence of crystals or negative images in the lumen of the bile ducts and occasionally in the cytoplasm of hepatocytes (Riet-Correa et al. 2011, Mustafa et al. 2012). Characteristic foamy macrophages of poisoning by *Brachiaria* spp. are located mainly in the centrilobular zones. Foamy macrophages can also be seen in hepatic and mesenteric lymph nodes (Lemos et al. 1996, Riet-Correa et al. 2002, Gomar et al. 2005), spleen (Driemeier et al. 1998, Riet-Correa et al. 2002) and small intestine (Riet-Correa et al. 2002).

Spontaneous poisoning by *Brachiaria* spp. in goats is still little described in Brazil (Lemos et al. 1998). The aim of the current study is to describe a spontaneous outbreak of poisoning by *Brachiaria decumbens* in state of Mato Grosso do Sul that resulted in death of seven goats and present the epidemiological, clinical, necropsy and histopathology aspects of this outbreak.

MATERIALS AND METHODS

The cases described of this report occurred in a particular goat herd, and were seen as a part of a study of causes of death of goats in progress in this property. The epidemiological and clinical data were obtained in a visit to the farm with the owner and with the veterinarian responsible for the animals. Seven

goats were necropsied in these opportunities at the farm or at the Laboratory of Pathology of the Federal University of Mato Grosso do Sul. Fragments of several organs were collected and fixed in 10% formalin. The material was routinely processed for histopathology and stained with hematoxylin and eosin (HE). Lung fragments of three goats which also had pneumonia were collected, stored under refrigeration and sent to the bacteriology laboratory for bacterial culture and isolation. The goats were identified through the numbers 1-7.

RESULTS

Epidemiological and clinical findings

The outbreak occurred in Bandeirantes, a city near the Campo Grande, in Mato Grosso do Sul, in the months of May and June 2015. The goats were from a farm with herd of about a thousand goats of different categories. The property is exclusively focused on meat production and animals are from several regions of Brazil, especially the Northeast. The property is divided into 13 lots and goats are raised in a semi-intensive system, they remain in the pasture during the day and are grouped in the late afternoon and receive supplemental feed. The pasture of every lot consists exclusively of *Brachiaria decumbens*. The seven goats were between five months and five years old and were mixed breed. Clinical signs developed by goats included anorexia, lethargy, diarrhea, dehydration, weight loss, sternal recumbency followed by lateral recumbency and death. Three goats showed signs of photosensitivity characterized by facial edema, ulcers and crusts on the nose, nasal plan and tips of ears, swelling of the vulva and perineum and alopecia in the periocular and perioral regions and dorsum.

Pathology

Necropsy findings included alteration in liver staining, marked dehydration and serous atrophy of fat in all cases. The liver was orange in two goats and two were yellow with accentuation of lobular pattern. One of these four goats had shallow depressions randomly distributed in the parenchyma and capsular surface. The gallbladder was distended in these four animals. Two goats had a liver markedly decreased in size, gray and with numerous punctate areas, linear or stellate, depressed and whitish on the capsular surface. To the cut surface was markedly firm. Cutaneous lesions, previously described, were observed in three cases. In the three goats that developed respiratory signs was observed right unilateral suppurative bronchopneumonia characterized by blackening and consolidation of the parenchyma mainly in right cranial lobe with adhesion to the costal pleura. Other necropsy findings included hydropericardium (2/7) and ascites (2/7). Histologically, in all cases the main lesions found were limited to the liver and consisted of varying degree of periportal fibrosis and periportal mononuclear inflammatory infiltrate, neutrophilic and lymphoplasmacytic cholangitis and pericholangitis, bile duct proliferation, refringent crystals or acicular negative images of these crystals in the lumen of bile ducts and in the cytoplasm of hepatocytes. Foamy macrophages were observed in all cases, randomized, solitary or in groups, predominantly in centrilobular area. Occasionally they were fused to form multinucleated giant cells. Multifocally there were also slight swelling and finely granular cytoplasmic vacuolization of hepatocytes. Individual necrosis of hepatocytes was rare. In goats that developed photosensitive signs, vulva and skin lesions were characterized by areas of necrosis or loss of epithelium with replacement by cellular debris and sometimes by crusts. The dermis and submucosa of the vulva were markedly thickened by edema and neutrophil and also had vasculitis and hemorrhage in the submucosa. In goats that had unilateral pneumonia, this was characterized by extensive multifocal areas of infiltrate of neutrophils and histiocytes that filled the airspaces (bronchi, bronchioles and alveoli). Amid this infiltrated had areas of necrosis, hemorrhage and basophilic coccoid bacterial aggregates. In one case the pleura was markedly thickened by fibrous connective tissue and infiltration of neutrophils and macrophages. Bacteria were not grown in bacteriological examination. This fact is possibly due to antibiotics administered to the goats. In two cases there was multifocal infiltration of foamy macrophages in the hepatic and mesenteric lymph nodes, characterized by large groups of cells with abundant and finely granulated cytoplasm located both in the cortical and medullar zones. Fragments of brain, spleen, kidney and small intestine were analyzed in all cases and did not show lesions. Data relating to clinical signs and lesions presented by each goat are shown on Table 1.

DISCUSSION

The diagnosis of poisoning by *Brachiaria decumbens* was based on epidemiological and clinical-pathologic findings, which were very similar to those described in spontaneous and experimental cases of this poisoning in ruminants in Brazil and other parts of the world (Abas-Mazni et al. 1985, Opasina 1985, Lemos et al. 1998, Brum et al. 2007, Castro et al. 2011, Lemos et al. 2011, Mustafa et al. 2012, Oliveira et al. 2012). In Brazil, although the problem is often reported in cattle and sheep, there is only one report of poisoning by *Brachiaria* spp. in goats. In that study were reported two outbreaks of photosensitization associated with cholangitis in goats grazing *Brachiaria* spp. (Lemos et al. 1998). Others rare studies report the occurrence of the disease in goats in other parts of the world (Abas-Mazniet al. 1985, Opasina 1985).

Beef goat production is a recent activity in Mato Grosso do Sul, as well as throughout the cerrado region, where pastures consist almost exclusively of *Brachiaria* spp. is fed to ruminants. Cases of poisoning by plants of this genus are frequent in the region, which makes this study relevant also for goat breeders. Animals affected by poisoning reported in this study were born on the property, i.e., always fed of this plant. However his parents are from the Brazilian Northeast, where there are no reports of poisoning by *Brachiaria* spp. in goats. Recent studies in sheep show that there is genetic resistance to poisoning (Castro et al. 2011, Riet-Correa et al. 2011), i.e. animals from herds that have never had contact with the *Brachiaria* grasses are more susceptible than those created in this pastures (Lemos et al. 1996, Castro et al. 2011, Riet-Correa et al. 2011, Oliveira et al. 2012). Perhaps this genetic resistance observed in animals raised in *Brachiaria* pasture is due to reducing of the mechanism of the toxic effect of the plant such as greater metabolization capacity and degradation of toxic principle (Castro et al. 2011) and adaptation of ruminal microbiota (Albernaz et al. 2010). Moreover, it has been proven the existence of individual sensitivity to intoxication (Saturnino et al. 2010, Castro et al. 2011). For these reasons, explained a relatively small number of animals have been affected by intoxication in this property. In addition, there are probably cases of subclinical poisoning or cases where the clinical signs are unnoticed. It is further believed that the poisoning is underreported, as is well known by clinicians and owners and these don't send material for diagnostic laboratories.

The *Brachiaria* poisoning occurs at all times of the year, but most outbreaks have been recorded at the beginning of the rainy season (Lemos et al. 1996, Riet-Correa et al. 2011), when the pasture is greener and vigorous and the plants are young, because there are more amount of saponins (Tokarnia et al. 2012). However in a retrospective study of *Brachiaria* poisoning in cattle there was a greater number of outbreaks in June and July, i.e. in the dry season (Souza et al. 2010). The cases described here occurred in May and June, after months in which there were high rainfall in the region. However it was not possible to associate the outbreak to the recent rains even in the dry season. As for the susceptibility of goats to intoxication, there is disagreement in the literature when compared to sheep. There have been outbreaks where only goats were affected where there were sheep in the pasture (Lemos et al. 1998) and another in which only sheep showed clinical signs (Graydon et al. 1991). In the outbreak reported here it was not possible to determine this relationship, because the property was exclusively focused on beef goat production.

Clinically, the animals intoxicated in this outbreak showed signs similar to those previously reported for goats (Opasina 1985, Lemos et al. 1998) and also to sheep (Mustafa et al. 2012) and bovine (Souza et al. 2010). The clinical course and the age of the animals were quite variable. The most characteristic clinical signs of *Brachiaria* spp. poisoning is photosensitive, characterized by erythema and cutenous edema followed by necrosis with detachment of the skin in the most affected areas, which include the depigmented and thinner skin (Tokarnia et al. 2012). Besides edema, erythema and crusting on the face and ears, similarly to sheep, goats which have shown the photosensitization in this outbreak had skin lesions of the vulva and vagina most likely due to sunlight exposure of these sites, as tail is facing upward in goats. Photosensitization was observed in three of the seven affected goats, two of them had a depigmented skin and one had a pigmented skin. It is believed that the pathogenesis of photodermatitis is related to increased skin sensitivity due to the deposition of photodynamic filoeritrin pigment (Tokarnia et al. 2012). Four goats showed no photosensitizing signals. In such cases the major clinical signs were weight loss, dehydration and death in poor body condition that have been assigned to liver failure. A similar clinical presentation characterized by progressive weight loss and death without photosensitivity, has been reported in cattle in pastures of *B. decumbens* (Riet-Correa et al. 2002). In such cases foamy macrophages were viewed in liver, spleen, lymph nodes and small intestine submucosa; so it was suggested that wasting was due to intestinal granulomatous lesion (Riet-Correa et al. 2002). Foamy macrophages were not observed in the small intestine in all the goats of this study. Therefore, more detailed studies are needed to determine the epidemiology and pathology of this form of *B. decumbens* poisoning and its probable relationship with the intake of lithogenic saponins. Three goats showed respiratory signs due to bronchopneumonia, which, in association with liver damage also contributed to the death of the animals. At all cases the bronchopneumonia was unilateral in right side, this kind of presentation is characteristic of aspiration pneumonia. The ruminal contents are highly irritating and cause a fibrinous, necrotizing

pneumonia. The right cranial lung lobe tends to be more severely affected because the right cranial bronchus is the most cranial branch and enters the ventrolateral aspect of the trachea (López 2012). The severe malnutrition observed in these goats could have contributed to aspiration pneumonia. Neurological signs due to hepatic encephalopathy and associated with photosensitization caused by *Brachiaria spp*. ingestion have been described in cattle (Souza et al. 2010) and sheep (Salam-Abdullah et al. 1989). These signs were not observed in this study. There are reports of a subclinical form of the disease in cattle that have less weight gain; this has been related to a negative correlation between the number of foamy macrophages in the liver and body weight in a slaughterhouse (Fioravante 1999).

The macroscopic and microscopic lesions observed in goats in this study is consistent of *Brachiaria decumbens* poisoning (Lemos et al. 1998, Riet-Correa & Méndez 2007, Souza et al. 2010, Mustafa et al. 2012, Tokarnia et al. 2012). The main lesion found in the necropsy is the discoloration of the liver, which can be yellow or orange. Hepatomegaly and distension of the gallbladder can also occur (Riet-Correa & Méndez 2007). These findings were present in five of the seven goats in this study. In two goats the liver was reduced in size and firm at the cut surface, with multiple punctuate to starry areas in the capsular surface. This pattern had been described in sheep (Tokarnia et al. 2012), but never in goats. Besides the liver lesion, photodermatitis and jaundice are also characteristics of *Brachiaria* poisoning. These lesions coexisted in three goats. Different degrees of jaundice are often described in poisoning in ruminants (Riet-Correa et al. 2011). In sheep, jaundice is an infrequent finding probably due to acute clinical course of the disease, different from that observed in cattle (Mustafa et al. 2012). Jaundice was frequently observed in other outbreaks in goats (Abas-Mazni et al. 1985, Opasina 1985, Lemos et al. 1998). The cause of jaundice in this disease is an obstruction of the bile ducts by crystals formed by aggregation of saponins, featuring a post-hepatic jaundice (Cross et al. 2001). At necropsy of animals showing clinical signs of weight loss and dehydration there were emaciation, marked serous atrophy of fat and varying degrees of cavity edema. These findings are related to anorexia and liver failure (Driemeier et al. 1999).

The main histological lesions observed in this outbreak were the presence of crystals in the lumen of the bile ducts and in the cytoplasm of hepatocytes, periportal fibrosis associated with mononuclear cell infiltration, cholangitis, proliferation of bile ducts and the presence of foamy macrophages. These lesions were responsible for hepatomegaly and changes in liver staining observed during autopsy. All these findings are typical of *Brachiaria spp*. poisoning and were also observed in sheep and cattle (Lemos et al. 2011). In two cases the liver was reduced in size, gray and firm due to extensive fibrosis observed histologically. In this case, there was periportal fibrosis, rather than desiccant and there was not megalocytosis, typical lesions of pyrrolizidine alkaloids poisoning (Riet-Correa & Mendez 2007). It is interesting to note that lesions directly induced by saponins are foamy macrophages and crystals in the bile ducts with subsequent cholangitis (Tokarnia et al. 2012). Hepatic fibrosis and proliferation of bile ducts are generic responses to aggression. In cattle, the finding of foamy macrophages only indicates that the animal ingested *Brachiaria spp*. or other plant which contains steroidal saponins and not necessarily who has developed the disease. Foamy macrophages are seen histologically in the liver of healthy slaughtered cattle (Driemeier et al. 1998). The formation of foamy macrophages takes time, so cannot be seen in the liver of animals that die quickly by hepatic insufficiency and not by photosensitization (Tokarnia et al. 2012). In sheep, the foamy macrophages are not present or are inconspicuous and less obvious, perhaps by the greater sensitivity to poisoning of these animals, which die more quickly, or their formation demand more time in this species (Tokarnia et al. 2012). The accumulation of crystals in the bile ducts in sheep is also infrequent (Mustafa et al. 2012), unlike in bovine (Souza et al. 2010) and goats in this study, which had great amount of crystals in the bile duct lumen. In goats of this study foamy macrophages were very obvious, formed large clusters and were randomized, but mainly in the centrilobular zone. It cannot be concluded, however, that goats are more resistant to *Brachiaria spp.* poisoning than sheep based only on this information. It is believed that the cytoplasm of the macrophages becomes foamy due to the presence of crystals. These crystals are formed from the connection of the saponin to glucuronic acid which, in turn, binds to calcium salts forming insoluble crystals (Driemeier et al. 2002, Santos 2008). Foamy macrophages are also described in hepatic and mesenteric lymph nodes, spleen and small intestine in cattle (Riet-Correa et al. 2011). In this report, however, these cells were seen in two cases in hepatic and mesenteric lymph nodes, in both cortical and medullary zones. Possibly remnants of the substances described above are excreted in the bile and reabsorbed by hepatic and mesenteric lymph nodes (Driemeier et al. 2002).

This study emphasizes the aspects of spontaneous poisoning by *Brachiaria decumbens* in goats, demonstrating that goats are susceptible to poisoning and this may be an important cause of death in this species. Through the results obtained with this study, it is concluded that: goats poisoned by *B. decumbens* may or may not have photosensitivity; the main clinical signs were dehydration, anorexia, lethargy and weight loss; have macroscopic changes in liver staining, which can become orange, yellow or gray, and in size, with a predominance of hepatomegaly; and have in all cases, microscopic lesions in the liver which

include the presence of crystals in the lumen of bile duct and in the cytoplasm of hepatocytes, periportal fibrosis associated with mononuclear infiltrates, cholangitis, bile duct proliferation and the presence of foamy macrophages.

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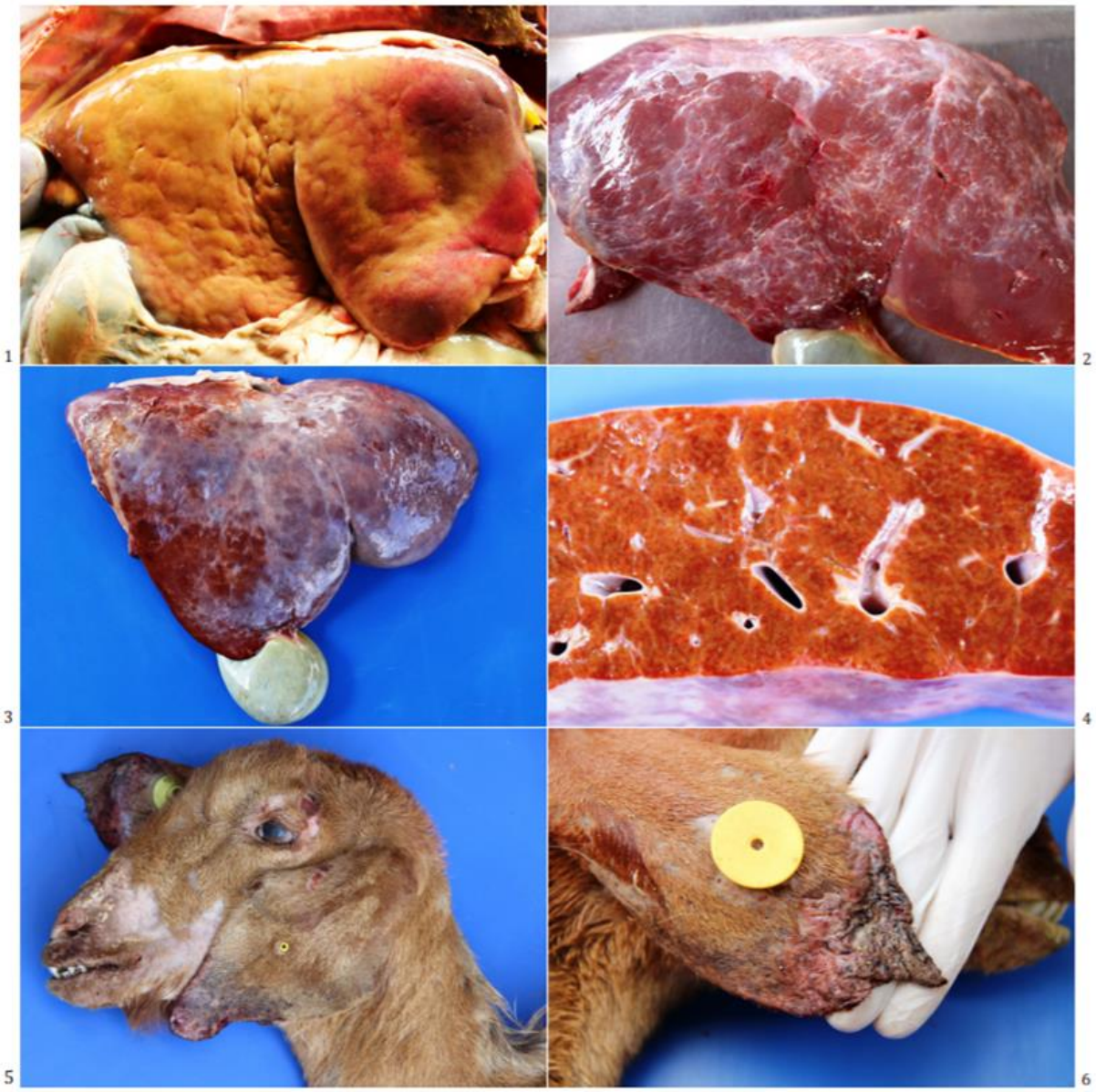

Fig.1. Liver increased in size, diffusely yellowish with irregular surface in Goat 6, succumbed from *Brachiaria decumbens poisoning*. Fig.2. Liver decreased in size and with multiple punctate, linearorstellate, depressed whitish areas on the capsular surface of Goat 1, succumbed from *Brachiaria decumbens* poisoning. Fig.3. Liver decreased in size with marked fibrosis, irregular capsular surface and atrophy of the left lobe in Goat 7, succumbed from *Brachiaria decumbens* poisoning. Fig.4. Cut surface of the liver showing marked fibrosis in Goat 7, ucumed from *Brachiaria decumbens* poisoning. Fig.5. Facial and periocular alopecia, crusting of the muzzle and necrosis of the ear tips in Goat 5, with photosensitization due to *Brachiaria decumbens* poisoning. Fig.6. Necrosis of the tip of the ear in Goat 5 poisoned by *Brachiaria decumbens*, with photosensitization due to *Brachiaria decumbens* poisoning.

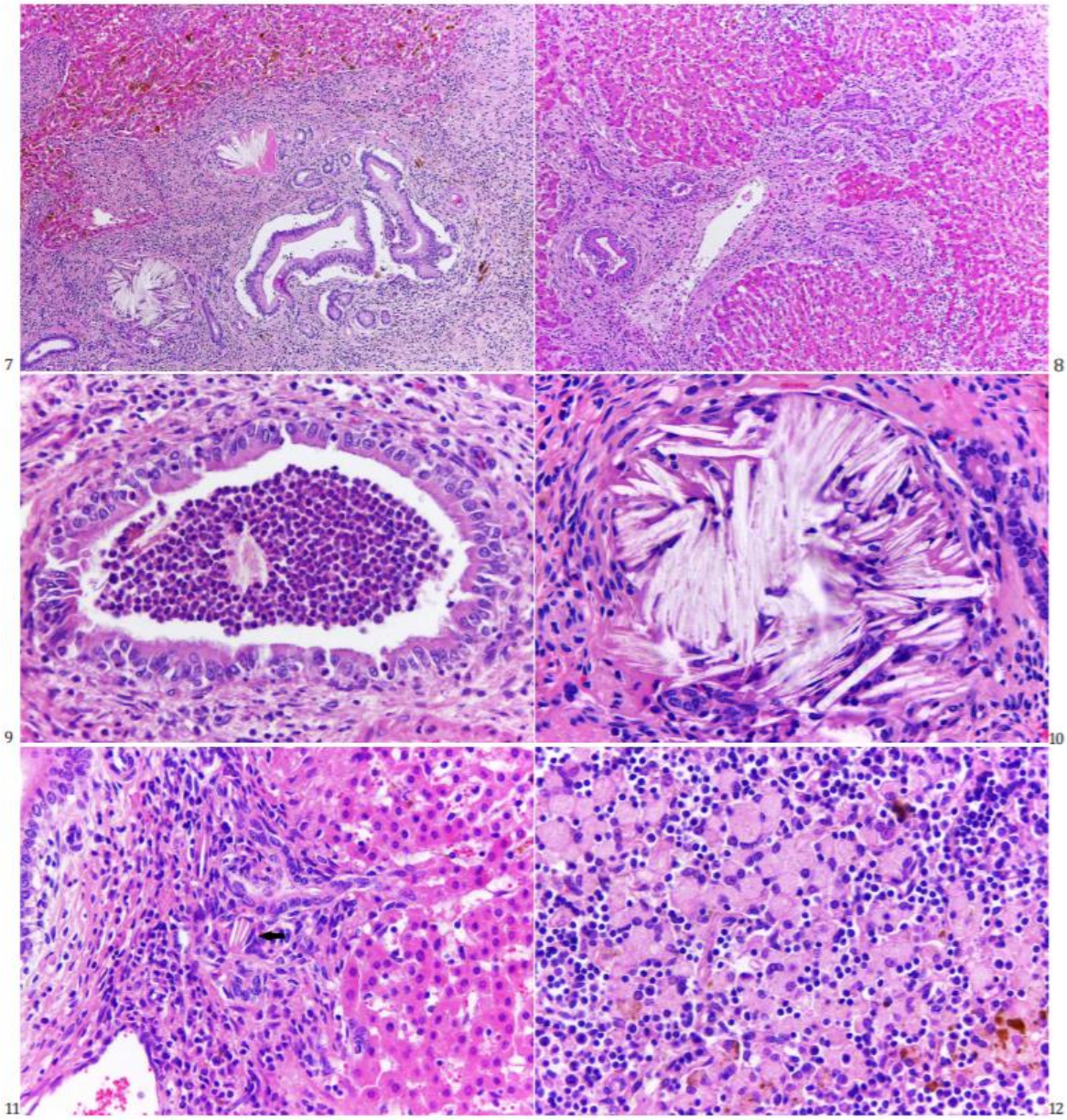

Fig.7. Histology of the liver of Goat 5, succumbed from *Brachiaria decumbens* poisoning. There is a focally extensive area of severe fibrosis, biliary duct proliferation and mononuclear infiltrate. Some biliary ducts are distended by refringent crystals. HE, obj.20x. Fig.8. Histology of the liver of Goat 7, succumbed from *Brachiaria decumbens* poisoning. Bridging fibrosis characterize portal tracts distended by fibrous connective tissue which extends from a portal triad to another portal area, associated biliary duct proliferation. HE, obj.20x. Fig.9. Histology of the liver of Goat 2, succumbed from *Brachiaria decumbens* poisoning. Biliary duct lumen with numerous neutrophils and a refringent crystal. HE, obj.40x. Fig.10. Histology of the liver of Goat 5, succumbed from *Brachiaria decumbens* poisoning. There is a biliary duct completely obliterated by refringent crystals or negative images of such crystals. HE, obj.40x. Fig.11. Histology of the liver of Goat 3, succumbed from *Brachiaria decumbens* poisoning. Negative images of the crystals within the cytoplasm of one macrophage (arrow). HE, obj.40x. Fig.12. Histology of hepatic lymph node from Goat 5, succumbed from *Brachiaria decumbens* poisoning. Numerous foamy macrophages in the lymph node parenchyma. HE, obj.40x.

Table 1. Age, main clinical signs and necropsy findingsin seven goats dead from poisoning by *Brachiaria decumbens*.

#	Age	Main clinical signs	Main necropsy findings
1	4 years-old	Dehydration, anorexia, apathy, weight loss, diarrhea.	Liver: decrease in size, grayish discoloration, multiple punctate, linear or stellate depressed whitish areas on the capsular surface. Distended gall bladder. Hydropericardium. Fat deposits: serous atrophy.
2	3 years-old	Dehydration, anorexia, apathy, weight loss, jaundice, photosensitization.	Liver: increase in size, diffuse yellowish discoloration. Unilateral bronchopneumonia. Fat deposits: serous atrophy.
3	5 month-old	Dehydration, anorexia, apathy, weight loss, cough, nasal discharge.	Liver: diffuse orange discoloration. Distended gall bladder. Unilateral fibrinosuppurative bronchopneumonia. Fat deposits: serous atrophy.
4	5 month-old	Dehydration, anorexia, apathy, weight loss, cough, nasal discharge, jaundice, photosensitivity,	Liver: increased in size, diffuse orange discoloration, accentuation of lobular pattern. Distended gall bladder, ascites. Fat deposits: serous atrophy.
5	4 years-old	Dehydration, anorexia, apathy, weight loss, jaundice, photosensitization.	Liver: increased in size, diffuse orange discoloration, accentuation of lobular pattern. Distended gall bladder, ascites. Fat deposits: serous atrophy.
6	8 month-old	Dehydration, anorexia, apathy, weight loss, cough, nasal discharge, jaundice.	Liver: increase in size, diffuse yellowish discoloration. Unilateral fibrinosuppurative bronchopneumonia. Fat deposits: serous atrophy.
7	5 years-old	Dehydration, anorexia, apathy, weight loss.	Liver: decrease in size, grayish discoloration, multiple punctate, linear or stellate depressed whitish areas on the capsular surface. Fat deposits: serous atrophy.

5 ARTIGO 2 – Renal encephalopathy due to acute renal failure in a goat

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Renal encephalopathy due to acute renal failure in a goat

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Abstract

Renal encephalopathy was diagnosed in a 7-month-old male goat with a history of diarrhea and dehydration due to *Eimeria* sp. infection. The goat was treated with sulfadiazine before developing central nervous system (CNS) signs characterized by severe anorexia, salivation, tremors, inability to stand and depression. Biochemical parameters revealed high levels of blood urea nitrogen (BUN) and creatinine, 263.6 mg/dl and 2.9 mg/dl respectively. No gross pathological changes were observed at necropsy. Histopathological examination of the brain revealed large irregular empty spaces (status spongiosus) in the white matter of the brainstem, cerebellum, thalamus, basal nuclei and in the interface of white and grey matter in the cerebrum. There was severe multifocal renal tubular necrosis characterized by abundant deposits of basophilic granular material, frequently forming crystals that replaced the lost tubular epithelial cells and filled the lumina. The clinical-pathologic findings support to a diagnosis of encephalopathy due to acute renal failure.

Key words: diseases of goats, renal encephalopathy, status spongiosus, acute renal failure, sulfadiazine.

Introduction

In humans, an encephalopathy subsequent to the development of severe renal failure is well recognized, but in the veterinary literature very few cases have been described (4, 7). Renal encephalopathy has been observed in cattle, goats, dogs, horses and woodchucks (1, 2, 5, 11, 14, 16). Although this is a diffuse encephalopathy, CNS signs are chiefly referable to the prosencephalon including various behavioral changes – staring into space, inappropriate vocalization, aggression, agitation, propulsive walking or circling. Other clinical signs included lethargy, head pressing, ataxia, blindness, collapse, and coma. Classically these signs wax and wane from day to day, and in many cases can be precipitated by feeding the animal with a high-protein diet (13). The nervous dysfunction is associated with extensive and well-developed vacuolation of myelin sheaths, (spongy degeneration or status spongiosus) which tends to be most intense at the interface of the cerebral cortex and adjacent white matter, and around the deep cerebellar nuclei. Spongy degeneration is found also in the internal capsule, thalamus, hypothalamus, cerebellar medulla and peduncles, and pons and medulla oblongata (9, 13, 14).

Numerous toxic substances used as therapeutic agents can cause acute tubular lesion in domestic species resulting in acute renal failure. Some of these agents are no longer important as nephrotoxins, even with the new formulations more soluble and less toxic, the sulfonamides still are important nephrotoxins (12). The toxicity of

these nephrotoxic agents is exacerbated by several systemic states, such as dehydration or shock, which concomitantly impaired renal function. Sulfonamides are a well-known cause of severe nephropathy when administrated in excessive doses and especially if treated animal is dehydrated (8). This substance leads to crystalline nephropathy characterized tubular necrosis and deposits of crystals in the tubular lumen (8, 10, 12, 15). In the past, the toxicity was much more common when only less-soluble forms of the drug were available, e.g., sulfapyridine, sulfathiazole, and sulfadiazine. Currently, due to new short-acting sulfonamides with greater solubility, sulfonamide toxicosis is a rare event (8).

Renal encephalopathy has rarely been previously reported in small ruminants. This study describes a case of renal encephalopathy with status spongiosus associated with severe acute renal failure in a goat.

Case report

A 7-month-old male goat was part of a group of 30 goats used in a metabolic experimental study at the Veterinary Hospital of FAMEZ/UFMS in the state of Mato Grosso do Sul, Brazil. The goat initially developed diarrhea and dehydration for two days. Due to a high count of *Eimeria* sp. oocysts per gram of feces the goat was treated with intramuscular sulfadiazine 20 mg/kg daily for five days. During these five days, the goat presented CNS signs characterized by severe salivation, tremors, inability to stand and apathy. BUN was 263.6 mg/dl (reference value 21.04-42.8 mg/dl) (6) and serum creatinine was 2.9 mg/dl (reference value 1.0- 1.8 mg/dl) (6). Albumin, aspartate aminotransferase and gama glutamiltransferase seric values were within normal reference values. At postmortem examination, no gross lesions were observed. Tissues from multiple organs, including the brain, heart, liver, lung, spleen, kidney, intestine, were sampled and fixed in buffered 10% formalin. Paraffin sections, 3 µm thick, were prepared and processed routinely and stained with hematoxylin and eosin (H&E) for histopathologic examination. Histopathological findings in the brain consisted of large empty intramyelinic spaces in the white matter in the brain stem, cerebellum (Fig. 1), thalamus, basal nuclei (Fig. 2) and in the interface of the cortical grey matter and subcortical white matter of the cerebrum. The kidney had severe tubular necrosis characterized by abundant deposits of basophilic granular material that replaced the lost tubular epithelial cells and filled the tubular lumina (Figs. 3 and 4). Frequently this material formed basophilic to amphophilic crystals. There were no significant microscopic changes in other organ systems.

Discussion

The diagnosis of renal encephalopathy due to acute renal failure induced by administration of sulfadiazine was based on clinical-pathologic findings. Renal encephalopathy is uncommon in domestic animals and there is only one report of this condition in goats, caused by diffuse, severe tubular and glomerular necrosis and degeneration of undetermined cause (11).

The pathophysiology of renal encephalopathy is complex and poorly understood. Accumulation of metabolites, hormones, disturbance in the intermediary metabolism and imbalance between excitatory and inhibitory neurotransmitters have been pointed as contributing factors (3). Renal failure results in accumulation of numerous organic substances that may act as uremic neurotoxins, but no single metabolite has been identified as the sole cause of renal failure (4). Some of the metabolites that accumulate include urea, guanidino compounds, uric acid, several amino acids and polypeptides (3, 4). As in this current case where high level of creatinine were found, in uremic human patients the creatinine was highly increased in serum, cerebrospinal fluid and brain (7). It is postulated that these compounds may contribute to the clinical signs accompanying uremic encephalopathy (3, 4, 7).

Histologically, the distribution of spongy degeneration in the brain of this goat is characteristic both of renal and hepatic encephalopathy in ruminants (13). No hepatic lesions were observed in the goat of this report, it's been assumed that the severe renal lesions were responsible for the renal encephalopathy. The typical histological lesion observed in these cases is polymicrocavitation or status spongiosus of the white matter and typically involves myelinated bundles of fibers that are interspersed with gray matter (13). Vacuolated myelin appears to be stable, does not incite microglial response, and can be viewed as a form of cytotoxic edema. The spongiotic change results from intramyelinic edema (vacuolation) or results from swelling of the outer tongue of oligodendrocyte cytoplasm (9). In humans and horses another typical change are solitary or small groups of astrocytes with clear, swollen nuclei, known as Alzheimer type II cells (9, 13). No such cells were present in this case. In the horses, changes are limited to the development of Alzheimer type II cells and were reported associated with renal encephalopathy (5). In other species, such as ruminants the polymicrocavitation is the most obvious alteration, and sometimes it is the sole lesion observed (13).

The severe renal tubular lesions in this case were most likely responsible for induction of renal encephalopathy. Sulfonamide toxicity was concluded to be the cause of toxic tubular necrosis. Nephrotoxicity associated with sulfonamides is rare as most of the current pharmaceutical preparations are relatively highly

soluble at the normal renal pH (12). In this case the kind of sulfonamide used was sulfadiazine. A major side effect of sulfadiazine therapy is the occurrence of crystallization in the urinary collecting system (12). A case of an AIDS patient with toxoplasmic encephalitis treated with sulfadiazine who developed acute renal failure is reported (10). Severe nephropathy may be caused by an overdose of sulfonamide or when the animal is dehydrated (8), as was the case in this goat which was dehydrated due to diarrhea caused by *Eimeria* spp. Animals with toxic nephropathy will show elevated levels of BUN and creatinine (15), as seen in this case. In some animals sulfonamide crystals may be observed grossly in the renal pelvis (12). However, no gross lesions were observed in this case. It's supposed that the renal lesions are due both to local toxic and mechanical (tubule-obstructive) effects (12). Although acute renal failure is a common affection in domestic animals, rarely it develops in renal encephalopathy.

The differential diagnoses for CNS disease in goats included scrapie, caprine arthritis encephalitis, rabies, heavy-metal toxicosis (lead), copper deficiency, storage diseases (mannosidosis), polioencephalomalacia, listeriosis, *Histophilus somni* infection, hepatic and renal encephalopathy and pregnancy toxemia (9).

Renal encephalopathy is rarely reported in small ruminants. We report here, a case of renal encephalopathy associated with status spongiosus due to severe acute renal failure in a goat treated with sulfadiazine

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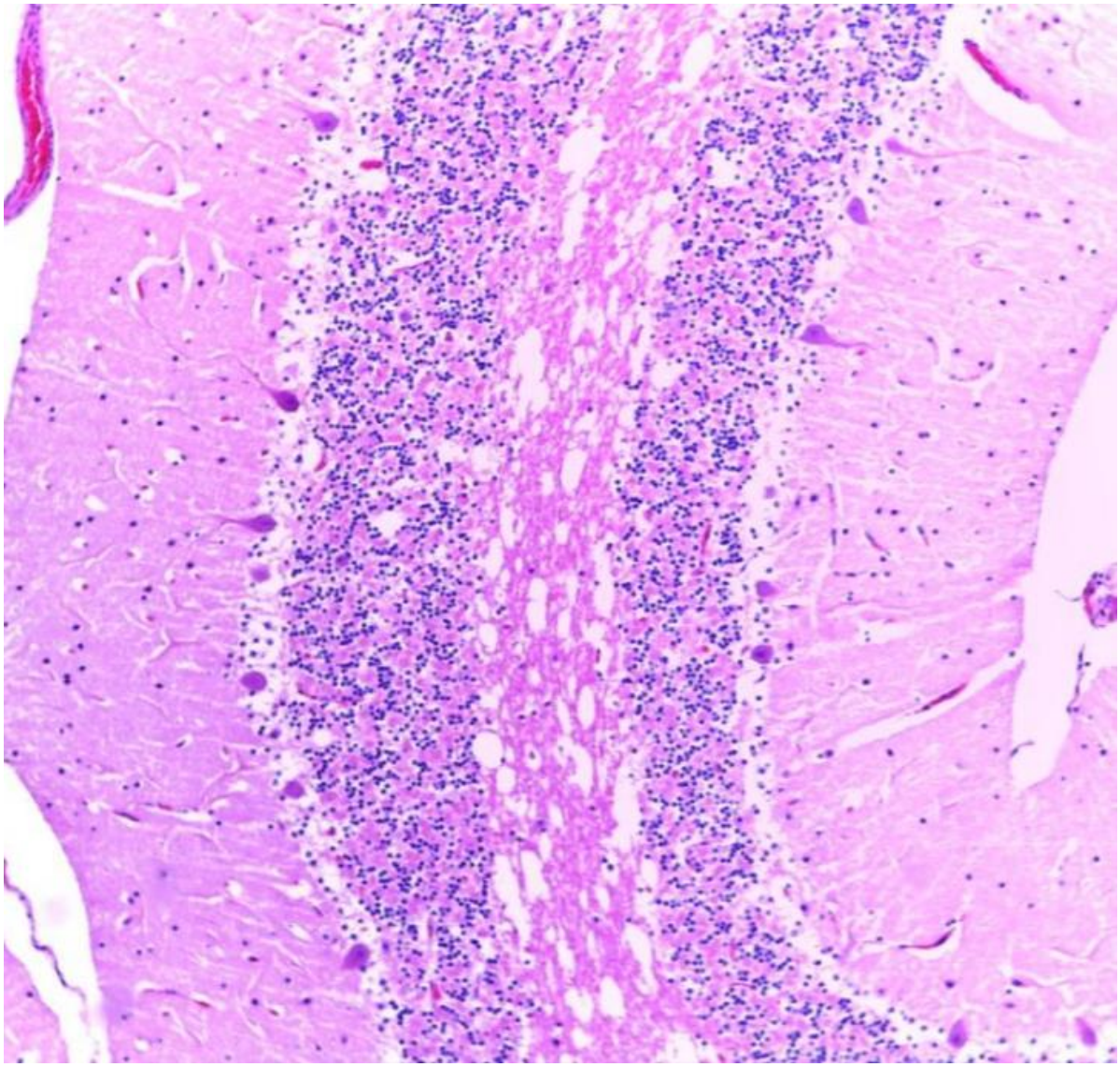

Figure 1. Histology of the brain of a goat dead from renal encephalopathy. Cerebellar medulla. Marked spongy degeneration of the white matter. HE, obj.20x.

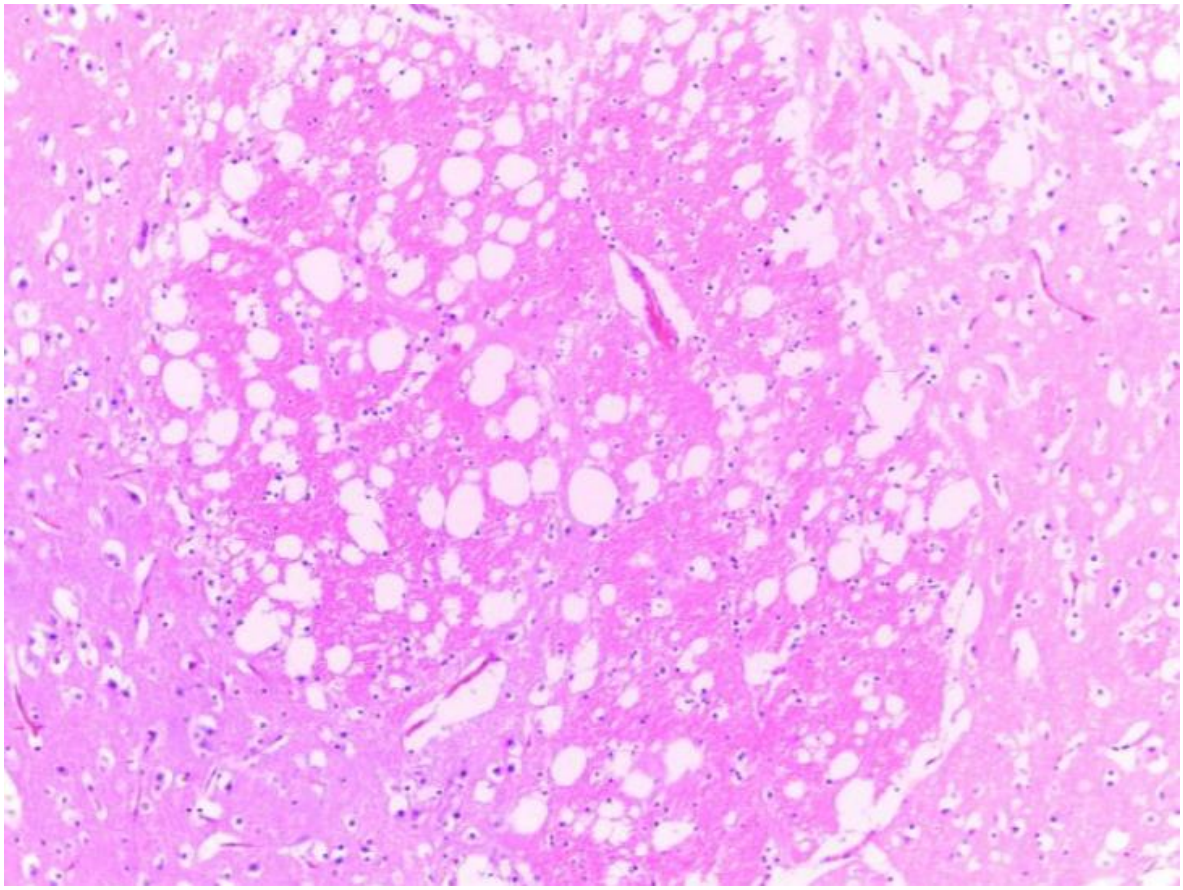

Figure 2. Histology of the brain of a goat dead from renal encephalopathy. Basal nuclei and internal capsule. Severe spongy degeneration in the white matter of the internal capsule. HE, obj.40x.

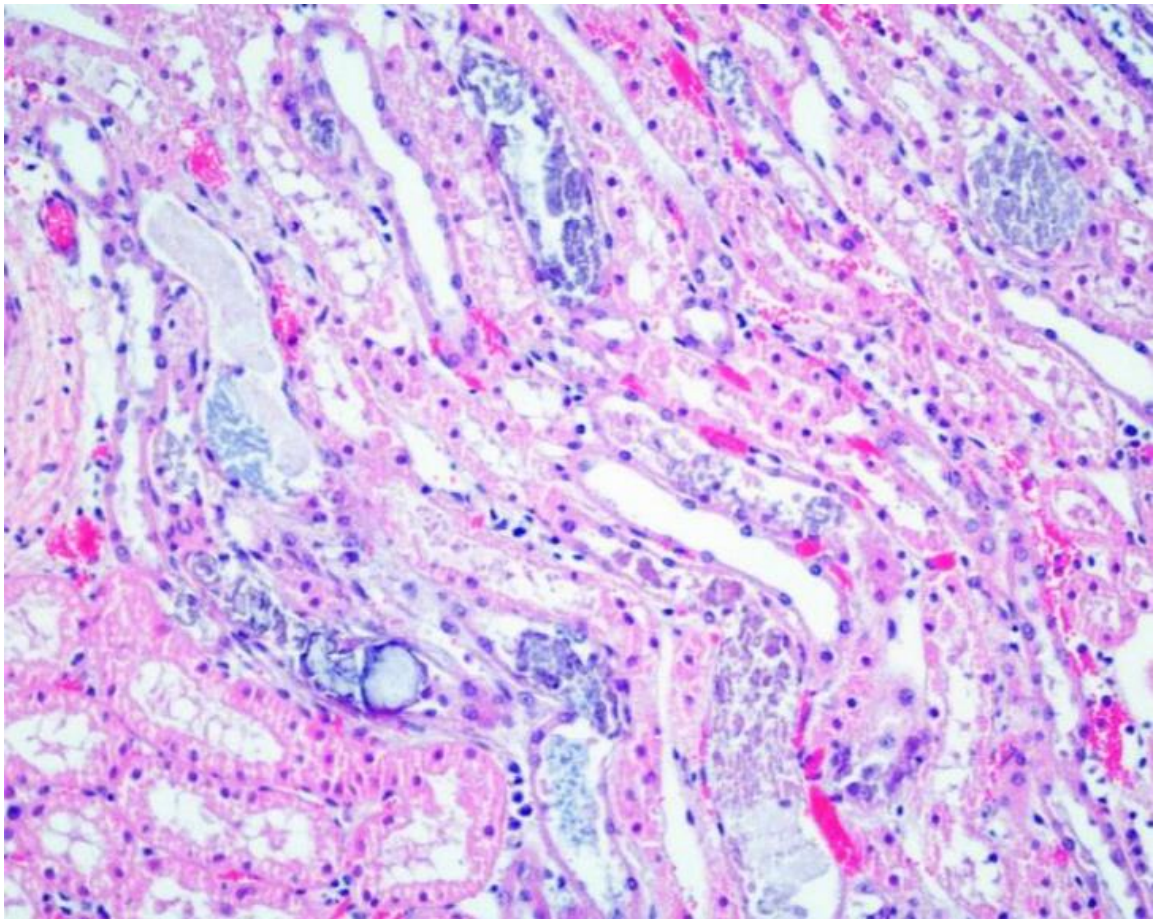

Figure 3. Histology of the kidney of a goat dead from renal encephalopathy. Severe multifocal tubular necrosis with intratubular crystals. HE, obj.20x.

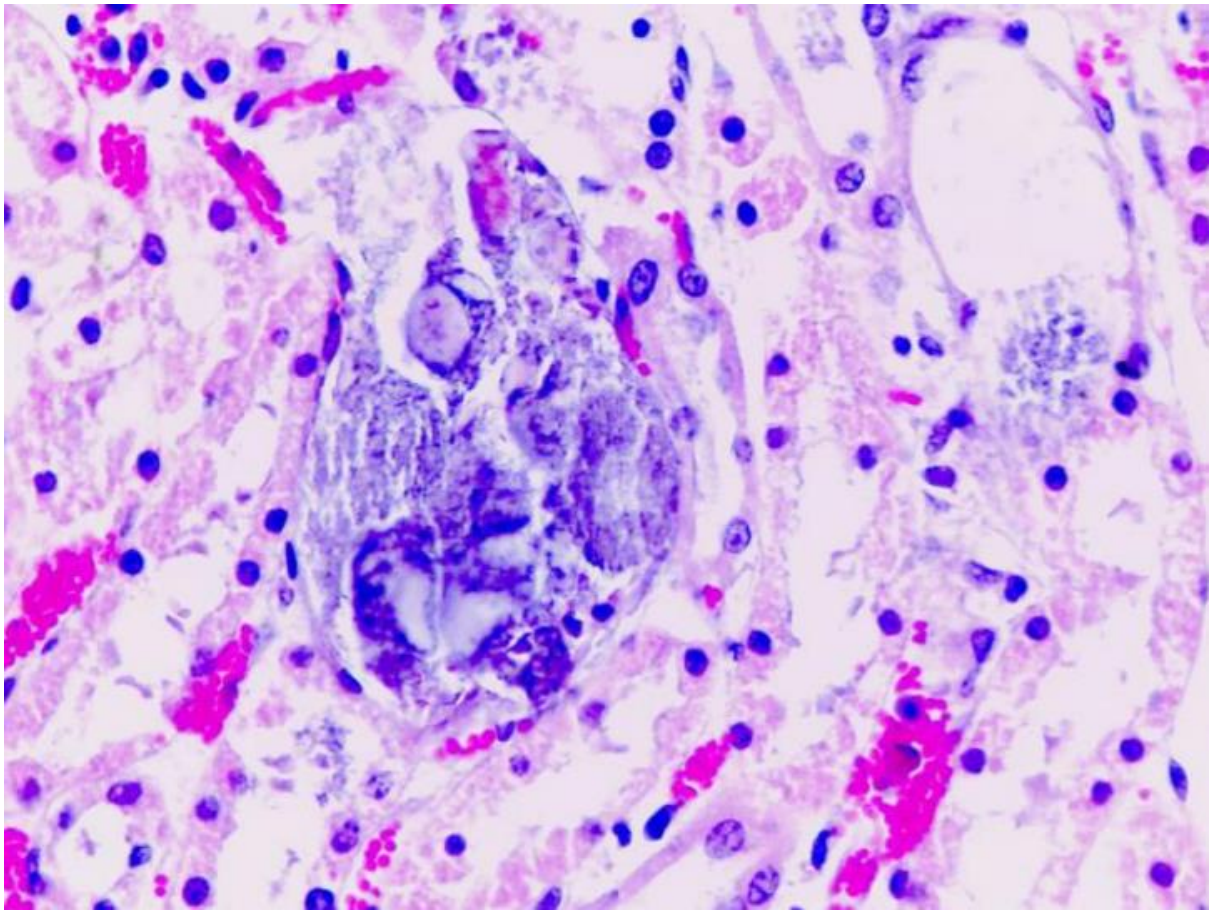

Figure 4. Histology of the kidney of a goat dead from renal encephalopathy. Replacement of lost epithelial tubular cells by crystals that obliterate the tubular lumen. HE, obj.40x.

6 ARTIGO 3 – Granulomatous leptomeningitis in a goat associated with infection by *Cryptococcus neoformans*

Fabio B. Rosa; Mara I. B. Rubin; Roberio G. Olinda; Paula V. Leal; Stephanie C. Lima; Rayane C. Pupin; Danilo C. Gomes; Ricardo A. A. Lemos; Tessie B. Martins; Aline Rodrigues-Hoffmann; Claudio S. L. Barros

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Granulomatous leptomeningitis in a goat associated with infection by *Cryptococcus neoformans*

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Abstract

A case of granulomatous leptomeningitis caused by *Cryptococcus neoformans* is described in a 2-year-old mixed-breed, female goat which presented acute neurological signs including ataxia, nystagmus, bilateral blindness, opisthotonus, hyperesthesia, and spastic paresis of forelimbs. Granulomatous inflammation was detected in the pia arachnoid covering the frontal, parietal and occipital lobes of the telencephalon; cerebellum, thalamus, mesencephalon, pons, medulla, and cervical and thoracolumbar segments of the spinal cord. Yeast-like organisms with morphology compatible with *Cryptococcus* spp. were observed. A 350 base pair sequence was amplified from DNA extracted from the formalin fixed paraffin embedded (FFPE) tissue. The amplified sequence matched 100% *Cryptococcus neoformans*. It is suggested that cryptococcal meningitis should be included in the differential diagnosis list of goat diseases with neurological signs such as ataxia, opisthotonus, nystagmus, hyperesthesia and spastic paresis.

Key words: Goat diseases, central nervous system, leptomeningitis, mycosis, *Cryptococcus neoformans*

Introduction

Cryptococcosis is a systemic mycosis more frequently found in cats and to a lesser extent in dogs (12). The disease is less frequently reported in several other animal species including horses (9,11), cattle (16), sheep (13), goats (118,19), and human beings (4).

Infection occurs when cryptococcal spores are inhaled and enter the alveolar lumina. In most, but not all, immunocompetent individuals, this infection either is cleared or remains dormant until an immune compromise leads the development of lesions induced by the yeast (2). When affecting individual with already compromised immune system, the organism disseminates with particular tropism for the central nervous system (18).

Although affecting both human beings and animals, cryptococcosis is considered neither a zoonosis, nor an anthropozoonosis since the organisms cannot disseminated in the environment from tissue lesions neither of animals nor human beings (7).

Cryptococcosis is caused by two fungal species: (a) *Cryptococcus neoformans*, with the varieties *grubii* (serotype A) and *neoformans* (serotype D) and (b) *Cryptococcus gattii* (3). *C. neoformans* is commonly isolated from avian excreta particularly that of pigeons, occurs worldwide, and primarily cause disease in immunocompromised patients (3). *C. gattii* primarily affects otherwise healthy individuals. Typically, *Cryptococcus* spp. entry the body through the respiratory system, by the inhalation of infectious spores. In human beings, the agent can cause symptomatic or asymptomatic pulmonary infection followed by invasion of the central nervous system via the blood (16). Although both species of *Cryptococcus* may infect the central nervous system, *C. gattii* appears to do that more frequently than *C. neoformans* (3). The infection can disseminate to affect other organs causing cutaneous manifestations, osteomyelitis, septic arthritis, chorioretinitis, myocarditis, hepatitis, peritonitis, renal abscesses, prostatitis, myositis, mastitis and gastroenteritis (16).

C. neoformans has a worldwide distribution. It is isolated frequently from the droppings of birds, especially pigeons (1). *C. gattii* has not been isolated from bird droppings. Plant debris (especially *Eucalyptus* spp. but also from other plants) seems to be the natural reservoir for *C. gattii* (6). The report describes a case of caprine cryptococcal meningoencephalitis caused by *C. neoformans*.

Case report

A 2-year-old mixed-breed, female goat was presented to the Anatomic Pathology Laboratory (LAP) of the FAMEZ/UFMS with neurological signs of one-day duration that included ataxia, nystagmus, bilateral blindness, opisthotonus (Fig. 1), hyperesthesia, and spastic paresis of thoracic members. The goat was from a farm in the municipality of Bandeirantes (19º55'04" S 54°21'50" W), in the central part of the state of Mato Grosso do Sul, Midwestern Brazil. The goat was part of a herd of 1,500 goats most of which were originally raised in the State of Bahia in the Northeastern Brazil, and then transported from there into the Bandeirantes farm in two occasions. The first movement of goats from Bahia to the Bandeirantes was in 2013 and the other on July 2014, the same time frame in which this case occurred. However, the owner was unable to inform to which of those two lots this affected goat belonged. By the time the goat got sick, the farm in Bandeirantes was going through a long series of sanitary and husbandry problems such as, parasitism (haemonchosis), caseous lymphadenitis, and type D enterotoxaemia. These ailments claimed heavy losses in the heard.

The goat was euthanized for humanitarian reasons and necropsied on the same day of arrival at the APS. At necropsy, it was in good body conditions with well-preserved fat deposits. The leptomeninges of the brain and some segments of the spinal cord were mildly edematous, thicken and slightly opaque.

Several tissue samples (including the whole brain and spinal cord) were fixed in 10% formalin solution, processed routinely for histopathological examination, and stained with hematoxylin and eosin (HE). Sections from several regions of the central nervous system (CNS) were additionally stained with Mayer's mucicarmine, Grocott's methenamine silver, periodic acid-Schiff (PAS), Alcian blue and Fontana Masson stains. Fresh samples from the encephalon were submitted to FA technique for rabies, and to the biological assay for rabies detection (intracerebral inoculation in neonate mice); both tests resulted negative.

Microscopic lesions were observed in the leptomeninges covering the frontal, parietal and occipital lobes of the telencephalon, thalamus, mesencephalon, pons, medulla, and cervical and thoracolumbar segments of the spinal cord. In these regions, the leptomeninges displayed moderate inflammatory reaction consisting of macrophages, few multinuclear giant cells, lymphocytes, and plasma cells (Fig. 2). Numerous ovoid to spherical, thick-walled, yeast-like structures were observed amidst the cellular reaction and occasionally within the cytoplasm of macrophages. These organisms measured 5-20 µm in diameter, occasionally had a single, narrow base budding and abundant non-staining capsular material corresponding to a clear halo surrounding the organisms which lends a "soap bubble" appearance to the lesion. (Fig. 2).

The capsules stained positively with Alcian Blue (Fig.4), Mayer's mucicarmine, and PAS. The wall of the yeast-like organisms stained strongly with Grocott's and Fontana Masson stains.

Fifty micrometers scrolls from a formalin fixed paraffin embedded (FFPE) tissue block were used for DNA extraction and polymerase chain reaction (PCR), as previously described (14). DNA was extracted and isolated from FFPE tissues using the BiOstic FFPE Tissue DNA isolation kit (MoBio Laboratories, Carlsbad, CA). PCR was performed using ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers targeting the Internal Transcribed Spacer (ITS) region (found in all eukaryotes). Positive bands were submitted for sequencing at the Eton Bioscience Lab (San Diego, CA). Received sequences were trimmed for quality, joined as contigs using Sequencher®, and classified at >97% identity match using the basic local alignment search tool (BLAST) against the GenBank database of ITS sequences.

The panfungal PCR amplified an approximately 350 base pair fragment from DNA extracted from the FFPE tissue block. The amplified sequence matched 100% to *Cryptococcus neoformans*. Further subclassification

between *C. neoformans* var. *neoformans* and *C. neoformans* var. *gatii* was not possible, as the amplified sequence matched equally to both varieties.

Discussion

The diagnosis in the present case was based in the characteristic morphology of the organism which is consistent with descriptions for *Cryptococcus* spp. (7). Species was confirmed by panfungal PCR as *Cryptococcus neoformans.* The true polysaccharide capsule outside the organism cell wall is important for the evasion of the organism from the immune system (2,10). This capsule does not stain with HE giving the characteristic clear halo surrounding the organism (7).

Caprine cryptococcal disease is uncommon. Published cases include pneumonia by *C. gatii* in Spanish goats (1), pneumonia caused by *C. neoformans* associated with *Mycobacterium bovis* infection (8), experimental and natural occurrence of mastitis by *C. neoformans* (15,17), obstructive granulomatous rhinitis by *C. neoformans* (5), and granulomatous dermatitis and panniculitis by *C. gatii* (19); however, there is only one published case of cryptococcal meningitis in goats (18).

In caprine cryptococcal disease, *C. gatii* (1,19) or *C. neoformans* may be involved (18). As confirmed by panfungal PCR analyses the latter was involved in the case of this report.

The immune status of the goat of this report was unknown. However general sanitary conditions in the premises were less than adequate, and although no lesions of caseous lymphadenitis were detected at necropsy, the disease was endemic in this herd. *Corynebacterium tuberculosis* (18) and *Mycobacterium bovis* (8), have been described to facilitate cryptococcal disease in goats. Transportation can be a cause of stress and resulting immune compromise; there was a possibility that transportation had been a feature in the setting of this particular case.

The infection of *Cryptococcus* spp. is by inhalation of air-borne organisms and that systemic cryptococcosis will start in the nasal cavity or the lungs, although respiratory involvement usually does not result in clinical signs (18). Then, the infection results in dissemination via the bloodstream and spread to the CNS. No lung lesions were observed in the goat of this report, but the extension to the CNS can occur hematogenous or through the cribform plate from a nasal asymptomatic focus (18). Again, no lesions were found in the nasal cavity of this goat, but we cannot rule out for sure that a tiny asymptomatic lesion in the nasal cavity could have been neglected at necropsy.

The blindness in this presented in the goat of this resort could be cortical due to pressure of meningeal cellular exudate or due to primary eye lesions, as chorioretinitis is frequently described in association with cryptococcosis (20). Although no, clinical or gross evidence of eye lesions were observed, the globes were not submitted for histopathological examination.

In conclusion, cryptococcal meningitis should be included in the differential diagnosis list of goat diseases with neurological signs such as ataxia, opisthotonus, nystagmus, hyperesthesia and spastic paresis.

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Figure 1. Goat. Opisthotonus associated with granulomatous leptomeningitis by *Cryptococcus neoformans*.

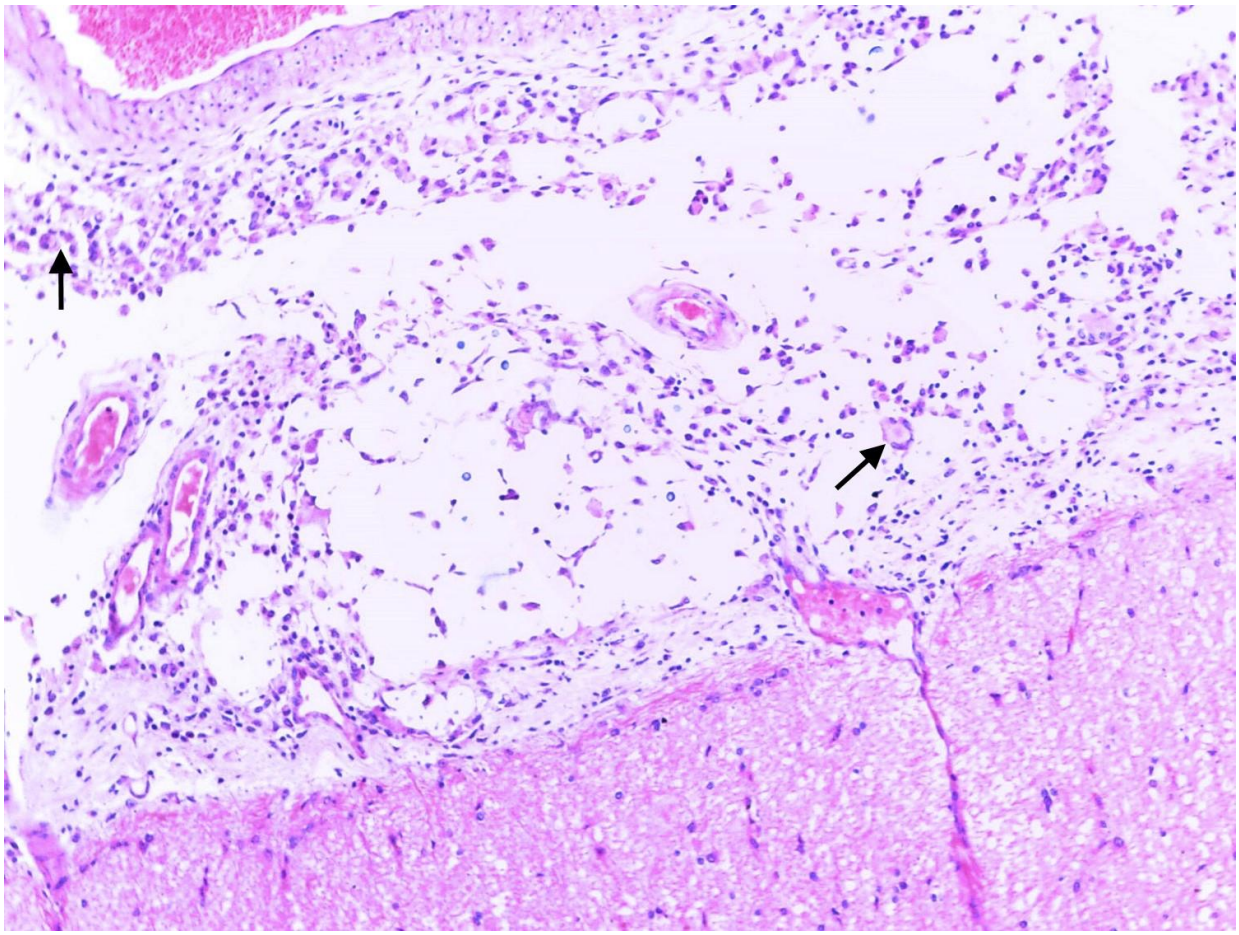

Figure 2. Goat. Leptomeninges. Cerebral cortex. Granulomatous leptomeningitis by *Cryptococcus neoformans*. Leptomeninges display moderate inflammatory reaction consisting of macrophages, lymphocytes, plasma cells, and multinucleated giant cells (arrows). Numerous, ovoid to spherical, 5-20 µm in diameter thick-walled, yeast-like structures are observed amidst the cellular reaction (HE stain).The abundant nonstaining capsular material corresponds to clear halo surrounding the organism which lends a “soap bubble” appearance to the lesion.

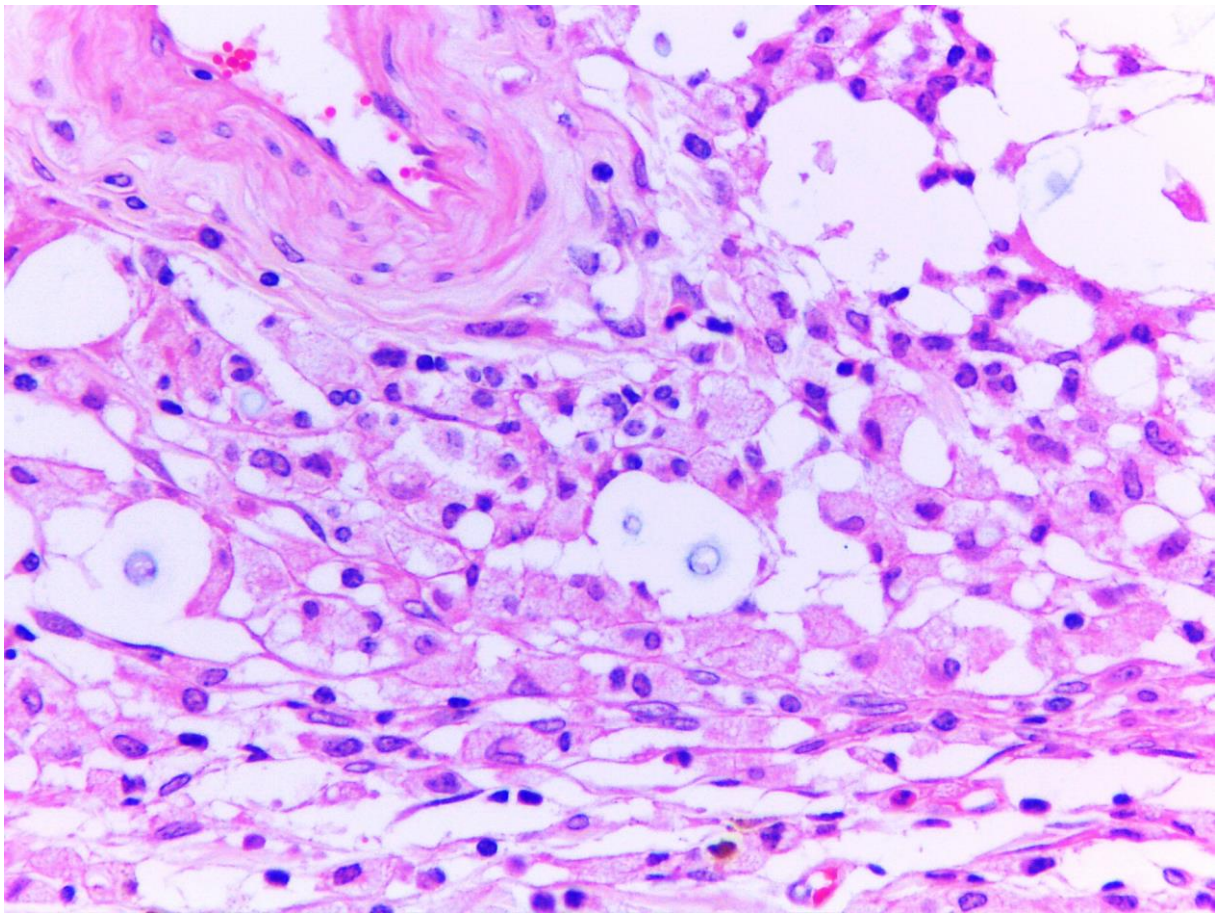

Figure 3. Goat. Leptomeninges. Granulomatous leptomeningitis cause by *Cryptococcus neoformans*. Higher magnification of Fig. 2. Intralesional C. neoformans organisms can be seen displaying a distinct clear halo (capsule) amidst the cellular infiltrate and occasionally with the cytoplasm of macrophages (HE stain).

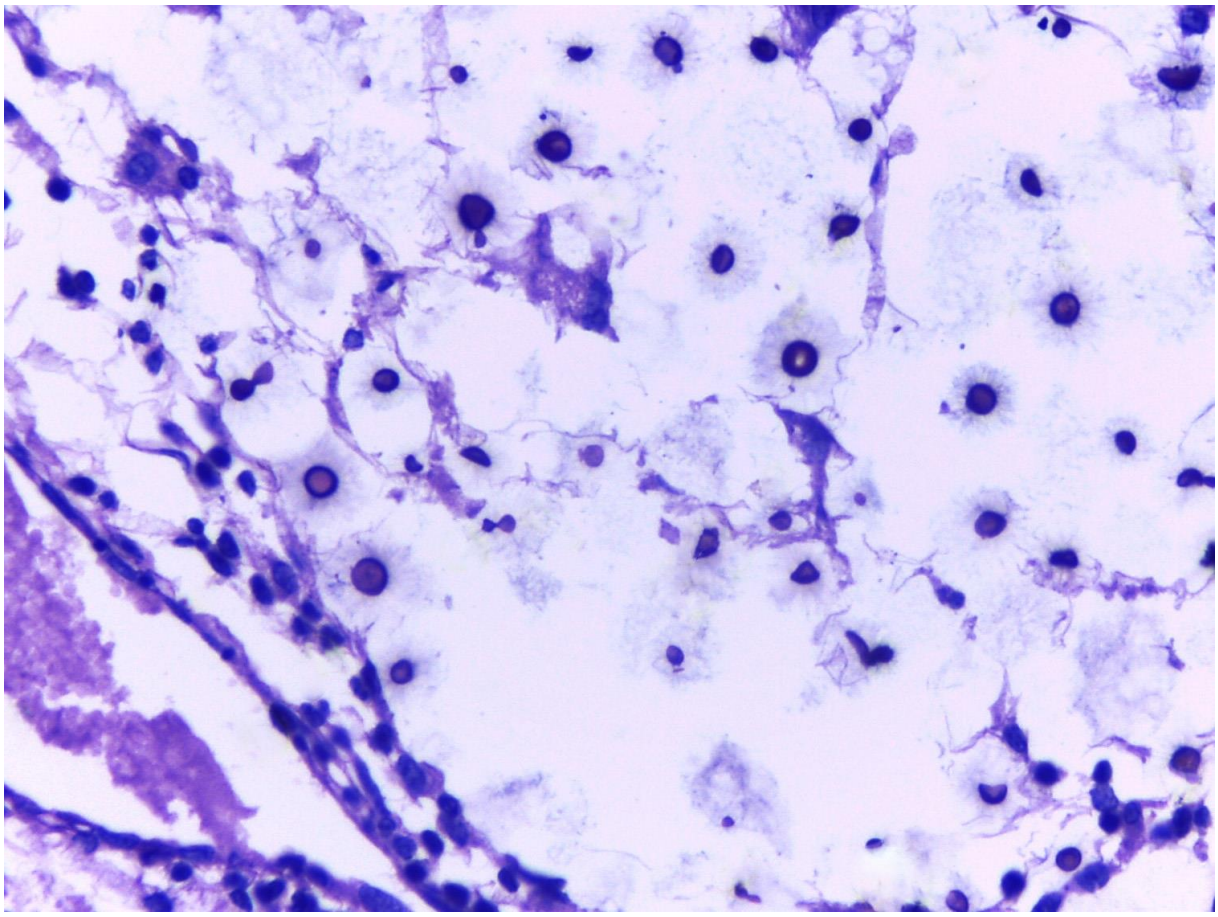

Figure 4. Granulomatous leptomeningitis in a goat. *Cryptococcus neoformans* observed in the leptomeninges by Alcian Blue stain.

7 ARTIGO 4 – Hepatogenous chronic copper toxicosis associated with grazing *Brachiaria decumbens* in a goat

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Hepatogenous chronic copper toxicosis associated with grazing *Brachiaria decumbens* in a goat

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Abstract

A case of hepatogenous chronic copper toxicosis associated with ingestion of *Brachiaria decumbens* in a 4-year-old female is reported in a goat from a herd of approximately 1,000 goats of different categories, all grazing in a pasture consisting exclusively of *B. decumbens.* The goat had chronic weight loss, dehydration, and apathy. Just prior to death it developed anemia, icterus and hemoglobinuria. Necropsy findings included marked icterus, enhanced lobular pattern and orange discoloration of the liver, pulmonary edema, distention of the gall bladder and hemoblobinuric nephosis. Histopathological examination of the liver revealed marked random degeneration and necrosis of individual hepatocytes, marked bilestasis, intracytoplasmic hemosiderin in hepatocytes and Kupffer cells, birefringent crystals with bile staining in the lumen of bile ducts, and sparse, randomly distributed foamy macrophages. Severe multifocal tubular degeneration and necrosis associated with multiple hyaline and coarsely granular hemoglobin casts were observed in the kidneys. Copper levels determined in liver and kidney samples by atomic absorption spectrophotometry were 410 ppm of liver dry matter and 34.4 ppm (kidney, dry matter). The gross, histopathological findings and copper analysis in the tissues of this goat led to a final diagnosis of hepatogenous chronic copper toxicosis associated with grazing of *B. decumbens*.

Key words: Diseases of goats, liver diseases, toxicosis, copper, poisonous plants.

Introduction

Copper toxicosis in livestock is described in acute and chronic forms. The latter is very common, mainly in sheep, and the former is rare. These two forms refer to the duration of copper exposure, as opposed to the onset of clinical signs (22). Acute toxicity occurs when large quantities of copper are ingested at once, e.g. from footbath, which triggers gastrointestinal disorders due to copper caustic properties; this is characterized by abdominal pain,

diarrhea, emesis, anorexia, dehydration and shock. Chronic toxicity is the most important in domestic animals and can be (a) primary, caused by the consumption of feed containing high levels of copper; (b) phytogenous, associated with the consumption of pasture containing normal copper and reduced molybdenum levels; and (c) hepatogenous, when the copper accumulation results from liver damage caused by toxic plants (3,14). The chronic form is characterized by a subclinical phase, during which copper accumulates within hepatocytes, for weeks or months, followed by an acute phase resulting from the release of the accumulated copper into the bloodstream, causing intravascular hemolysis, anemia, icterus and hemoglobinuria (11,16). There is a significant variation in species susceptibility to copper toxicosis. Sheep is rather susceptible due to reduced biliary excretion of copper (9,11,16). Toxicosis in cattle and pigs are less common and due to abnormally high intake of the copper (24). Goats are reportedly more resistant to copper intoxication than sheep (22). Consequently, there are few published reports of copper toxicosis in goats (1,2,7,20).

In sheep, species in which the toxicosis is more common, there are reports of hepatogenous chronic copper poisoning associated with the ingestion of toxic plants containing pyrrolizidine alkaloids such as *Senecio* spp., *Echium plantagineum* and *Crotalaria retusa* (10, 19). However, there is no report of hepatogenous copper toxicosis in goats.

It is well known that ingestion of *Brachiaria* spp. by ruminants cause hepatogenous photosensitization and crystal-associated cholangiopathy (11). There is scarce documentation of naturally occurring chronic copper toxicosis in goats and to the authors' knowledge, this is the first report of caprine chronic hepatogenous copper toxicosis secondary to ingestion of *Brachiaria decumbens*. The current report describes the pathological aspects of this toxicosis.

Case report

A 4-year-old female mixed-breed goat died after presenting chronic weight loss, dehydration, mild icterus, and apathy; two days before its death the goat developed acute severe icterus and hemoglobinuria. The goat was part of a herd of 1,000 goats of different categories. The farm is exclusively focused on meat production and the goats graze in a pasture consisting exclusively of *B. decumbens.* Necropsy changes included severe yellow discoloration (icterus) of oral, ocular and vaginal mucous membranes, subcutaneous tissue, muscle fascia and visceral fat; diffuse orange discoloration (Fig. 1) and enhanced lobular pattern in the liver; distension of the gallbladder; kidneys markedly and diffusely dark brown, with metallic appearance throughout the cut surface, and markedly yellow pelvic fat (Fig. 2). The bladder contained dark brown urine; the spleen was slightly increased in volume; and moderate amounts of foamy fluid oozed from the trachea (pulmonary edema). Samples from multiple organs, including brain, heart, liver, lung, spleen, kidney and intestine were fixed in buffered 10% formalin and processed routinely for histopathology and stained with hematoxylin and eosin.

Formalin-fixed fragments of liver and kidney were submitted to copper determination by atomic absorption spectrophotometry, and resulted in 410 ppm and 34.4 ppm, respectively. Copper concentration reference values in the liver and kidney of goats are much lower than those reported for sheep. They may be as low as 25–150 ppm for the liver and 3–6 ppm for the kidney and 230 mg/kg in the liver and 12 mg/kg in the kidney are consistent with copper toxicosis in goats (7). In an outbreak of 3 goats with chronic copper poisoning, copper concentration for the 3 goats (given in ppm) were 436, 378, and 23.4 for the liver and 22.2, 17.6, and 17.1 for the kidney. Thus the levels of copper in the goat o this report is in accordance with a diagnosis o chronic copper poison ing.

Histological findings in the liver consisted of multiples bile casts in bile canaliculi and bile-stained birefringent crystals in the lumen of bile ducts (Fig. 3), random hepatocellular degeneration and necrosis and randomly distribute foamy macrophages (Fig. 4), intracytoplasmic hemosiderin in the cytoplasm of hepatocytes and Kupffer cells. The renal cortex had severe diffuse tubular degeneration and necrosis associated with multiples hyaline and coarsely granular hemoglobin casts. Multifocally, epithelial tubular cells contained intracytoplasmic dark brown pigment (hemosiderin) and there was tubular ectasia (Fig. 5). The spleen had numerous hemosiderin-laden macrophages. There were no microscopic changes related to the intoxication in other organs.

Discussion

Diagnosis of hepatogenous chronic copper toxicosis induced by liver damage due to ingestion of *B. decumbens* was based on excluding exposure to exogenous sources of copper, and in clinical-pathologic findings and levels or copper in liver and kidney. Although feedstuffs were not tested for copper levels, there was no history of administration of copper containing hematinics, copper oxide-containing boluses, exposure to swine or poultry litter, copper-containing footbaths, copper plumbing, or chemicals. The only hepatoxic, or toxic for that matter, plant present was *Brachiaria decumbens*. Ideally, all components of the diet should be analyzed for trace element content in instances of suspected copper toxicosis, and a careful assessment should be made of the proportions of

those components in the diet. However, there were logistical and financial constraints on the number of possible sources of copper and/or mineral imbalances that could be investigated.

In most reports of copper toxicosis in goats, there is an association with high intake of dietary copper or the cause is undetermined (1,2,7). There are few reports regarding caprine chronic copper intoxication in goats (1,2,7,8) and there is no uniformly accepted maximum tolerable levels of dietary concentration of copper for goats (15). Similar to what occurs in sheep, it is known that caprine susceptibility to chronic copper toxicosis varies with breed (5,6,15). Adult goats and Boer crosses are generally considered resistant, especially to the hemolytic stage of the toxicosis (2) which is at odds with the results of the current report which describes a case of copper toxicosis in an adult goat.

Hepatogenous chronic copper toxicosis is rare in sheep (10) and it has never been documented in goats. Reports of hepatogenous chronic copper toxicosis in sheep are associated with the ingestion for several months of toxic plants containing pyrrolizidine alkaloids such as *Senecio* spp., *Echium plantagineum* and *Crotalaria retusa* (10,19). These plants predispose to excessive liver uptake of copper. It has been postulated that pyrrolizidine alkaloids increase the avidity of liver cells for copper, ultimately leading to the hemolytic crisis of chronic copper toxicity (10,12). In such cases, despite the chronic liver injury, animals die from loss of kidney function and anemia due to acute hemolytic crisis. Clinical signs may include photosensitization, chronic weight loss, hepatic encephalopathy followed by icterus and hemoglobinuria (12). With exception of photosensitization and hepatic encephalopathy, all of these clinical signs were observed in the case of this report. But then, a clinical syndrome of progressive weight loss and death, without photosensitization, has been reported in cattle poisoned by *B. decumbens* (17), and probably the goat of this report is included in this syndrome.

The liver damage observed in this goat was due to ingestion of *B. decumbens.* Protodioscin is the toxic principle of *Brachiaria* spp. This substance is a lithogenic steroidal saponin that induces pathologic changes in liver parenchyma and biliary ducts, disturbing clearance of phylloerythrin, a photodynamic pigment (4). The sequence of chemical transformations of the lithogenic steroidal saponins (LSS) resulting in liver damage is as follows. The LSS ingested in the forage go through hydrolysis in the rumen yielding the sapogenins diosgenin and yamogenin. These two sapogenins are respectively converted to smilagenin and sarsasapogenin. Following this conversion these compounds go through epimerization resulting in their respectively isomers epismilagenin and episarsapogenin (13). These isomers are absorbed and transported via blood to the liver where they conjugate with glucuronic acid giving rise to epismilagenin and episarsapogenin glucoronides that bound to calcium ions forming insoluble calcium salt crystals which precipitate in the hepatic parenchyma and in the bile ducts damaging those structures (14). Multiple crystals were observed within bile ducts in this current case. We believe that the normal flow of bile was compromised by the crystals, resulting in diminished excretion of copper through the bile. When the hepatocytes die (either spontaneously or in response to environmental stress or dietary changes), the release of large amounts of copper in the bloodstream ensues (9). However, the mechanism that relates stressful events to the release of copper by hepatocytes is unknown. The excess of copper in circulation causes denaturation of hemoglobin and direct oxidative damage to the cell membrane of red blood cells with subsequent intravascular hemolysis. The abrupt destruction of erythrocytes (hemolytic crisis) causes necrosis of hepatocytes, which releases even more copper in circulation, creating a vicious cycle of hemolytic crises and hepatic necrosis (23).

Gross and histopathological findings in the goat of this case are typical and similar to those previously described for chronic copper toxicosis in small ruminants (1,2,7,8,10,11,18). The hepatic lesion induced by *B. decumbens* described above may have contributed to the mild icterus initially observed but the icterus suddenly noted was due to acute phase of copper release, i.e., hemolytic crisis. The bile stasis observed histologically may also have been induced by *B. decumbens* ingestion but certainly was most likely a sequel to hemolysis. Therefore, the gross lesions such icterus and orange discoloration of the liver (due to bile stasis and hemosiderin) are a superposition of both mechanisms. In addition, the bile stasis could have also contributed to the excessive hepatic copper accumulation because of impaired biliary excretion of copper (9). The kidney showed a dark brown discoloration due to the high amounts of intratubular hemoglobin casts and intracytoplasmic hemosiderin in tubular epithelial cells observed histologically. It is known that hemoglobin is not nephrotoxic itself. In copper toxicosis, animals affected generally have secondary renal ischemia due to hypovolemic shock or severe anemia and the hemoglobinuria may have additional deleterious effect on the tubular epithelium already compromised by ischemic necrosis (6). However, high concentrations of this element in blood serum and glomerular filtrate may exacerbate tubular necrosis occurring as a result of ischemia (6, 16).

The main differential diagnosis for chronic copper toxicosis is yellow lamb disease, caused by *Clostridium perfringes* type A, wherein both icterus and hemoglobinuria are present. The other differential diagnoses for hemoglobinuria in goats include leptospirosis (*Leptospira interrogans serovar pomona*, *icterohemorrhagica* and *hardjo*), babesiosis (*Babesia ovis* and *B. motasi*), bacillary hemoglobinuria (*Clostridium haemolyticum),* and poisoning by plants of *Brassica* genus (16). The differentiation from the listed diseases can be done by history, clinical signs, combination of gross and microscopic lesions of the kidney and other organs, demonstration of infectious agents when appropriate, by histological findings and, in most cases, by the determination of copper levels in affected animals tissues.

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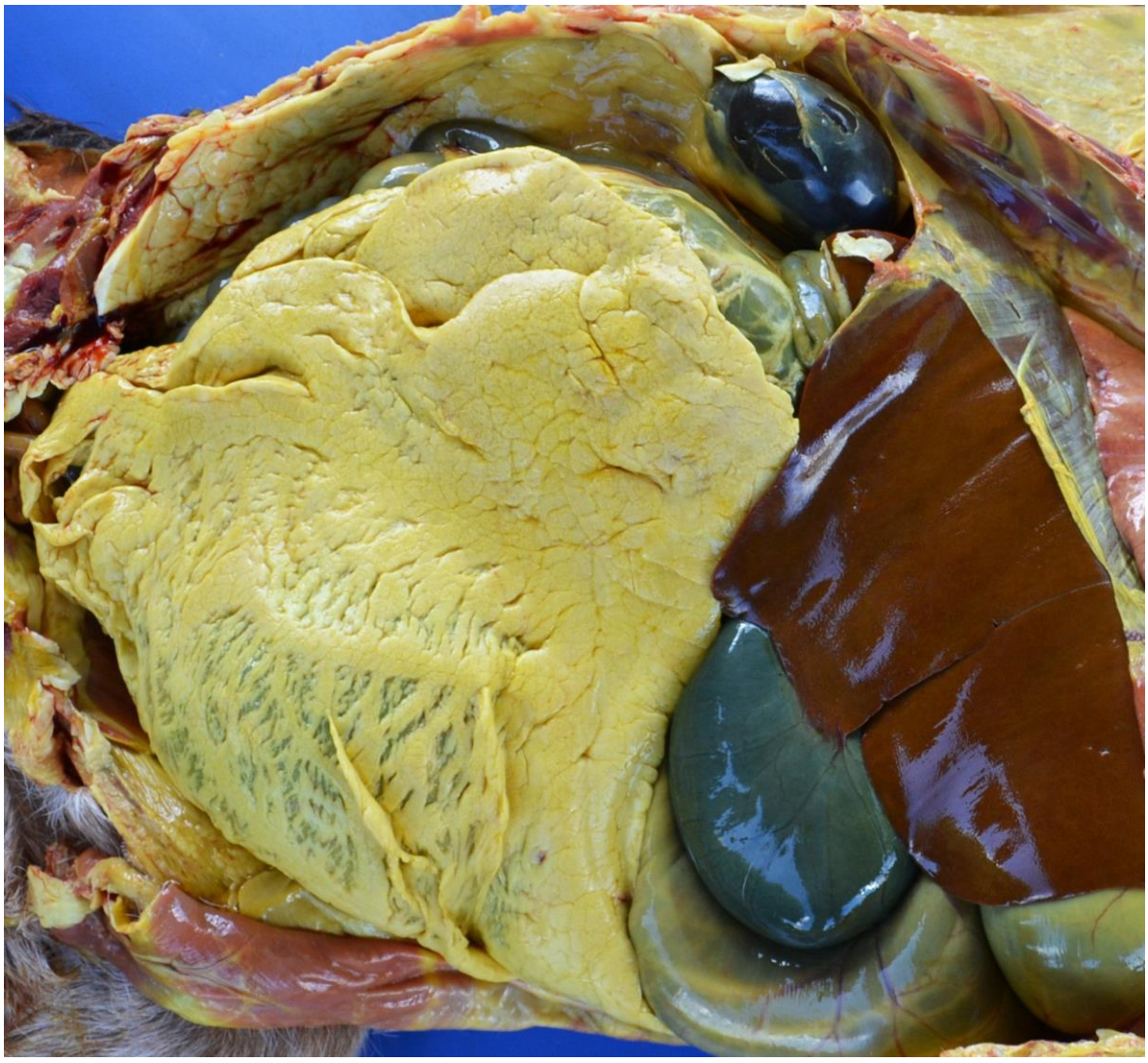

Figure 1. Goat. Chronic hepatogenous copper toxicosis. Exposed abdominal viscera. Severe icterus, dark brown kidneys, and orange discolored liver. The fat of the omentum is markedly yellow (icterus). All these are signs of hemolytic crisis.

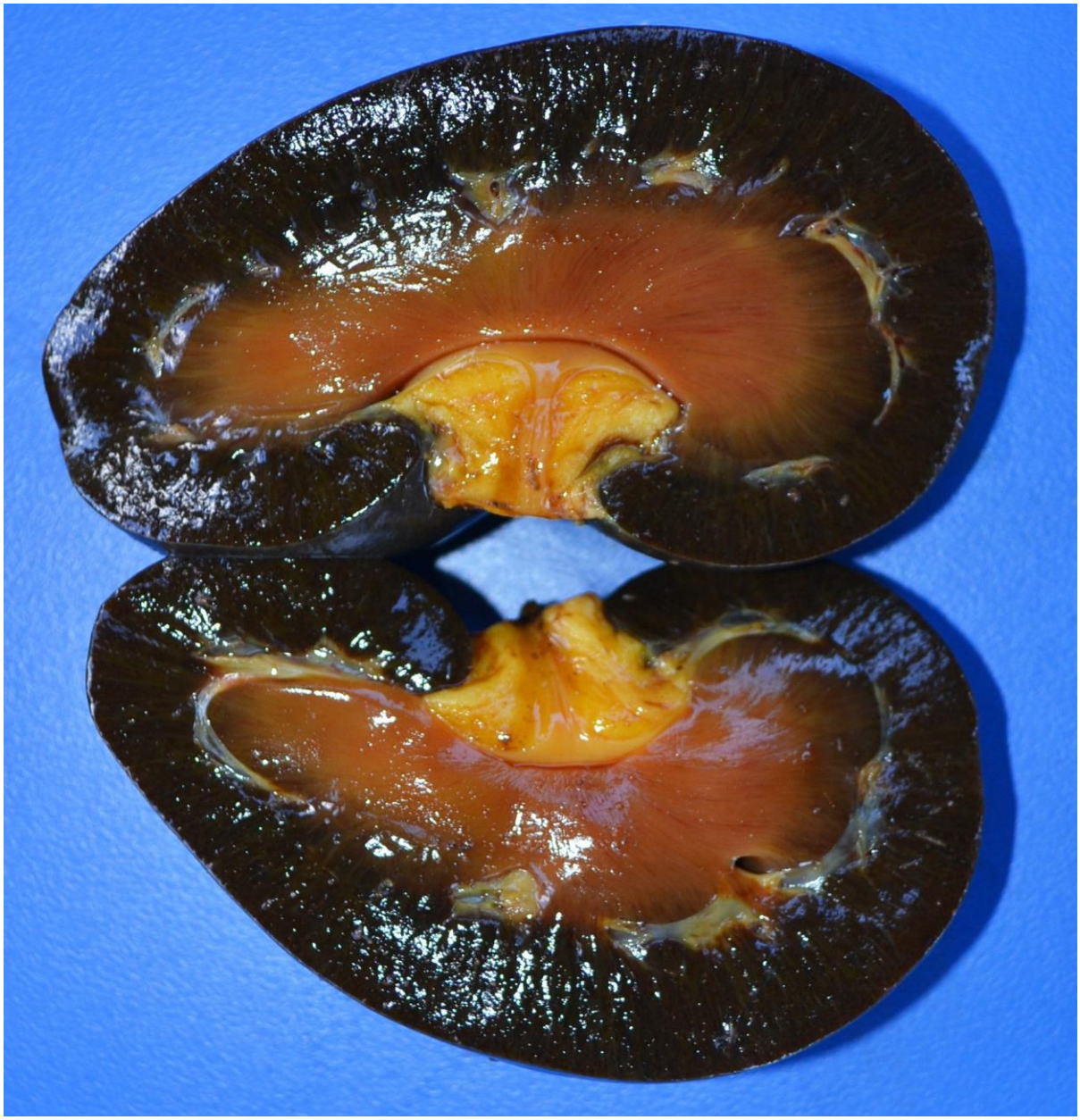

Figure 2. Goat. Chronic hepatogenous copper toxicosis. Kidney. Cut surface. The parenchyma is dark brown with metallic appearance. The fat of renal pelvis is markedly yellow (icterus).

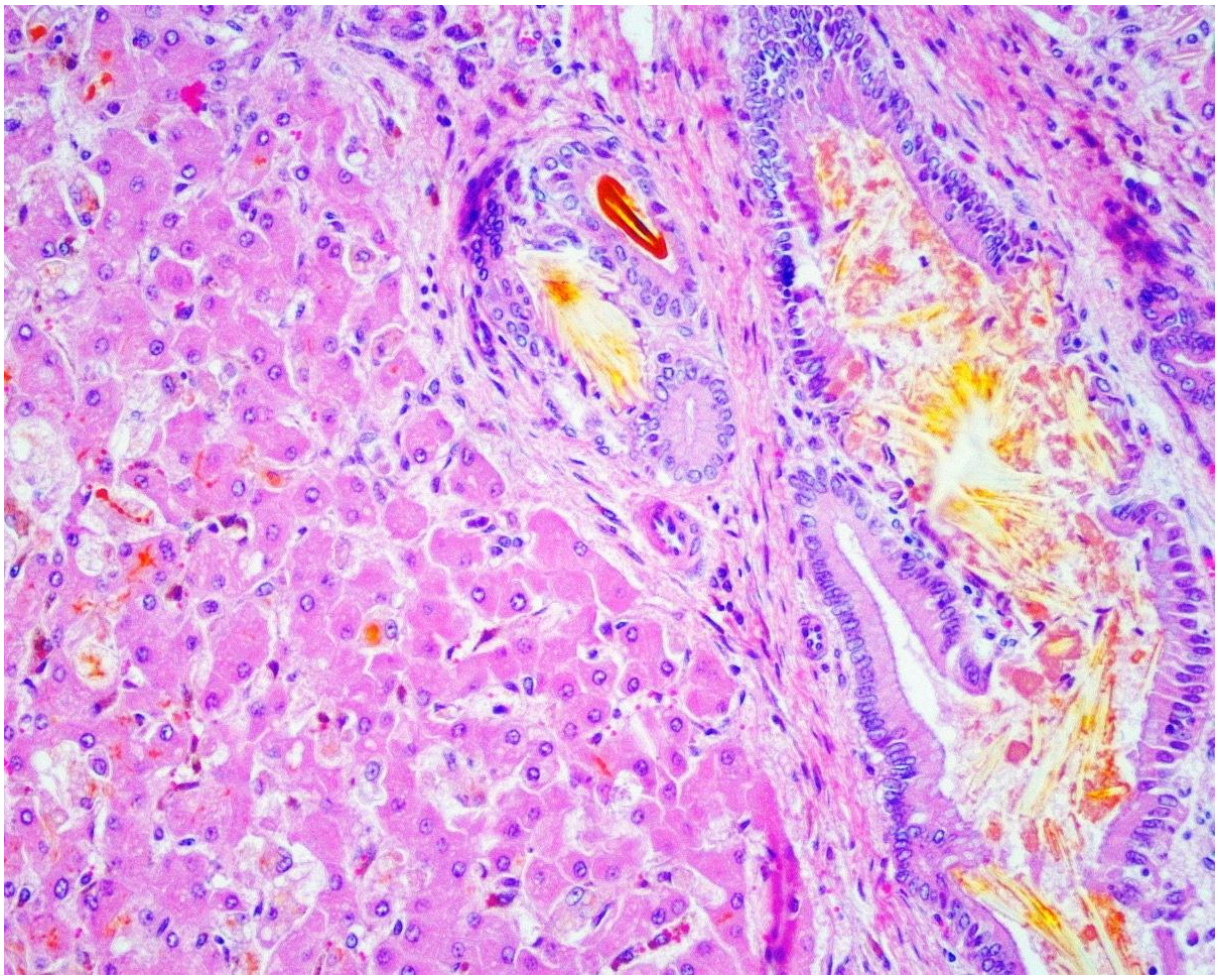

Figure 3. Goat. Chronic hepatogenous copper toxicosis. Histology of the liver. Multiple bile ducts filled with bilirubin-embedded refringent crystals. Mild hepatocellular degeneration and marked cholestasis. Hematoxylin and eosin, obj.40x.

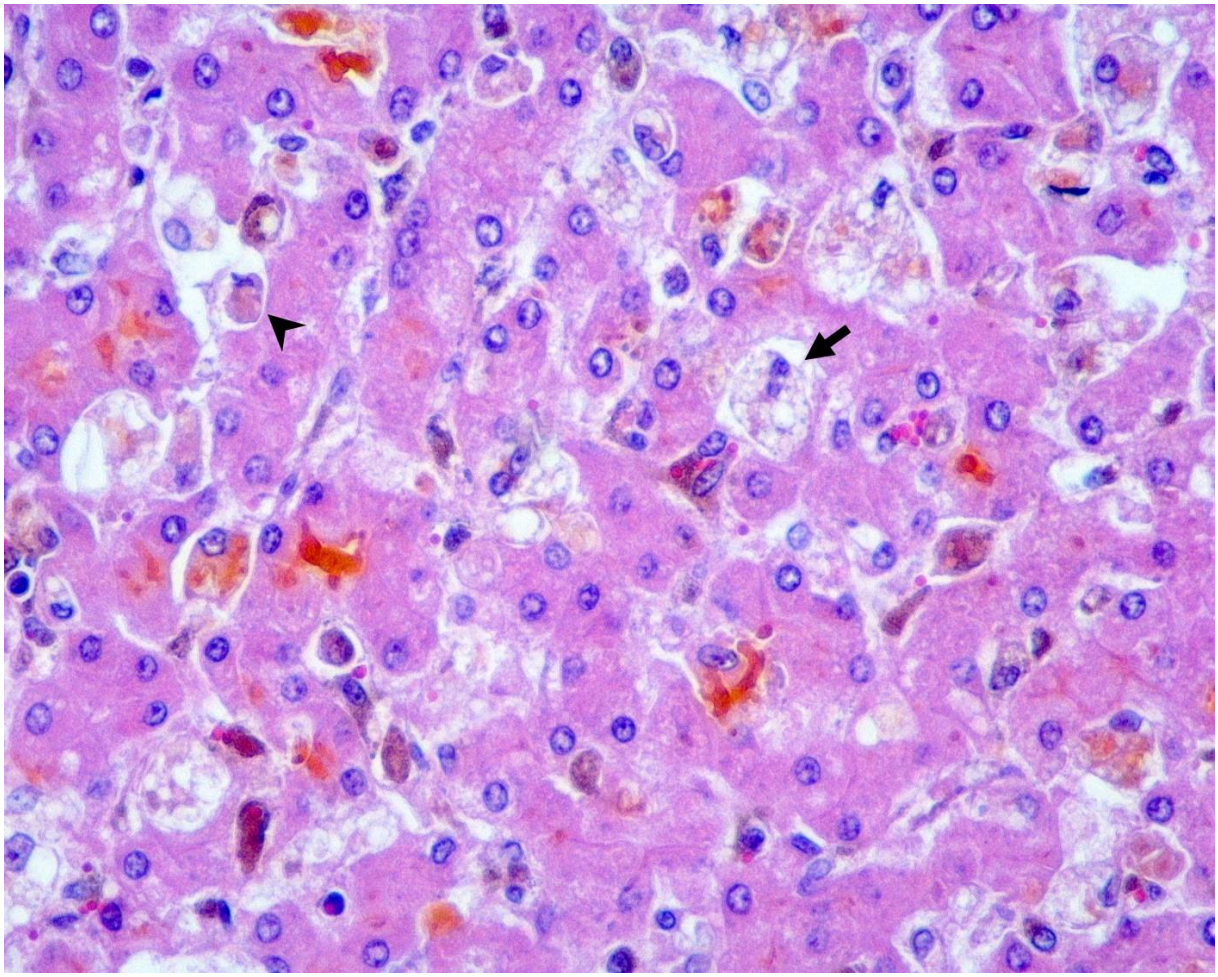

Figure 4. Goat. Chronic hepatogenous copper toxicosis. Histology of the liver. Individual hepatocellular necrosis (arrow head) and marked cholestasis. Foamy macrophages (arrow) are seen within sinusoids. Hematoxylin and eosin, obj.40x.

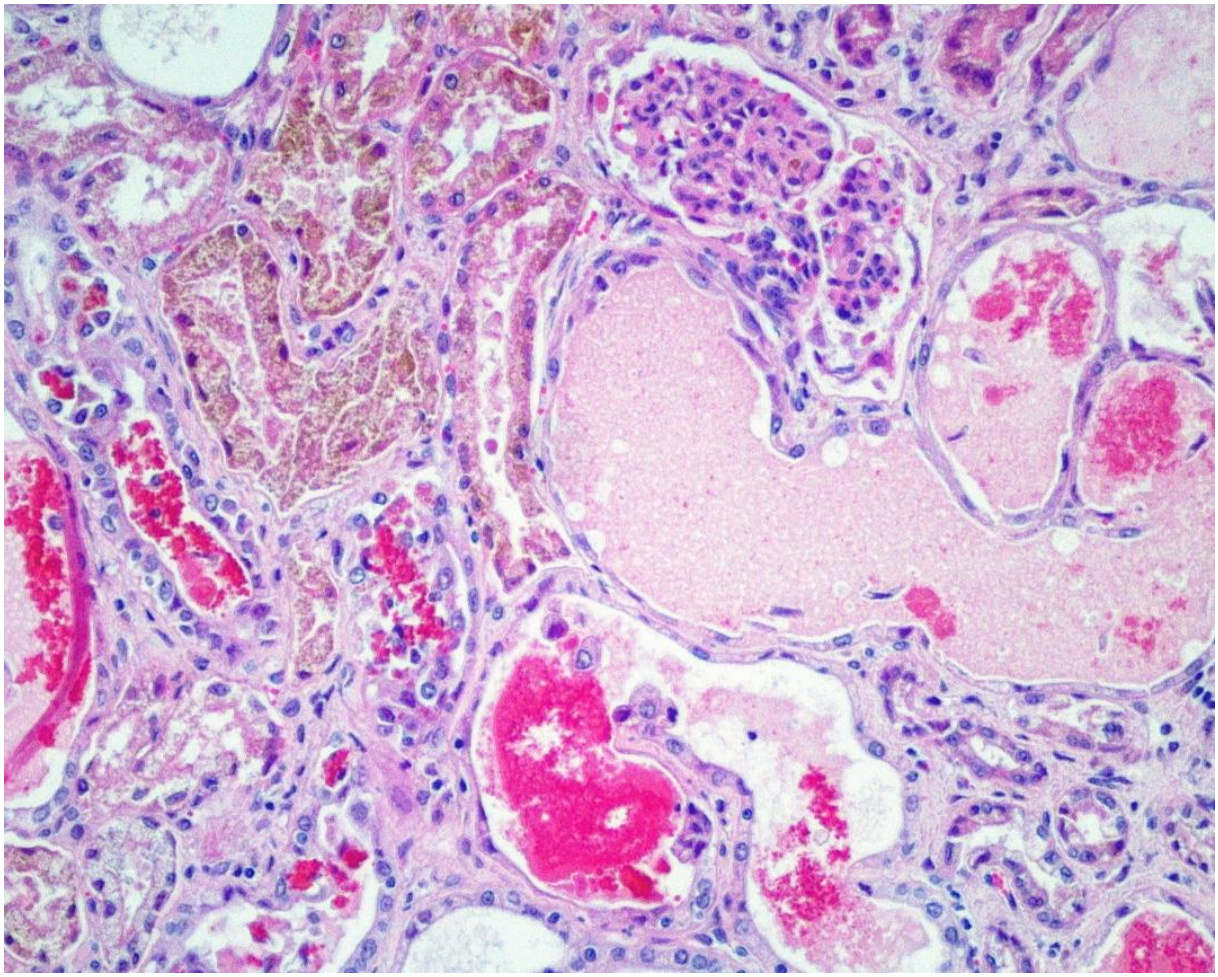

Figure 5. Goat. Chronic hepatogenous copper toxicosis. Histology of the kidneys. Some tubules are dilated and filled by granular (hemoblobin) and homogenous eosinophilic casts. Multifocally, the tubular epithelial cells contain intracytoplasmic golden-brown pigment (hemosiderin). Tubular ectasia can also be observed. Hematoxylin and eosin, obj.40x.

8 CONCLUSÕES

- Os diagnósticos mais prevalentes foram: hemoncose, broncopneumonia fibrinopurulenta bilateral com pleurite e intoxicação por *Brachiaria decumbens*.

Artigo 1:

- Cabras intoxicadas por *B. Decumbens* podem ou não ter fotossensibilização. Os principais sinais clínicos foram desidratação, anorexia, letargia e perda de peso.
- Apresentaram alterações macroscópicas na coloração do fígado, que pode se tornar laranja, amarelo ou cinza, e em tamanho, com predominância de hepatomegalia.
- Em todos os casos as lesões microscópicas no fígado foram: presença de cristais no lúmen de ductos biliares e no citoplasma dos hepatócitos, fibrose periportal associada a infiltrado mononuclear, colangite, proliferação dos ductos biliares e presença de macrófagos espumosos.

Artigo 2:

- Administração de sulfadiazina em animais desidratados pode causar insuficiência renal.
- A insuficiência renal neste caso levou à encefalopatia renal.
- Microscopicamente observou-se necrose tubular renal, multifocal, acentuada com cristais intratubulares. Degeneração esponjosa na substância branca no tronco cerebral, cerebelo, tálamo, núcleos basais e na interface da substância cinzenta cortical e da substância branca subcortical.

Artigo 3:

- Meningite criptocócica deve ser incluída na lista de diagnósticos diferenciais de doenças caprinas com sinais neurológicos como ataxia, opistótono, nistagmo, hiperestesia e paresia espástica.

Artigo 4:

- Lesão hepática induzida por intoxicação por *B. Decumbens* pode levar à intoxicação crônica hepatógena por cobre em cabras.
- A bilestase contribuiu para o acúmulo excessivo de cobre no interior dos hepatócitos devido

à diminuição da excreção biliar do cobre. A bilestase pode ter sido iniciada pelo dano hepático causado pela intoxicação por *B. Decumbens* e agravada pela crise hemolítica.

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