UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA

Marcella Barrella Ambrosio

# DOENÇA RENAL CRÔNICA E UREMIA EM GATOS DOMÉSTICOS

Santa Maria, RS
2020

**Marcella Barrella Ambrosio**

# DOENÇA RENAL CRÔNICA E UREMIA EM GATOS DOMÉSTICOS

Dissertação apresentada ao Curso de Pós-Graduação em Medicina Veterinária na Universidade Federal de Santa Maria (UFSM, RS), como requisito para a obtenção do título de **Mestre em Medicina Veterinária.**

Orientadora: Profª Drª. Glaucia Denise Kommers

Santa Maria, RS

2020

**Marcella Barrella Ambrosio**

# DOENÇA RENAL CRÔNICA E UREMIA EM GATOS DOMÉSTICOS

Dissertação apresentada ao Curso de Pós-Graduação em Medicina Veterinária na Universidade Federal de Santa Maria (UFSM, RS), como requisito para a obtenção do título de **Mestre em Medicina Veterinária.**

**Aprovado em 18 de fevereiro de 2020:**

_____

**Glaucia Denise Kommers, PhD (UFSM)**
(Presidente/Orientador)

_____

**Maria Andréia Inkelmann, Dra. (UNIJUÍ)**

_____

**Mariana Matins Flores, Dra. (UFSM)**

Santa Maria, RS
2020

## AGRADECIMENTOS

Agradeço em primeiro lugar minha família por estar comigo em todos os momentos, nem sempre fisicamente, mas com certeza em pensamento. Desejando sempre o melhor e torcendo por mim, apoiando meus sonhos, mesmo quando o mundo todo me fazia pensar em desistir.

Aos amigos, tanto do LPV-UFSM, quanto os de Santa Maria, São Paulo, do mundo e de outras galáxias, pelo companheirismo e por colocarem sal e açúcar na minha vida. Da vida nada se leva, apenas as memórias dos bons momentos que passamos juntos.

Agradeço à minha orientadora Glaucia Kommers pelas oportunidades que me proporcionou, pela confiança, apoio e pelo aprendizado. Por me fazer me sentir um ser-humano único, e não apenas um número, com compreensão das minhas virtudes e defeitos.

Agradeço à professora Mariana Flores, pela igual oportunidade de aprendizado, pela paciência e confiança. A convivência com ela permitiu que todo o caminho fosse menos árduo.

Agradeço ao Programa de Pós-Graduação e a CAPES pelo apoio estrutural e financeiro.

Aos médicos-veterinários, professores, pós-graduandos, residentes e estagiários que pude compartilhar experiências desde que iniciei meu trajeto na Patologia Veterinária.

Deixo uma singela homenagem às famílias brasileiras que não puderam estudar em uma universidade federal, nem ver seus filhos, nem ninguém da sua família com um diploma. Não por falta de merecimento ou esforço, mas por viverem em uma sociedade não igualitária, em que a educação não é oferecida de forma igual a todos. Que tudo que está sendo produzido nessa universidade retorne de alguma forma a essas pessoas e que votemos nos nossos representantes com sabedoria. No momento que vendermos nossa educação, venderemos, portanto, nossa alma ao capital.

"*Que nada nos defina. Que nada nos sujeite.*
*Que a liberdade seja a nossa própria substância*."

(Simone de Beauvoir)

## RESUMO

## DOENÇA RENAL CRÔNICA E UREMIA EM GATOS DOMÉSTICOS

AUTORA: Marcella Barrella Ambrosio
ORIENTADORA: Glaucia D. Kommers

Esta dissertação de mestrado foi dividida em duas partes, resultando em dois artigos científicos. O primeiro artigo consistiu em um estudo comparativo entre as alterações macroscópicas, histológicas e características imuno-histoquímicas de 25 gatos com doença renal crônica (DRC) com fibrose intersticial. O diagnóstico morfológico de nefrite túbulo intersticial crônica (NTIC) foi o mais comumente observado (20/25) e cinco gatos (5/25) apresentaram doença glomerular primária. Foi observada redução do tamanho dos rins em 22 dos 25 casos e os rins diminuídos de tamanho apresentaram graus mais elevados de fibrose intersticial ($p=0.021$) e a redução do tamanho renal foi correlacionada à severidade da inflamação crônica ($p=0.0039$) e da fibrose intersticial ($p<0,001$). O aspecto macroscópico arredondado dos rins, presente apenas na NTIC foi atribuído à fibrose intersticial, atrofia tubular, obsolescência glomerular e redução da espessura da camada cortical lateralmente ao hilo renal, região compatível com os polos renais. A imunomarcação celular para actina-alfa de músculo liso (α-SMA) foi observada em todos os estágios da DRC, demonstrando a importância dos miofibroblastos nos diferentes processos e graus de intensidade da DRC com desenvolvimento de fibrose intersticial. O segundo estudo teve como objetivo determinar a prevalência e os aspectos clínicos da uremia em gatos, além dos aspectos patológicos e a distribuição anatômica das lesões extrarrenais da uremia. No período estudado (janeiro de 2000 a outubro de 2019) foram necropsiados 1.330 gatos, dentre os quais 78 apresentaram lesões extrarrenais de uremia (5,8%). Em 75% dos casos, a azotemia prolongada e uremia foram consequência de doenças renais. A DRC na forma de NTIC foi o processo renal mais comumente observado. As principais lesões extrarrenais de uremia observadas nos gatos foram o edema pulmonar e a gastrite hemorrágica e/ou ulcerativa. Mineralização de tecidos moles e hiperplasia das paratireoides foram achados incomuns, e osteodistrofia fibrosa não foi observada. A apresentação multissitêmica de lesões extrarrenais de uremia foi observada em apenas 24% dos casos, e algumas lesões normalmente encontradas em cães urêmicos não foram observadas nos gatos deste estudo.



**Palavras-chave:** Gato. Doença renal crônica. Uremia. Histoquímica. Imuno-histoquímica

## ABSTRACT

### CHRONIC KIDNEY DISEASE AND UREMIA IN DOMESTIC CATS

AUTHOR: Marcella Barrella Ambrosio
ADVISOR: Glaucia Denise Kommers

This masters dissertation was composed of two different parts, resulting in two scientific articles. The first article consisted of a comparative study between macroscopic, histological and immunohistochemical features changes in 25 cats with chronic kidney disease (CKD) with interstitial fibrosis. The morphological diagnosis of chronic tubular interstitial nephritis (CTIN) was the most commonly observed (20/25) and five cats (5/25) had primary glomerular disease. Reduction of kidney size was observed in 22 of the 25 cases and the reduced kidneys had higher degrees of interstitial fibrosis ($p = 0.021$) and the reduction in kidney size was correlated with the severity of chronic inflammation ($p = 0.0039$) and interstitial fibrosis ($p < 0.001$). The rounded gross appearance of the kidneys, observed only in CTIN, was attributed to interstitial fibrosis, tubular atrophy, glomerular obsolescence and reduced thickness of the cortical layer laterally to the renal hilum, a region compatible with the renal poles. Cellular immunostaining for α-smooth muscle actin (α-SMA) was observed in all stages of CKD, demonstrating the role of myofibroblasts in the different processes and degrees of severity of CKD with the development of interstitial fibrosis. The second study aimed to determine the prevalence and clinical aspects of uremia in cats, in addition to the pathological aspects and the anatomical distribution of nonrenal uremic lesions. During the studied period (from January 2000 to October 2019), 1,330 cats were necropsied in LPV-UFSM, of which 78 had nonrenal uremic lesions (6%). In 75% of the cases, prolonged azotemia and uremia were the result of kidney disease. CKD in the form of NTIC was the most commonly observed renal process. The most frequente nonrenal uremic lesions observed in cats were pulmonary edema and hemorrhagic and/or ulcerative gastritis. Soft tissue mineralization and parathyroid hyperplasia were uncommon findings and fibrous osteodystrophy was not observed. The multisystemic presentation of nonrenal uremic lesions was observed only in 24% of the cases, and some uremic lesions usually observed in uremic dogs were not observed in the cats of this study.



**Keywords:** Cat. Chronic kidney disease. Uremia. Histochemistry. Immunohistochemistry.

**SUMÁRIO**

## 1 INTRODUÇÃO

A doença renal crônica (DRC) é a forma mais comum de doença renal em gatos domésticos (O'NEILL et al., 2014; POLZIN, 2017; SOSNAR et al., 2003) e sua prevalência varia entre os estudos, podendo chegar até 50% (MARINO et al., 2014). Apesar de acometer gatos de todas as idades, a maior parte dos animais com DRC consiste em adultos e idosos (BARTGES, 2012; BROWN et al., 2016; DIBARTOLA et al., 1987). A DRC como causa de morte de gatos é mais expressiva em animais idosos, quando comparada a animais jovens (HAMILTON; HAMILTON; MESTLER, 1969; TOGNI et al., 2018).

Apesar de a DRC ser frequentemente observada em gatos na rotina diagnóstica do Laboratório de Patologia Veterinária da Universidade Federal de Santa Maria (LPV-UFSM), há características morfológicas (macroscópicas e microscópicas) nas lesões renais crônicas de gatos que chamam a atenção, principalmente quando comparadas à espécie canina. Exemplificando, durante a necropsia de gatos com DRC, é frequentemente observada a acentuada redução do tamanho, com perda do formato normal, observando-se rins encolhidos e arredondados (uni ou bilateralmente). O aspecto histológico observado na DRC da espécie felina conta com comprometimento predominante do compartimento túbulo-intersticial.

De fato, as DRCs de origem glomerular não são tidas como a principal etiologia da DRC nos gatos, diferentemente do que ocorre em humanos (SUMNU et al., 2015) e no cão (CIANCIOLO et al., 2016). A forma de DRC mais comumente observada em gatos é a nefrite túbulo-intersticial crônica (NTIC) sem causa específica, com evolução para fibrose intersticial acentuada, na qual a etiologia, na maior parte dos casos, ainda é pouco compreendida (BROWN, et al., 2016; CHAKRABARTI et al., 2013; DIBARTOLA, 1987; MCLELAND et al., 2015; SCHERK, 2015). Assim, os objetivos deste estudo foram caracterizar detalhadamente os aspectos macroscópicos, histológicos e imuno-histoquímicos de rins de gatos em diferentes estágios de DRC, com ênfase no estudo da fibrose intersticial como a alteração comum predominante na DRC.

Além da DRC, outras doenças do trato urinário, tanto inferior quanto superior, são importantes na medicina felina. Visto que há uma escassez de informações sobre as lesões extrarrenais de uremia em gatos na rotina diagnóstica do LPV-UFSM e a literatura utiliza o cão como o principal modelo das lesões de uremia, objetivou-se também estudar a prevalência, epidemiologia, manifestações clínicas e distribuição anatômica das lesões extrarrenais de uremia em gatos.

# 2 REVISÃO BIBLIOGRÁFICA

Nesta revisão de literatura será abordada, de maneira sucinta, a DRC em gatos, o desenvolvimento da fibrose renal e a origem dos miofibroblastos, e por fim, aspectos da insuficiência renal, azotemia e uremia.

## 2.1 DOENÇA RENAL CRÔNICA

A DRC é definida por anormalidades estruturais e funcionais envolvendo um ou os dois rins, com período de desenvolvimento de pelo menos três meses. (POLZIN, 2017). Apresenta um amplo espectro de abrangência de lesões histológicas, podendo variar de uma lesão leve e unilateral, até comprometer parte significativa do parênquima de ambos os rins (POLZIN, 2011).

A DRC é a forma mais comum de doença renal observada em gatos (O'NEILL et al., 2014; POLZIN, 2017; SOSNAR et al., 2003). A prevalência foi estimada em estudo realizado por Lund et al. (1999), sendo de 1,9 % considerando todos os gatos atendidos por um ano em clínicas particulares do Estados Unidos. Segundo estudo de Sosnar et al. (2003), a prevalência foi de 16,4% em 164 felinos analisados, podendo chegar até 50% (considerando população de 100 animais) de acordo com Marino et al. (2013).

A DRC pode afetar animais de todas as faixas etárias (BARTGES, 2012; DIBARTOLA et al., 1987), entretanto, é mais comum em gatos idosos, afetando 49% dos gatos com mais de 15 anos (CHAKRABARTI et al., 2013). Em estudo realizado com 74 gatos, o intervalo etário de animais com DRC foi de nove meses a 22 anos de idade, demonstrando que gatos de todas as idades podem manifestar a doença (DIBARTOLA et al., 1987). Mesmo assim, a prevalência é demonstrada como maior em animais mais velhos, sendo de 28% em gatos com mais de 12 anos e de 31% em gatos com mais de 15 anos (LAWSON et al., 2015).

Em estudo realizado entre 1964 e 2013 acerca da causa de morte ou razão para eutanásia de gatos necropsiados no LPV-UFSM, a DRC estava entre as principais causas de morte em gatos, totalizando 2,64%. Considerando apenas os indivíduos idosos (com idade superior a 10 anos), esse valor subiu para 53,5% (TOGNI et al., 2018).

Apenas em uma minoria dos casos é possível atribuir as lesões renais a doenças específicas (CHAKRABARTI et al., 2013; MINKUS et al., 1994). A principal forma

histológica da DRC observada em gatos é a NTIC (CHAKRABARTI et al., 2013; DIBARTOLA, 1987; MCLELAND et al., 2015; SCHERK, 2015). Nessa doença os rins normalmente se apresentam diminuídos de tamanho, firmes e com a superfície irregular (BROWN et al., 2016; SCHERK, 2015). A lesão histológica consiste em infiltrado inflamatório principalmente linfocítico no ambiente túbulo intersticial, acompanhado de fibrose intersticial, degeneração, necrose e perda do epitélio tubular e obsolescência glomerular (MCLELAND et al., 2015). A distribuição da lesão varia de multifocal a segmentar (BROWN et al., 2016). O grau de comprometimento do tecido renal está relacionado ao estágio clínico da doença (MCLELAND et al., 2015).

A causa da NTIC observada em gatos com DRC ainda não foi completamente estabelecida pela literatura, é possível que fatores genéticos, individuais e ambientais possam estar envolvidos no seu desenvolvimento (JEPSON, 2016). A idade avançada, exposição à nefrotoxinas, hipotensão, desidratação e a ocorrência de eventos isquêmicos ao longo da vida do animal, que possibilitem episódios brandos e recorrentes de insuficiência renal aguda (IRA), são considerados como os principais fatores envolvidos na patogênese da DRC na forma de NTIC (BROWN et al., 2016; JEPSON, 2016). Adicionalmente, períodos de obstrução do trato urinário por nefrólitos ou ureterólitos são potencialmente capazes de causar lesão aguda ao parênquima renal, o que pode evoluir para NTIC (JEPSON, 2016).

A glomerulonefrite crônica (GNC), considerada a principal causa de DRC em cães (MACDOUGALL et al., 1986; MULLER-PEDDINGHAUS; TRAUTWEIN, 1977; VADEN, 2017), é menos frequentemente observada em gatos (DIBARTOLA et al., 1987, VADEN, 2017). De acordo com estudo realizado com biópsias renais de gatos com DRC, 70,4% dos 120 animais apresentavam NTIC, com médio a alto grau de severidade, com quantidade variável de fibrose renal. Em segundo lugar, em termos de prevalência, estavam as GNCs, incluindo todas as suas variedades, representando 14,8% dos casos (MINKUS et al., 1994).

Há dois possíveis mecanismos de desenvolvimento da GNC. O primeiro envolve a formação de anticorpos contra antígenos da membrana basal glomerular e o segundo consiste na formação de imunocomplexos na corrente sanguínea e deposição nos glomérulos. Nesse caso, os antígenos que compõem os imunocomplexos podem ser endógenos, no caso de doenças autoimunes, ou exógenos quando originados de infecções sistêmicas por bactérias, vírus ou parasitas (ALPERS; FOGO, 2013, BRESHEARS; CONFER, 2017, SLAUSON; LEWIS, 1979). Qualquer estímulo antigênico persistente é potencialmente capaz de causar uma GNC (CIANCIOLO; MOHR, 2016). No caso da não identificação da doença primária, a

GNC é considerada idiopática (GRANT; FORRESTER, 2001). Na Tabela 1 estão listadas as doenças que podem estar associadas às GNCs nos gatos.

Tabela 1 – Doenças sistêmicas associadas às GNCs em gatos

| **Doenças sistêmicas** | |
|---|---|
| Neoplasias | Distúrbios mieloproliferativos |
| | Linfoma |
| Infecciosas | Infecções bacterianas crônicas |
| | Peritonite infecciosa felina |
| | Imunodeficiência felina |
| | Vírus da leucemia felina |
| | Poliartrite por *Mycoplasma gatae* |
| Inflamatórias | Pancreatite |
| | Colângio-hepatite |
| | Poliartrite crônica progressiva |
| | Lupus eritematoso sistêmico |
| Outros | Idiopática |
| | Toxicidade por mercúrio |
| | Glomerulopatia familiar (gatos da raça Abissínio) |

Fonte: (VADEN; GRAUER, 2011; SCHERK, 2015; WHITE et al., 2008).

Gatos com GNC podem desenvolver síndrome nefrótica, caracterizada por hipoproteinemia, proteinúria, hiperlipidemia, edema subcutâneo ou generalizado, perda de peso e atrofia muscular (CIANCIOLO; MOHR, 2016; PRESSLER, 2011; WHITE et al.; 2008) ou diferentes graus de severidade de insuficiência renal crônica (IRC) (SCHERK, 2015).

Embora menos frequentes, existem outras possíveis etiologias envolvidas no desenvolvimento da DRC na espécie felina. Dentre as doenças congênitas, a mais comumente observada é a doença dos rins policísticos, que afeta principalmente gatos da raça Persa. Outras doenças congênitas já identificadas são a amiloidose renal dos gatos Abissínios e Siameses, doença glomerular dos gatos Abissínios e a displasia renal (REYNOLDS; LEFEBVRE, 2013). As doenças adquiridas que podem levar à DRC são as nefrolitíases,

pielonefrites bacterianas, infecções pelos vírus da leucemia felina (FelV) e da imunodeficiência felina (FIV), recuperação incompleta do tecido renal após necrose tubular aguda de origem tóxica, além das causas neoplásicas, que compreendem os linfomas e carcinomas renais (CHAKRABARTI et al., 2013; REYNOLDS; LEFEBVRE, 2013).

## 2.2 FIBROSE RENAL E MIOFIBROBLASTOS

A fibrose se caracteriza pelo depósito excessivo de colágeno e outros componentes da matriz extracelular (MEC) em um determinado tecido ou órgão (KUMAR; ABBAS; ASTER, 2013). A fibrose renal é a substituição do parênquima renal por tecido conjuntivo fibroso maduro (BRESHEARS; CONFER, 2017).

Os rins são potencialmente capazes de se regenerar (LAWSON et al., 2015) e a fibrose ocorre como mecanismo alternativo à não regeneração dos componentes do néfron, quando as nefrotoxinas não são completamente removidas, quando não há manutenção da integridade da membrana basal tubular após lesões isquêmicas, ou quando o epitélio tubular não resiste às agressões dos agentes agressores. Pode envolver os glomérulos, túbulos e interstício, e infrequentemente trata-se de um evento primário (BRESHEARS; CONFER, 2017).

A fibrose é o evento que marca o estabelecimento da DRC (MACK; YANAGITA, 2014). Quando a fibrose é acentuada e extensa há destruição do parênquima renal, levando à perda estrutural e prejuízos funcionais ao órgão, e é comum o desenvolvimento de insuficiência renal crônica e uremia (BRESHEARS; CONFER, 2017; SUN et al., 2016). O mecanismo da progressão da fibrose não é completamente elucidado, mas acredita-se que proteinúria, inflamação crônica, hipóxia, idade avançada dos animais e hiperfosfatemia estejam envolvidas no estabelecimento e aceleração desse processo (LIU, 2006).

Os fibroblastos são o principal constituinte celular do interstício renal. São células mesenquimais quiescentes, fusiformes e alongadas, conhecidas pela expressão de marcadores celulares como receptor β do fator de crescimento derivado de plaquetas (PDGFR- β) e CD73 (MACK; YANAGITA, 2014; STRUTZ; ZEISBERG, 2006). Promovem sustentação do tecido renal e são responsáveis pela homeostase do ambiente renal mediante a produção e degradação equilibrada de MEC (STRUTZ; ZEISBERG, 2006; SUN et al., 2016). Atuam também na organização do ambiente intersticial, promovendo comunicação celular com o epitélio tubular, endotélio vascular e células hematopoiéticas circulantes (SUN et al., 2016).

Miofibroblastos são células responsáveis pela produção de MEC nos rins com fibrose intersticial, e compartilham características estruturais com fibroblastos, porém expressam a proteína citoplasmática actina-alfa de músculo liso (α-SMA) e possuem alta atividade proliferativa. São células contráteis, alongadas, com projeções citoplasmáticas constituídas de microfilamentos, retículo endoplasmático rugoso bem desenvolvido e contam com hemidesmossomos (STRUTZ; ZEISBERG, 2006). Foram identificadas em humanos e em diversos modelos animais de fibrose renal, principalmente no interstício e em menor quantidade nos glomérulos (SUN et al., 2016). São consideradas as células responsáveis pela deposição excessiva de MEC nos processos de fibrose renal e podem ser detectadas pela expressão de α-SMA (STRUTZ; ZEISBERG, 2007).

Apesar de muito estudada, ainda é controversa a origem dos miofibroblastos no tecido renal, pois há marcada variedade entre os resultados de diferentes grupos de pesquisa. A dificuldade se dá principalmente pela da heterogeneidade das células identificadas nos estudos e pelo fato dessas pesquisas utilizarem diferentes modelos experimentais. De maneira sucinta, esses estudos consistem em rastreamento de linhagem celular *in vivo*, e o principal modelo utilizado é a obstrução ureteral unilateral em roedores, que reproduz a fibrose intersticial e lesão tubular em relativamente pouco tempo (MACK; YANAGITA, 2014). O consenso existente mais atual é que os miofibroblastos renais possuam múltiplas origens. As teorias mais aceitas são que a transdiferenciação de fibroblastos residentes e a migração de células precursoras hematopoiéticas para os rins sejam a fonte mais expressiva. Outra possibilidade é que uma menor parcela seja limitada aos pericitos, e aos processos de transição epitelial-mesenquimal das células epiteliais tubulares, e endotelial-mesenquimal das células endoteliais vasculares (MACK; YANAGITA, 2014, STRUTZ; ZEISBERG, 2006, SUN et al., 2016).

Em humanos, a expressão de α-SMA em biópsias de rins de pacientes com DRC está diretamente relacionada à intensidade de fibrose intersticial e, portanto, à gravidade da DRC (BOUKHALFA et al., 1996). O aumento da expressão de α-SMA em rins de felinos e caninos com DRC também já foi reportado (ARESU et al., 2007, SAWASHIMA et al., 2000; YABUKI et al., 2010).

Yabuki et al. (2010) reportou imunomarcação positiva para α-SMA no interstício de rins de gatos com DRC e a expressão foi correlacionada estatisticamente com o aumento dos valores da creatinina plasmática e ao grau de fibrose intersticial, indicando que as células α-SMA positivas, interpretadas como miofibroblastos, desempenham um papel fundamental no processo fibrogênico e na progressão da DRC. Considerando a origem dos miofibroblastos nesse estudo, a expressão de vimentina, marcador celular expresso por células mesenquimais,

foi observada nos túbulos renais e foi correlacionada com a fibrose intersticial e a expressão de α-SMA, sugerindo que a transição epitelial-mesenquimal seja um possível mecanismo envolvido no surgimento dos miofibroblastos na DRC do gato.

Em estudo imuno-histoquímico em gatos com nefrite túbulo-intersticial crônica realizado por Sawashima et al. (2000), foi observada imunomarcação para α-SMA no interstício peritubular e periglomerular nas áreas de fibrose intersticial. Além disso, a expressão de α-SMA foi correlacionada positivamente com o aumento de ureia e creatinina plasmáticas. Torna-se interessante ressaltar que nesse estudo a expressão de α-SMA foi observada em estágios iniciais da lesão renal, antes mesmo da colagenização tecidual, indicando que α-SMA pode ter utilidade na detecção precoce da fibrose renal em gatos.

## 2.3 INSUFICIÊNCIA RENAL, AZOTEMIA E UREMIA

Insuficiência renal ocorre quando aproximadamente 75% da capacidade funcional dos rins está comprometida (BRESHEARS; CONFER, 2017; BROWN et al., 1997). IRA é caracterizada por redução súbita da função renal (KHAN; KHAN, 2015) com oligúria ou anúria repentinas e azotemia (CIANCIOLO; MOHR, 2016).

As principais causas de IRA são a necrose tubular aguda de origem infecciosa (bacteriana ou viral), tóxica (medicamentosa, plantas tóxicas ou metais pesados), isquemia renal, eventos obstrutivos, processos neoplásicos do trato urinário inferior ou descompensação de uma lesão renal crônica. A IRA costuma ser um processo reversível (ALPERS; FOGO, 2013; BREASHEARS; CONFERS, 2017; CIANCIOLO; MOHR, 2016; SCHERK, 2015; SERAKIDES; SILVA, 2016). As manifestações clínicas e alterações bioquímicas costumam ser mais severas na IRA, pois mecanismos compensatórios sistêmicos ainda não foram estabelecidos (DIBARTOLA; WESTROPP, 2015).

A IRC é o resultado comum das doenças renais crônicas, normalmente de caráter irreversível, caracterizada por poliúria, polidipsia, perda de peso, anemia arregenerativa, e sinais prolongados de uremia (CIANCIOLO; MOHR, 2016; DIBARTOLA;WESTROPP, 2015). Algumas doenças renais crônicas com curso clínico silencioso e insidioso podem resultar em manifestações clínicas de IRA, pela ocorrência repentina (CIANCIOLO; MOHR, 2016).

Azotemia é a elevação do nível plasmático de pequenas moléculas hidrossolúveis, compostos ligados a proteínas e moléculas de peso molecular intermediário (BRESHEARS;

CONFER, 2017; DIBARTOLA; WESTROPP, 2015; ROSS, 2011), devido à retenção causada por doenças renais ou causas extrarrenais (CIANCIOLO; MOHR, 2016). Dentre essas moléculas a ureia e a creatinina são os compostos mais estudados e melhor caracterizados, e identificados rotineiramente nos exames bioquímicos dos animais (DIBARTOLA; WESTROPP, 2015; ROSS, 2011).

A azotemia pré-renal ocorre devido à menor perfusão dos rins por desidratação severa, perda de volemia por hemorragias graves ou insuficiência cardíaca congestiva. A azotemia pós-renal ocorre quando há obstrução do trato urinário inferior (ureteres, vesícula urinária ou uretra) (ALPERS; FOGO, 2013; CIANCIOLO; MOHR, 2016).

A uremia consiste em uma síndrome clínica resultante do acúmulo de toxinas urêmicas na circulação, e envolve manifestações clínicas e lesões multissistêmicas (BRESHEARS; CONFER, 2017; ROSS, 2011). Alterações bioquímicas, metabólicas e endócrinas estão envolvidas no seu desenvolvimento (BRESHEARS; CONFER, 2017; CIANCIOLO; MOHR, 2016). As doenças renais crônicas são as principais causadoras dessa síndrome, devido ao caráter progressivo e irreversível. No entanto, injúrias agudas ou eventos obstrutivos do trato urinário inferior também podem causar uremia (CLARKSON; THOMAS, 2011).

Os sinais clínicos observados em animais com uremia são múltiplos e variam de acordo com o indivíduo, grau e velocidade da perda da função renal, e tempo que o animal permaneceu vivo em estado urêmico (BRESHEARS; CONFER, 2017; ROSS, 2011). Dentre os principais estão anorexia, perda de peso, letargia, náusea, vômito, halitose urêmica, estomatite e glossite ulcerativas, diarreia, melena ou hematoquezia, dificuldade respiratória (associada ao edema pulmonar), alterações neurológicas e de estado de consciência (encefalopatia urêmica), fragilidade e fraturas ósseas (associadas à osteodistrofia fibrosa) e hemorragias (DIBARTOLA; WESTROPP, 2015; PETERS et al., 2005; POLZIN, 2017).

Os mecanismos envolvidos nas lesões da uremia são a necrose fibrinoide vascular resultando em aumento da permeabilidade vascular, vasculite, trombose, infarto e necrose tecidual; injúria cáustica dos epitélios devido à conversão de ureia salivar ou gástrica em amônia pela atividade bacteriana (BRESHEARS; CONFER, 2017); redução da resistência dos eritrócitos na circulação, redução da hematopoese (CHALHOUB, LANGSTON, EATROFF, 2011); e desbalanço entre cálcio e fósforo (CIANCIOLO; MOHR, 2016).

As lesões de uremia são mais comumente observadas na espécie canina (CIANCIOLO; MOHR, 2016). As principais lesões extrarrenais de uremia são a estomatite e glossite ulcerativas e necrosantes, mais observadas em cães e gatos; as lesões gástricas, que

ocorrem em cães e menos comumente em gatos urêmicos, e consistem em edema da mucosa gástrica, com necrose e ulcerações, além de mineralização das camadas médias e profundas da parede gástrica; colite ulcerativa e hemorrágica, mais comumente observada em bovinos e equinos; pericardite fibrinosa, e arterite envolvendo átrios, aorta e tronco pulmonar, também são lesões observadas na espécie canina (GOLDSTEIN et al., 1998; BOEDEC et al., 2012; BRESHEARS; CONFER, 2017; PETERS et al., 2005; POLZIN, 2017). A maior parte dos animais urêmicos apresenta edema pulmonar, e a pneumopatia urêmica, caracterizada por edema, mineralização dos septos alveolares com deposição de fibrina e inflamação granulomatosa, é geralmente observada na espécie canina (BRESHEARS; CONFER, 2017; CIANCIOLO; MOHR, 2016). Calcificação dos tecidos moles ocorre principalmente em cães, e o sítio anatômico mais afetado é o tecido conjuntivo subpleural dos espaços intercostais craniais; locais menos frequentes de mineralização são coração, aorta, laringe, diafragma, intestino, língua e traqueia (SILVEIRA et al., 2015). Adicionalmente, animais urêmicos podem apresentar anemia não regenerativa, hiperplasia das paratireoides e osteodistrofia fibrosa (BRESHEARS; CONFER, 2017; CIANCIOLO; MOHR, 2016; GEDDES et al., 2013; KING et al., 1992; POLZIN, 2017).

## 3 ARTIGO 1 –

# Chronic Kidney Disease with Interstitial Fibrosis in Cats: a Comparative Study between Macroscopic and Histological Changes and the Presence of Myofibroblasts

**M. B. Ambrosio*, E. C. Lamego*, L. A. S. Tondo*, S. M. P. de Melo*, L. M. Eisenhardt*, L. C. Binder°, M. M. Flores*, R. A. Fighera*, G. D. Kommers*.**

**Laboratório de Patologia Veterinária, Departamento de Patologia, Universidade Federal de Santa Maria, Av. Roraima 1000, Camobi, Santa Maria-RS, Brasil.*

*°Laboratório de Doenças Parasitárias, Departamento de Medicina Veterinária Preventiva e Saúde Animal, Universidade de São Paulo, Av. Prof. Dr. Orlando Marques de Paiva, 87, Cidade Universitária, São Paulo-SP, Brasil.*

Corresponding author: G. D. Kommers (e-mail: glaukommers@yahoo.com).

**Abstract**

Chronic kidney disease (CKD) is frequently seen in domestic cats, and chronic tubulointerstitial nephritis (CTIN) is the most common morphological form of the disease. Interstitial fibrosis is the main histological component in the kidneys of cats with CKD, and its role in the progression of the disease has been a recent focus of attention. Although the etiology behind the majority of the cases of CKD in cats is not completely understood, it is already well established that tubulointerstitial damage is the main event leading to kidney failure and to end-stage kidney disease. Thus, the early diagnosis of tubulointerstitial histological lesion would contribute to the development of therapies aiming to retard or even to prevent the progression of CKD in cats. The main objective of this study was to perform a gross, histological and immunohistochemical characterization of the kidneys of 25 cats with CKD, considering tissue loss and the development of interstitial fibrosis, aiming to contribute to the understanding of morphological changes in renal tissue of cats with CKD. The morphological diagnosis of CTIN was the most commonly observed (20/25), followed by chronic glomerulonephritis (CG) (5/25). The kidneys reduced in size had higher degrees of interstitial fibrosis ($p = 0.021$) and the reduction in renal size was correlated with the severity of chronic inflammation ($p = 0.0039$) and interstitial fibrosis ($p < 0.001$). The rounded gross appearance of the kidneys, present only in CTIN, was attributed to interstitial fibrosis, tubular atrophy, glomerular obsolescence and reduced thickness of the cortical layer laterally to the renal hilum, a region compatible with the renal poles. Cellular immunostaining for alpha smooth muscle actin (α-SMA) was observed in all stages of CKD, demonstrating the importance of myofibroblasts in the development of interstitial fibrosis in the kidneys of cats with CKD.

*Keywords:* chronic kidney disease; cat; myofibroblasts; interstitial fibrosis

# Introduction

Chronic kidney disease (CKD) is considered nowadays the most important metabolic disease in feline medicine (Brown *et al*., 2016; Jepson, 2016) and the prevalence can reach up to 50%, depending on the population of cats studied (Marino *et al*., 2014). Further, there is an increase in the prevalence of the disease considering aged cats (DiBartola *et al*., 1987; Minkus *et al*., 1994; Bartges, 2012). The disease presents a wide variety in the intensity of histological impairment, ranging from a mild and unilateral lesion to extensive damage affecting both kidneys (Polzin, 2011).

Although congenital and acquired diseases can lead to CKD in cats (Chakrabarti *et al*., 2013; Reynolds and Lefebvre, 2013), in most of the cases it is not possible to define the specific cause of the disease at the time of diagnosis (DiBartola *et al*., 1987, Minkus *et al*., 1994; Chakrabarti *et al*., 2013). The morphological diagnosis most commonly attributed to cats with CKD is chronic tubulointerstitial nephritis (CTIN) with interstitial fibrosis (DiBartola *et al*., 1987; Minkus *et al*., 1994; Chakrabarti *et al*., 2013; McLeland *et al*., 2015).

Renal fibrosis is considered the final common pathway of CKD (Liu, 2006; Lawson *et al*., 2015; Cianciolo and Mohr, 2016) and it is one of the main histological elements present in feline CKD (Chakrabarti *et al*., 2013; McLeland *et al*., 2015). Many studies have dedicated efforts to understand the relationship between interstitial fibrosis and the disease progression. It is already well established in human medicine that, regardless of the primary cause of CKD, changes in the tubulointerstitial environment are related to the reduction of renal function (Schainuck *et al*., 1970; Nath, 1992).

Studies point out to interstitial fibrosis as the histological component best related to the degree of severity of CKD in cats and to the worsening of clinical and laboratory findings

(Chakrabarti *et al*., 2013; McLeland *et al*., 2015). The development of renal fibrosis in CKD has been associated with the proliferation of myofibroblasts, cells of the renal interstice responsible for the excessive deposition of extracellular matrix (ECM) in chronic processes (Strutz and Zeisberg, 2007). These cells have already been detected in human CKD (Boukhalfa *et al*., 1996; Novakovic *et al*., 2012), and in the CKD of dogs (Aresu *et al*., 2007; Yabuki *et al*., 2010; Kutlu and Alcigir, 2019) and cats (Sawashima *et al*., 2000; Yabuki *et al*., 2010; Kutlu and Alcigir, 2019) through the expression of the alpha isoform of smooth muscle actin protein (α-SMA).

Kidneys with advanced CKD usually become small and fibrous, and the marked loss of renal mass is usually associated with reduction of functional capacity of the organ (Cianciolo and Mohr, 2016). In human medicine, studies looking for correlations between macroscopic renal changes (assessed by imaging exams) and histological lesions revealed that most patients (69%) with reduced kidney size had severe chronic kidney damage in histopathological analysis (Moghazy *et al*., 2005).

Although the specific etiology for many cases of CKD in cats is not already completely understood, the knowledge of histological events and cellular components involved in renal damage in cats CKD becomes useful to enable an accurate diagnosis of the disease and also to contribute to the development of therapies aiming to prevent or to slow down the progression of the injury. Thus, this study was designed to perform a macroscopic, histological and immunohistochemical characterization of kidneys of cats with different degrees of severity of CKD, aiming to contribute to the understanding of morphological changes in the renal tissue, taking into account tissue loss, the development of interstitial fibrosis, and its consequences on tissue morphology.

## Materials and Methods

For the morphological study of CKD, 25 necropsied cats were selected at the LPV-UFSM necropsy records between the years 2006 and 2019. The inclusion criteria of the study were the presence of chronic histological lesion, no evidence of “acute-on-chronic” disease, absence of obstructive episodes (urolithiasis) before the development of renal injury, and sufficient availability of tissue included in paraffin blocks to perform serial histological sections. Kidneys from ten cats also necropsied in the LPV-UFSM between the years 2018 and 2019, without gross and histological kidney changes and without clinical history of kidney disease were used as normal controls.

Information regarding the age, sex and breed of each case was obtained from the necropsy records. Kidney shrinkage (unilateral or bilateral) and changes in the kidney normal conformation (shrinking and rounding) were assessed, based on the anatomical characteristics and the normal size of the kidneys for the feline specie. In addition, other macroscopic changes such as increased consistency (firm kidneys), colour change, irregularity of the capsular surface; reduction of the cortical layer, presence of white streaks and areas of depression of the renal parenchyma (infarcts / scars) were also recorded.

The kidney samples of all cats had been collected during post-mortem examination, fixed in 10% neutral buffered formalin, and embedded in paraffin according to the standard histological procedure. Paraffin blocks were cut into serial and alternating 3mm sections, and subsequently stained with hematoxylin-eosin (HE) for histological evaluation of the kidney injury. Periodic Acid Schiff (PAS) and Masson's Trichrome stain (TM) were performed in all cases in order to confirm thickening of the glomerular basement membranes (of the glomerular capillaries and Bowman's capsule) and to better demonstrate the interstitial collagenized connective tissue, respectively.

In all cases the kidneys were assessed bilaterally, but in those where only one kidney showed histological lesion, only the affected kidney was considered in subsequent evaluations. In cases where the lesion was distinct between the two kidneys, both were considered.

Tissues stained with MT and HE were scored for interstitial fibrosis and interstitial inflammation, respectively, on a scale from 0 to 4, adapted from Chakrabarti *et al*. (2013) as follows: 1 = no fibrosis/inflammation to 25% of the section compromised; 2 = 26% to 50% of the section compromised; 3 = 51% to 75% of the section compromised; 4 = more than 75% of the section compromised by fibrosis/inflammation.

The thickness of the cortical layer was obtained considering the average of four consecutive measurements of renal cortex in a histological section of each kidney, obtained using a microscope Olympus BX5 with digital camera DP21, controlled by the Olympus CellScens Standard program. In cases where the cortical thickness was uniform, four random measurements were taken, and in cases of irregular reduction, the largest and the shortest distance were used.

The differences in the fibrosis and inflammation scores between kidneys with macroscopic reduction and those without macroscopic reduction were obtained using Mann-Whitney statistical test. Spearman's Correlation test was performed to verify the existence of a correlation between the fibrosis and inflammation scores and the thickness of cortical layer. Statistical tests were performed using the software R (R Development Core Team 2019).

Immunohistochemistry (IHC) as performed according to the following steps: deparaffinization and rehydration of silanized slides with 2µm to 3µm sections; blocking endogenous peroxidases with two treatments with 3% hydrogen peroxide ($H_20_2$) for 10 minutes each; antigen retrieval by heating the sample for ten minutes in a citrate buffer (pH 6.0) in a microwave; blocking unspecific reactions in 5% casein in PBST at 25 ºC for 30 min;

incubation with human anti-α-SMA antibody (clone 1 A4; α isoform of human smooth muscle actin, EasyPath [EP-12-52833]) diluted at 1:200, at 25ºC for 60 min; incubation with one step polymer (Kit EasyLink One [EP-12-20504]) at 25ºC for 30 min; washing was performed with PBTS; immunoreactivity was detected with 3, 3'diaminobenzidine (DAB; Dako, Carpinteria, CA) as chromogen for 3 to 5 min; and counterstaining was performed with Harris haematoxylin. For negative control sections, PBST was used instead of the primary antibody. For positive controls, sections of uterus of cats were used and smooth muscle cells from blood vessels of the kidneys were used as internal positive controls.

## Results

Renal histologic data were obtained from 25 cats with CKD of which age, sex and breed information are in Table 1. The renal lesion of twenty cats (20/25; 80%) was compatible with CTIN, and in five cases (5/25; 20%) the morphological diagnosis was of chronic glomerulonephritis (CG).

*Morphological aspects*

*Chronic tubulointerstitial nephritis (n=20)*

The most common gross finding was the marked shrinkage and rounding of the kidneys (Fig. 1). In most of the cases, capsular surface had a smooth and regular appearance, usually with a focal area of parenchyma retraction (scarring), often located bilaterally to the renal hilum (equivalent to the renal poles) (Fig. 2). The reduction in the size of rounded kidneys varied from bilateral (10/20) (Fig. 3), to bilateral asymmetric (3/20) and unilateral (5/20), with reduction of the right kidney in three cases, and of the left one in two cases. In two cases (2/20), there was no reduction in kidney size. In three cases of unilateral kidney

shrinkage, a less severe histological lesion was observed in the contralateral kidney, and in three cases of asymmetric bilateral kidney shrinkage the histological lesion was distinct between the two kidneys. In this way, 26 different histological lesions were observed in the cases of CTIN.

On histology, there was variation in the intensity of interstitial fibrosis and inflammation between cases and also in the cortical layer thickness (Table. 2). In general, CTIN was characterized by predominantly lymphocytic interstitial inflammation with interstitial fibrosis organized in bands, with radial orientation, and segmental multifocal distribution (Fig. 4). These bands consisting of inflammation and fibrosis coincided with the areas of reduced cortical thickness. All, or almost all of the glomeruli located within these bands showed marked shrinkage, hyalinization (PAS-positive) and fibrosis of the glomerular tuft (light blue colour on the TM) (Fig. 5) with capillaries loss, characterizing glomerular obsolescence.

Tubules in these areas showed atrophy and loss of tubular lumens. Tubular loss with tubular basement membrane disruption was frequently observed, with interstitial lipid present in the interstice. The lipid content was sometimes observed inside the cytoplasm of foamy macrophages. The areas of tissue retraction observed in the gross examination coincided with the areas of greatest histological lesion (accentuated cortical thinning, glomerular obsolescence, interstitial fibrosis and inflammation) (Fig. 6). In the bands consisting of inflammation and interstitial fibrosis, it was possible to observe a marked proliferation of small arteries usually surrounding the glomeruli with obsolescence. The morphologically viable glomeruli near to the segmental areas of lesion showed moderate to severe periglomerular fibrosis and Bowman's capsule thickening. Additional histological components observed were intratubular hyaline casts (20 cases), multifocal mineralization (18 cases), tubular epithelial necrosis (11 cases) and intratubular birefringent crystals (7 cases).

*Chronic glomerulonephritis (n=5)*

In four cases (4/5) the kidneys were reduced in size on gross examination, with maintenance of the natural shape of the kidneys in all cases. Delicate granulation of the capsular surface was observed, and at longitudinal section, the parenchyma showed variable amount of whitish streaks. On histology, two cases were diagnosed as membranous CG, two cases were diagnosed as membranoproliferative CG, and mesangioproliferative CG was observed in one case. The latter was characterized by expansion of the mesangial matrix associated with mesangial hypercellularity. Glomerular sclerosis was observed, characterized by obliteration of glomerular capillaries by extracellular matrix in areas of the glomerulus (segmental sclerosis), or of the entire glomerular tuft (global sclerosis). Sclerotic glomeruli were observed randomly and with multifocal distribution.

Cases of CG presented variable intensity of interstitial inflammation and fibrosis; cortical thinning was usually uniform (Table. 3). Multifocal lymphoplasmacytic inflammatory infiltrate was observed, mainly in the periglomerular region and there was a predominance of interstitial fibrosis in the corticomedullary junction, with uniform distribution throughout the histological section. Intratubular hyaline casts (5 cases), mineralization foci (3 cases), intratubular birefringent crystals (2 cases) and tubular epithelial necrosis (1 case) were observed.

*Statistic*

There was a significant difference of interstitial fibrosis scores between kidneys with macroscopic reduction than those kidneys without macroscopic diminution (p = 0,021) with

significance set at the 5% level. The kidneys reduced in size showed greater interstitial fibrosis scores than those without changes in size. Considering interstitial inflammation, there was no significant difference between both groups. The decrease of cortical layer thickness was found to be significantly correlated with interstitial inflammation score ($p = 0.0039$) and also strongly correlated with interstitial fibrosis ($p<0,001$).

*Immunohistochemistry*

*Control cats (n=10)*

Smooth muscle cells from blood vessels demonstrated strong intracytoplasmic immunostaining for α-SMA. In three cases, there were no other cells with positive immunostaining for α-SMA. In the other cases, few glomerular cells showed weak immunostaining for α-SMA, and a variable amount of strongly α-SMA positive spindle cells were observed in the interstice of the corticomedullary junction (Fig. 8).

*Chronic tubulointerstitial nephritis (n=20)*

In the cortical region corresponding to the radial bands of chronic inflammation, α-SMA immunostaining was markedly enhanced. The positive α-SMA cells ranged from spindle shape to slightly stellate (Fig. 9). In the interstice of the corticomedullary junction there was a marked α-SMA immunostaining and in the areas where interstitial fibrosis was more pronounced, the positive cells were in close contact with the atrophic tubules. The number of positive α-SMA cells was moderate in the external medulla, usually organized in radial orientation throughout the tissue (Fig. 10), and mild to sparse in the internal medulla. In fields free of histological lesion, immunostaining for α-SMA was similar to that observed in the control kidneys.

The morphologically healthy glomeruli, detected in the regions adjacent to the areas of glomerular obsolescence showed a moderate amount of cells strongly positive for α-SMA. In periglomerular spaces with deposition of fibrous tissue there were fusiform and delicate positive cells. The number of positive cells inside the glomeruli decreased as the glomeruli loss its cellularity and showed expansion of fibrous tissue (Fig. 11), until immunostaining became absent in glomerular obsolescence.

In the areas of marked tissue retraction, located mainly laterally to the renal hilum, it was possible to recognize a greater abundance of α-SMA-positive cells, with similar intensity in the cortical, corticomedullary junction and medullary regions. These regions showed proliferation of small arteries, which were evidenced by α-SMA positive smooth muscle cells.

*Glomerulonefrite crônica (n=5):*

Cells with positive immunostaining for α-SMA were located around the glomeruli, in the middle of the inflammatory infiltrate and in the regions of periglomerular fibrosis (Fig.12). In the corticomedullary junction positive α-SMA spindle cells were observed throughout the histological section. No positive immunostaining was observed in the inner medulla.

## Discussion

The systematic and comparative analysis carried out in this study between gross and histological aspects of the kidneys of cats with CKD allowed to the identification of a distinct pattern of lesion present in the kidneys with CTIN. Shrinkage and rounding aspect, observed in most cases of CTIN, especially in cases with more than 50% of the renal parenchyma compromised by chronic inflammation and interstitial fibrosis (scores III and IV), was not

observed in CG. The explanation for the round conformation of the kidneys with CTIN was the accentuation of the histological lesion bilaterally to the renal hilum, with greater narrowing of the cortical layer, greater intensity of inflammation and fibrosis and a higher number of α-SMA positive cell, compatible with myofibroblasts, in that specific region. Such changes resulted in flattening of renal poles, an alteration possibly similar to that previously described as "scars" in the renal poles (Lucke, 1968). Interestingly, in the study conducted by Lucke (1968), round kidneys had more marked histological lesion, while the cats had a greater decline in kidney function, suggesting that this is the presentation of the final stages of CKD in cats.

These areas of tissue loss, which led to the flattening of the renal poles, were compatible with areas of greater intensity of interstitial inflammation and fibrosis. It is known that CTIN, the most common presentation of CKD in cats (DiBartola *et al*., 1987; Chakrabarti *et al*., 2013; McLeland *et al*., 2019), has as its main components chronic inflammation and interstitial fibrosis, with multifocal to segmental distribution (Brown *et al*., 2016). These two histological elements were observed in all cases of CTIN of this study, reaching up to 75% of the renal parenchyma in some cases. The main recognized mechanism in the development of interstitial fibrosis is chronic inflammation (Lawson, 2015), in which the recruitment of inflammatory cells after tissue injury results in the production of pro-fibrotic cytokines, which lead to the activation of myofibroblasts, and to the production of ECM (Meng, 2019). This is one of the mechanisms recognized in the development of interstitial fibrosis in feline CTIN (Lawson *et al*., 2015) and a correlation between chronic inflammation and interstitial fibrosis has already been observed in the kidneys of cats with CKD (Chakrabarti *et al*., 2013).

Lymphocytes were the main cells present in the cases of CTIN in this study. Lymphocytic inflammation has been described in the early stages of CKD in cats, becoming more pronounced and accompanied by interstitial fibrosis in cases of greater impairment of

the renal parenchyma (McLeland *et al*., 2015). Granulomatous inflammation, observed in cases of more severe interstitial inflammation (score III and IV) in this study, was also observed by McLeland *et al*. (2015), especially in cats with more than 50% of renal tissue affected. In addition, a significant correlation has recently been established between the infiltration of macrophages in renal tissue, interstitial fibrosis and the progression of CKD in cats, indicating that macrophages also act in the development of interstitial fibrosis (Ohara *et* al., 2019). Granulomatous inflammation is these cases is considered to be secondary to ischemic tubular injury, with tubular rupture, and leakage of lipids from tubular epithelial cells to the renal interstice (Brown *et al*., 2016; Schmiedt *et al*., 2016).

Glomerular obsolescence is characterized by thickening and contraction of the glomerular basement membrane towards the vascular pole, and as the glomerulus undergoes retraction, there is deposition of acellular fibrous connective tissue between the glomerular tuft and the Bowman capsule, resulting in reduced size glomeruli (Hughson *et al*., 2002). This type of lesion, frequently observed in CTIN in this study, has been described as the predominant glomerular lesion in cats CKD (Chakrabarti *et al*., 2013). It is considered a lesion secondary to the primary tubulointerstitial process, and it is morphologically compatible with ischemic glomerular obsolescence (Brown *et al*., 2016). In humans, glomerular obsolescence is attributed to reduced glomerular perfusion by narrowing of interlobular arched artery secondary to vascular lesions (Hughson *et al*., 2012). In the case of cats, glomeruli with obsolescence are considered part of atubular nephrons, and have been associated with failure in the regeneration of atrophic tubules secondary to the expansion of interstitial fibrous connective tissue or secondary to tubules that undergo ischemic tubulorrhexis (Brown *et al*., 2019).

Current experimental studies have collaborated with the understanding of the pathogenesis involved in feline CTIN (Schmiedt *et al*., 2016; Brown *et al*., 2019). Changes

observed in the kidneys of cats six months after induced ischemic lesion (90 minutes of renal ischemia) consisted of inflammation and interstitial fibrosis, tubular atrophy and glomerular obsolescence with markedly compromised fields, interspersed with healthy areas (Brown *et al*., 2019). This set of alterations described is very similar to the histological pattern observed in spontaneous cases of CTIN in this study. The experimental findings have been compared to the histological lesion frequently observed in cats naturally affected by CKD (Brown *et al*., 2016; Brown *et al*., 2019). They support the hypothesis that multiple mild ischemic insults, or one single severe ischemic insult, followed by failures in tissue repair and propagation processes inherent to the feline specie, may be involved in the development of CKD in cats in the form of CTIN (Jepson, 2016).

In most of the cases of CKD in this study, it was notable the reduction of kidneys' size. The kidneys reduced in size had statistically higher scores of interstitial fibrosis than normal sized kidneys. It was also notable that, with the reduction in renal size, the degrees of inflammation were higher, but with no statistical difference for this parameter ($p = 0.054$). Additionally, the strong correlation between the increase in the scores of interstitial fibrosis and the reduction in renal cortex point to fibrosis as the main histological component present in the loss of renal tissue. Chronic inflammation was also correlated with renal cortex thinning, but there was a tendency for this parameter to show lower scores when compared to fibrosis, which probably reflects a chronological characteristic of chronic kidney injury. Inflammation is the initial response to tissue injury, and through the production of pro-fibrotic factors and activation of ECM-producing cells (myofibroblasts) there is a predominance of interstitial fibrosis in the most advanced terminal stages of CKD (Liu, 2006; Meng, 2019).

The detection of α-SMA in kidneys of cats with CKD allows the identification of interstitial cells with positive immunostaining for this antibody and these cells are considered to be myofibroblasts (Swashima *et al*., 2000; Yabuki *et al*., 2010). In fact, the anti-α-SMA

antibody is a reliable tool and is considered the most efficient in the identification of renal myofibroblasts, which by conventional histology cannot be differentiated from fibroblasts (Strutz and Zeisberg, 2006).

In the present study, it was possible to identify spindle cells positive for α-SMA in the renal interstice, both in CTIN and CG. Myofibroblasts were more evident in the interstice of kidneys with CTIN, which showed more severe histological lesion. In CTIN, the distribution of α-SMA-positive cells was compatible with the areas affected by chronic inflammation and interstitial fibrosis and, therefore, areas with greater tissue loss. It is possible that myofibroblasts are involved in the process of tissue loss in the kidneys of cats with CKD, as these cells have a contractile cellular apparatus (contractile proteins) in the composition of the cytoskeleton, which allows them to exert mechanical forces in the contraction of wounds and in the pathological conditions that culminate in tissue contraction and loss of delicate renal tissue architecture (Tomasek *et al*., 2002).

Glomerular mesangial cells act in the production of cytokines and growth factors involved in fibrosis, and are an expressive source of cell differentiation in myofibroblasts in the processes of glomerular sclerosis (Liu, 2016; Meng, 2019). Positive immunostaining for α-SMA in these cells has already been reported in human glomerular diseases (Boukhalfa et *al*., 1996). In the CTIN of the cats in this study, positivity for α-SMA was observed in morphologically healthy glomeruli located near to damaged areas, with subsequent reduction of the immunostaining in glomeruli undergoing obsolescence. This characteristic was observed by Sawashima *et al*. (2000) in cats with CKD and these findings may indicate a probable involvement of myofibroblasts originating from mesangial cells in the continuous process of glomerular obsolescence. The loss of mesangial cells with the progression of the lesion resulted in the absence of positive immunostaining in completely fibrous glomeruli.

Cases of CG demonstrated less involvement of the parenchyma by chronic inflammation and fibrosis (scores I and II) when compared to cases of CTIN. Even so, positive α-SMA cells were also observed, especially in areas of interstitial fibrosis such as periglomerular space corticomedullary junction. This finding demonstrates that, although primary glomerular processes are infrequent in cats (DiBartola *et al*., 1987; Minkus *et al*., 1994), they also culminate into tubulointerstitial injury. These consequences must be considered, since the progression of CKD with renal failure is related to the extent of tubulointerstitial involvement regardless of the primary cause (Nangaku, 2010).

Likewise, the observation of positive α-SMA cells in the renal interstice of cats with initial CTIN and CG (with less than 25% of the parenchyma compromised by the tubulointerstitial lesion) corroborates the findings of Sawashima *et al*. (2000), who reported increased expression of α-SMA in kidneys of cats in early stages of CKD, with absent or mild deposition of collagenous connective tissue in the renal interstice. The short interval between tissue damage and myofibroblast proliferation in renal tissue was also demonstrated by the presence of immunostaining for α-SMA in the initial repair phase after experimental ischemic injury (Schmiedt et al., 2016). These evidences are very relevant, considering that the expression of α-SMA may represent an early marker of tubulointerstitial damage in kidneys of cats with CKD.

The reduction in kidney size was a common aspect observed in the CKD in this study and it is suggestive that chronic inflammation with progression to interstitial fibrosis, together with proliferation of myofibroblasts would be involved in this process. The reduction in kidney size in CTIN occurred in a singular way, with frequent shrinkage and rounding of the kidneys. This unique characteristic was attributed not only to higher degrees of intensity of renal fibrosis, but also to the marked impairment of the glomeruli and tubules located in the

renal perihilar region. The results of this study reinforce the participation of myofibroblasts in the development of interstitial fibrosis in CKD in the feline species.

## Acknowledgements

This research was financially supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil. The student M. B. Ambrosio has a Master's scholarship from CAPES.

## Conflict of Interest Statement

The authors declare no conflicts of interest with respect to the research, authorship or publication of this article.

# References


Aresu L, Rastaldi MP, Scanziani E, Baily J, Radaelli E et al. (2007) Epithelial-mesenchymal transition (EMT) of renal tubular cells in canine glomerulonephritis. *Virchows Archiv: European Journal of Pathology*, **451,** 937–942.

Bartges JW (2012) Chronic kidney disease in dogs and cats. *Veterinary Clinics of North America - Small Animal Practice*, **42**, 669–692.

Boukhalfa G, Desmouliere A, Rondeau E, Gabbiani G, Sraer JD (1996) Relationship between alpha-smooth muscle actin expression and fibrotic changes in human kidney. *Experimental Nephrology*¸ **4**, 241–247.

Brown AB, Rissi DR, Dickerson VM, Davis AM, Brown SA (2019) Chronic Renal Changes After a Single Ischemic Event in an Experimental Model of Feline Chronic Kidney Disease. *Veterinary Pathology*, **56**, 536-543.

Brown CA, Elliot J, Schmiedt CW, Brown SA (2016) Chronic kidney disease in aged cats: clinical features, morphology, and proposed pathogeneses. *Veterinary Pathology*, **53**, 309-326.

Chakrabarti S, Syme HM, Brown CA, Elliot J (2013) Histomorphometry of feline chronic kidney disease and correlation with markers of renal dysfunction. *Veterinary Pathology*, **50**, 147–155.

Cianciolo RE, Mohr FC (2016) Urinary sistem. In: *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*, 6th Edit., MG Maxie, Elsevier, St Louis, pp. 377-463.

DiBartola SP, Rutgers HC, Zack PM (1987) Clinicopathologic findings associated with chronic renal disease in cats: 74 cases (1973 -1984). *Journal of American Veterinary Medical Association*, **190**, 1196–202.

Hughson MD, Johnson K, Young RJ, Hoy WE, Bertram JF (2002) Glomerular size and glomerulosclerosis: relationships to disease categories, glomerular solidification, and ischemic obsolescence. *American Journal of Kidney Diseases*, **39**, 679–688.

Jepson RE (2016) Current understanding of the pathogenesis of progressive chronic kidney disease in cats. *Veterinary Clinics of North America: Small Animal Practice*, **46**, 1015-1048.

Kutlu T, Alcigir G (2019) Comparison of renal lesions in cats and dogs using pathomorphological and ımmunohistochemical methods. *Biotechnic & Histochemistry*, **94**, 126-133.

Lawson J, Elliot J, Wheeler-Jones C, Symed H, Jepson R (2015) Renal fibrosis in feline chronic kidney disease: known mediators and mechanisms of injury. *Veterinary Journal*, **203**, 18–26.

Liu Y (2006) Renal fibrosis: new insights into the pathogenesis and therapeutics. *Kidney International*, **69**, 213–217.

Lucke VM (1968) Renal disease in the domestic cat. *Journal of Pathology and Bacteriology*, **95**, 67–91.

Marino CL, Lascelles BDX, Vaden SL, Gruen ME, Marks SL (2014) Prevalence and classification of chronic kidney disease in cats randomly selected from four age groups and in cats recruited for degenerative joint disease studies. *Journal of Feline Medicine and Surgery*, **16**, 1 –8.

McLeland SM, Cianciolo RE, Duncan CG, Quimby JM (2015) A comparison of biochemical and histopathologic staging in cats with chronic kidney disease. *Veterinary Pathology*, **52**, 524-534.

McLeland S, Quimby J, Lappin MR (2019) Alpha-enolase staining patterns in the renal tissues of cats with and without chronic kidney disease. *Veterinary Immunology and Immunopathology*, **212**, 23-26.

Meng XM (2019) Inflammatory mediators and renal fibrosis. *Advances in Experimental Medicine and Biology*, **1165,** p. 381-406.

Minkus G, Reusch C, Horauf P, Breuer W, Darbhs J et al. (1994) Evaluation of renal biopsies in cats and dogs — histopathology in comparison with clinical data. *Journal of Small Animal Practice*, **35**, 465–472.

Moghazi S, Jones E, Schroepple J, Arya K, McClellan W et al. (2005) Correlation of renal histopathology with sonographic findings. *Kidney International*, **67**, 1515–1520.

Nangaku M (2004) Mechanisms of tubulointerstitial injury in the kidney: final common pathways to end-stage renal failure. *Internal Medicine*, **43**, 9–17.

Nath KA (1992) Tubulointerstitial changes as a major determinant in the progression of renal damage. *American Journal of Kidney Diseases*, **20**, 1-17.

Novakovic ZS, Durvov MG, Puljak L, Saraga M, Ljutic D (2012) The interstitial expression of alpha-smooth muscle actin in glomerulonephritis is associated with renal function. *Medical Science Monitor*, **18**, 235-240.

Ohara Y, Yabuki A, Nakamura R, Ichii O, Mizukawa et al. (2019) Renal infiltration of macrophages in canine and feline chronic kidney disease. *Journal of Comparative Pathology*, **170**, 53-59.

Polzin, DJ (2011) Chronic kidney diseases in small animals. *Veterinary Clinics: Small Animal practice*, **41**, 15–30.

Reynolds BS, Lefebvre HP (2013) Feline CKD: pathophysiology and risk factors-what do we know. *Journal of Feline Medicine and Surgery*, **15,** 3–14.

Sawashima KO, Mizuno S, Mizuno-Horikawa Y, Shimada A, Kudo T et al. (2000). Expression of α-smooth muscle actin and fibronectin in tubulointerstitial lesions of cats with chronic renal failure. *American Journal of Veterinary Research*, **61**, 1080–1086.

Schainuck LI, Striker GE, Cutler RE, Benditt EP (1970) Structural-functional correlations in renal disease. II. The correlations. *Human pathology*, **4**, 631-641.

Schmiedt CW, Brainard BM, Hinson W, Brown SA, Brown CA (2016) Unilateral renal ischemia as a model of acute kidney injury and renal fibrosis in cats. *Veterinary pathology*, **53**, 87-101.

Strutz F. Zeisberg M (2006) Renal fibroblasts and myofibroblasts in chronic kidney disease. *Journal of the American Society of Nephrology*, **17**, 2992–2998.

Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA (2002) Myofibroblasts and mechanoregulation of connective tissue remodelling. *Nature Reviews Molecular Cell Biology*, **3**, 349-363.

Yabuki A, Mitani S, Fujiki M, Misumi K, Endo Y, et al. (2010) Comparative study of chronic kidney disease in dogs and cats: induction of myofibroblasts. *Research in Veterinary Science*, **88**, 294-299.

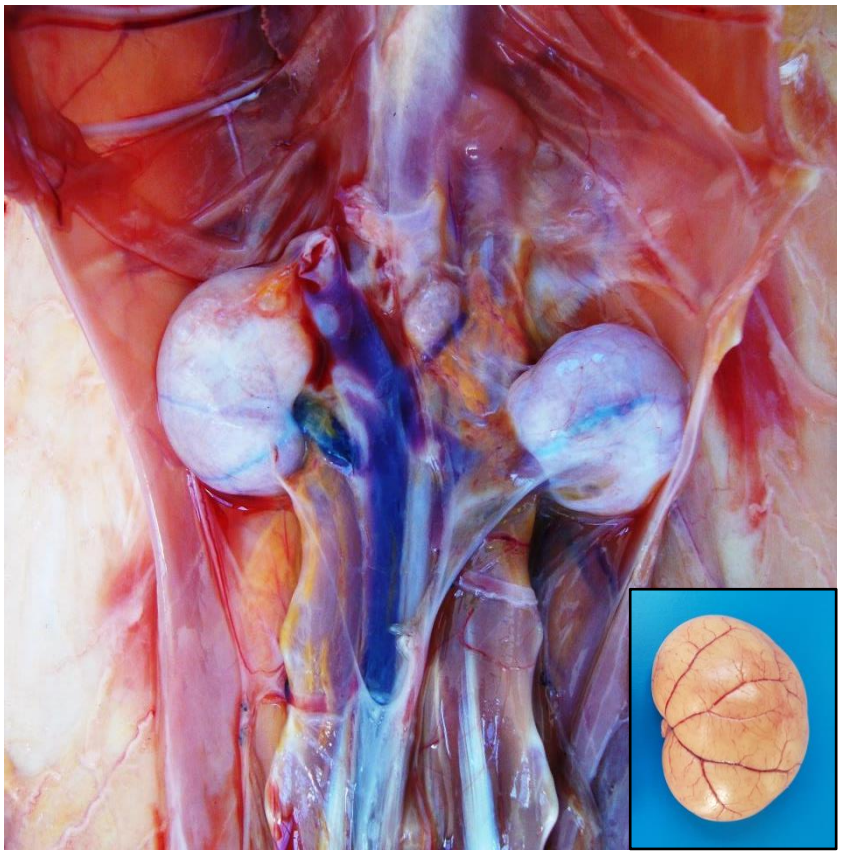

Fig. 1. CTIN, kidney, cat. Kidneys bilaterally reduced in size and markedly rounded, positioned dorsally in the retroperitoneum. In detail, the gross appearance of a control kidney.

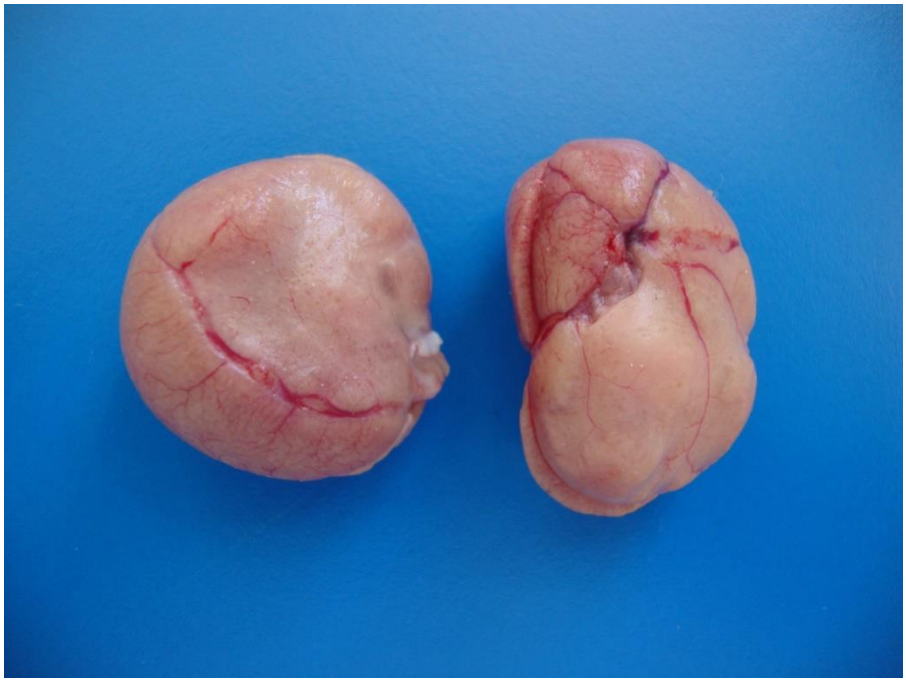

Fig 2. CTIN, kidney, cat. Left kidney rounded by flattening of the renal poles. Right kidney with focal area of tissue retraction (scar), located laterally to the renal hilum.

Fig 3. CTIN, kidney, cat. Bilaterally reduced in size and rounding kidneys. There is a greater narrowing of renal cortex bilaterally to the renal hilum, what turned into rounded gross appearence.

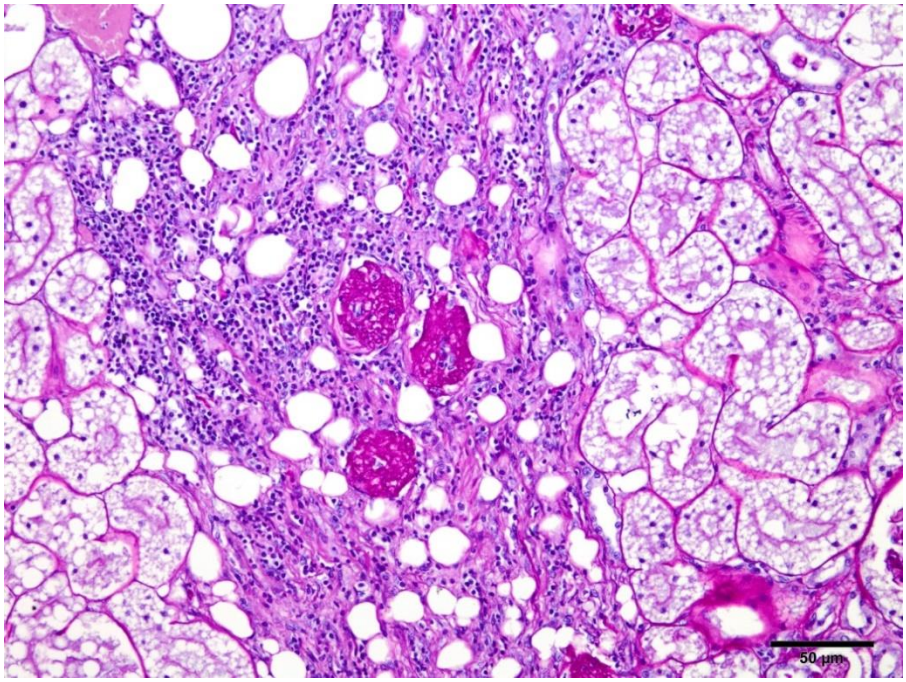


Fig 4. Light micrograph of a kidney of a cat with CTIN. Cortical lymphocytic inflammation is accompanied by interstitial fibrosis organized in bands with radial orientation. PAS. Bar, 50mm.

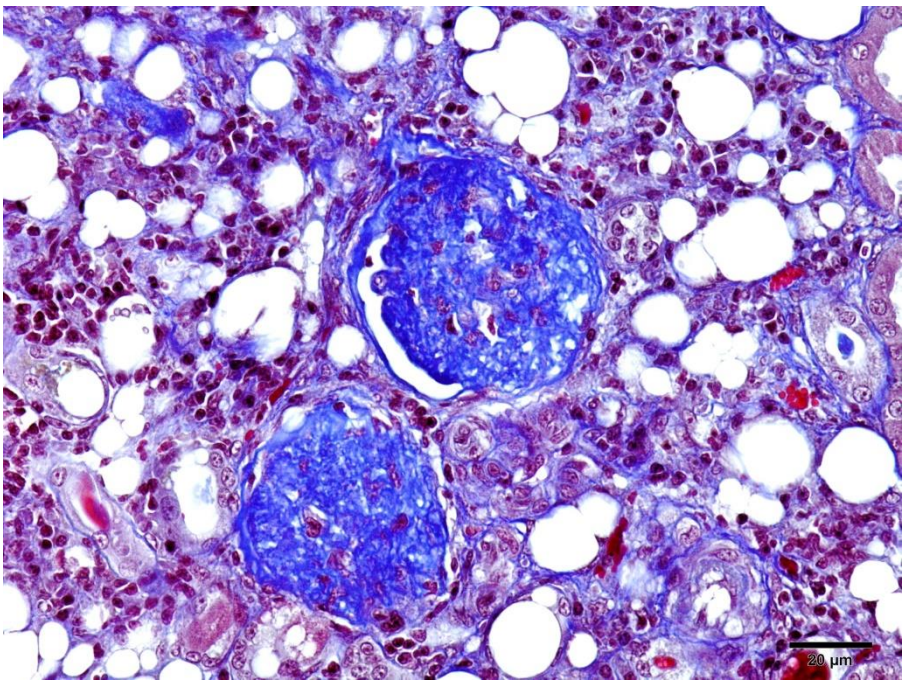


Fig 5. Light micrograph of a kidney of a cat with CTIN. The glomerulus is diminished in size and exhibits marked wrinkling of glomerular basement membrane, with capillary loss and deposition of fibrous connective tissue (ischemic obsolescence). Masson's trichrome stain. Bar, 20mm.

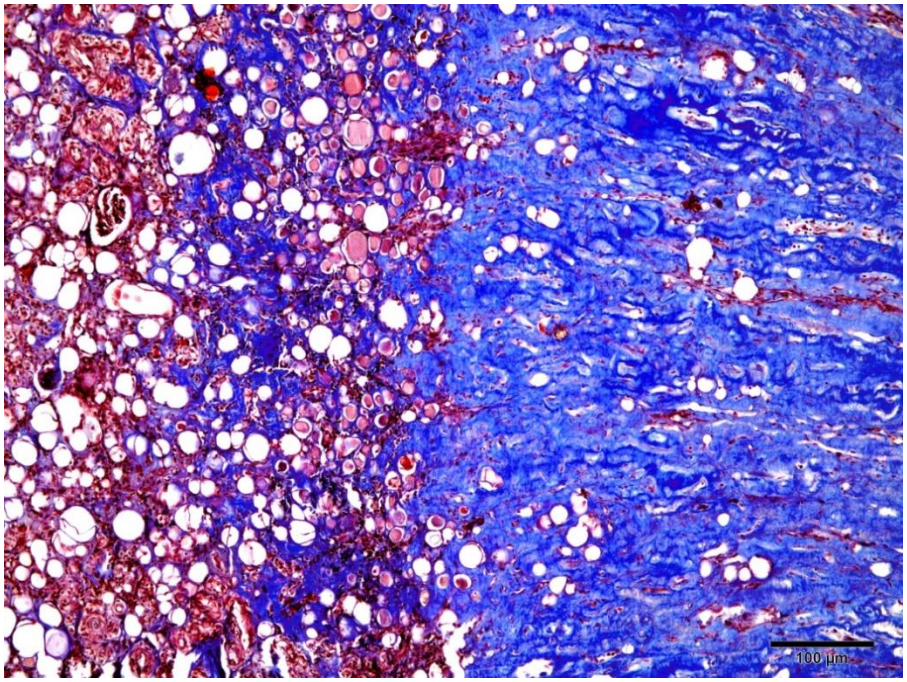


Fig 6. Light micrograph of a kidney of a cat with TICN. More than 75% of the renal parenchyma is affected by interstitial fibrosis (collagenous matrix stained in blue). There is collapse of cortical layer and marked tubular atrophy and loss. Masson's trichrome stain. Bar, 100mm.

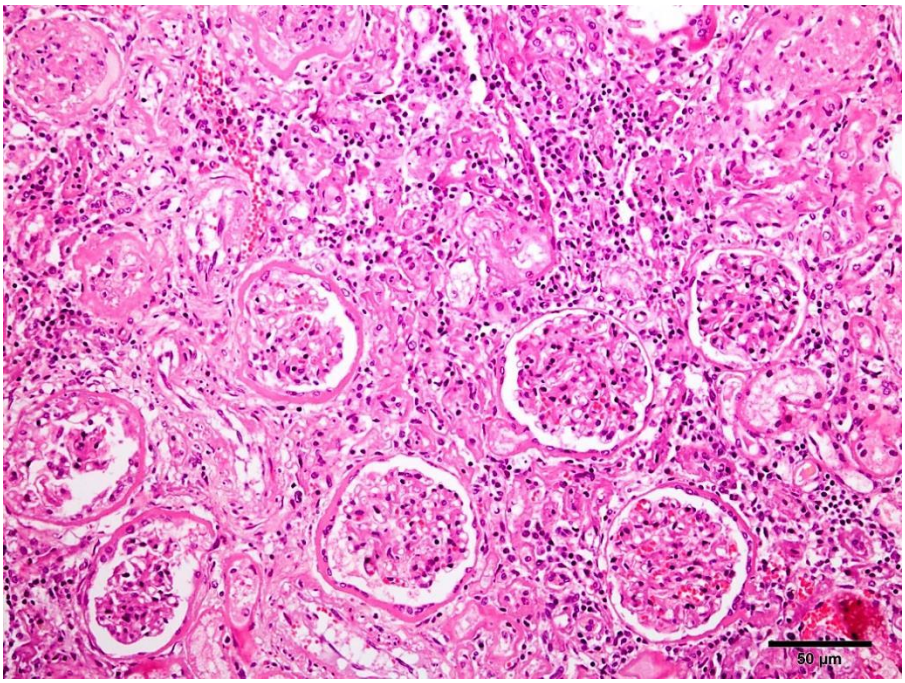


Fig 7. Light micrograph of a kidney of a cat with CG. There is periglomerular lymphoplasmocytic inflammation and segmental to global glomerular sclerosis with multifocal distribution. HE. Bar, 50mm.

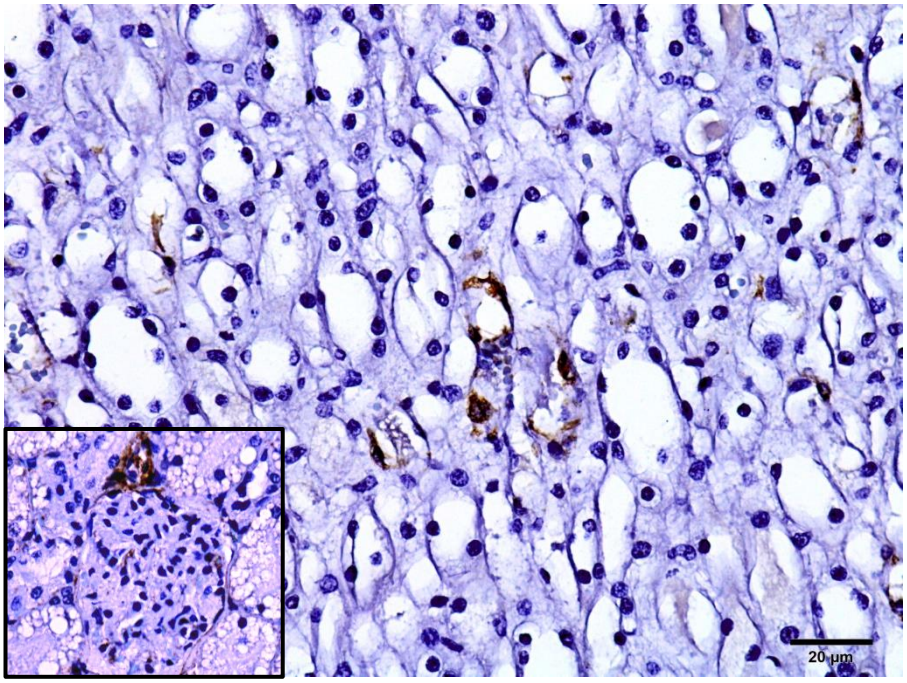


Fig 8. Immunohistochemical labelling for α-SMA in a control cat. Few myofibroblasts in the interstice surrounding the distal convoluted tubules. In detail, positive smooth muscle cells of the glomerular arteriole. Bar, 20mm.

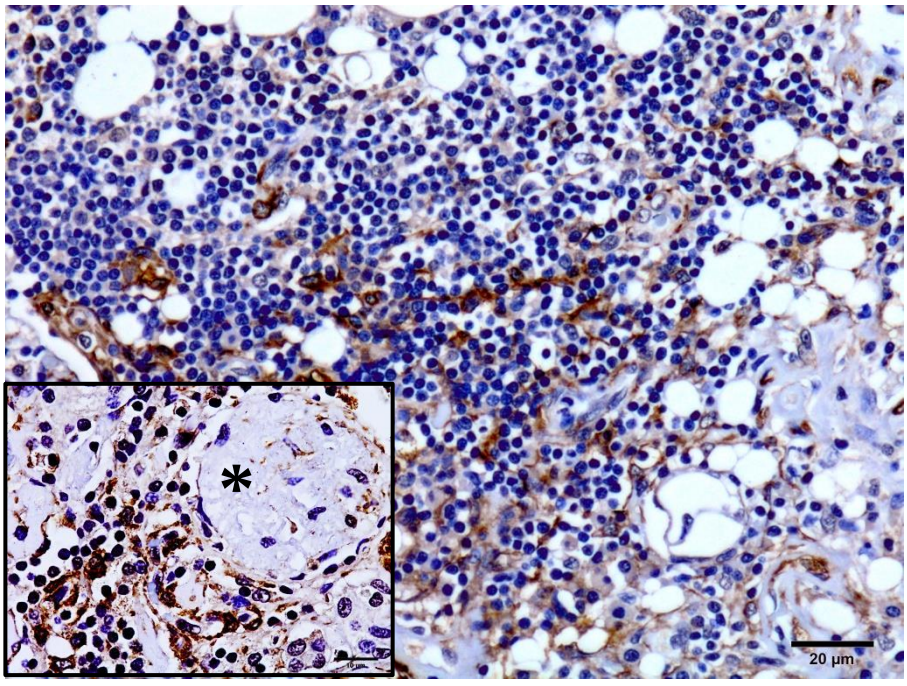


Fig 9. Immunohistochemical labelling for α-SMA, CTIN, cat. Positive spindle cells within the lymphocytic inflammatory infiltrate. In detail, myofibroblasts are visible surrounding a glomerular obsolescence. No positive cells inside glomerular corpuscle. Bar, 20mm.

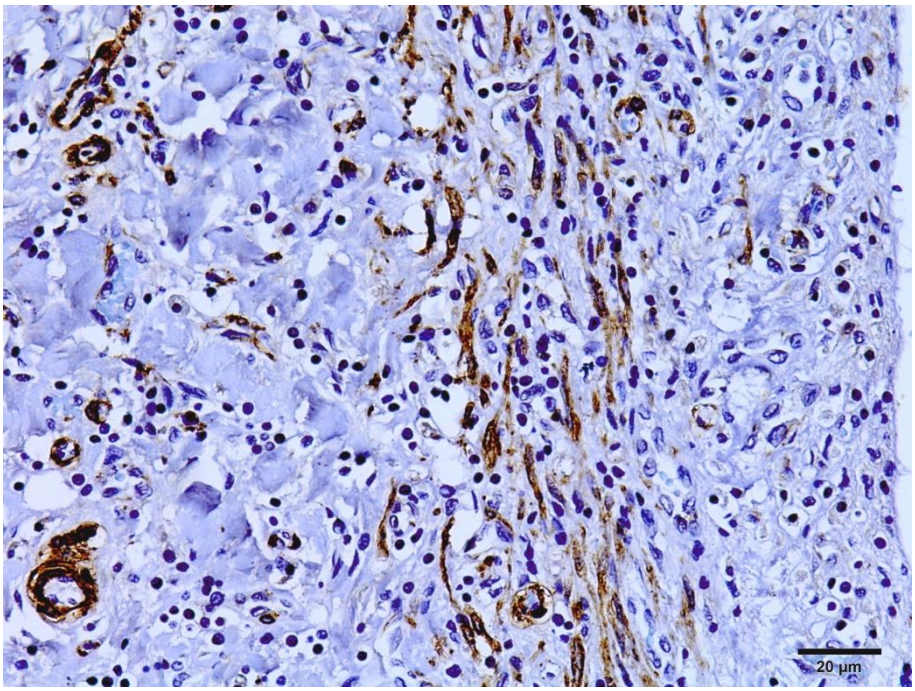


Fig 10. Immunohistochemical labelling for α-SMA. NTIC, cat. Positive spindle cells with radial orientation in outer medulla. Bar, 20mm.

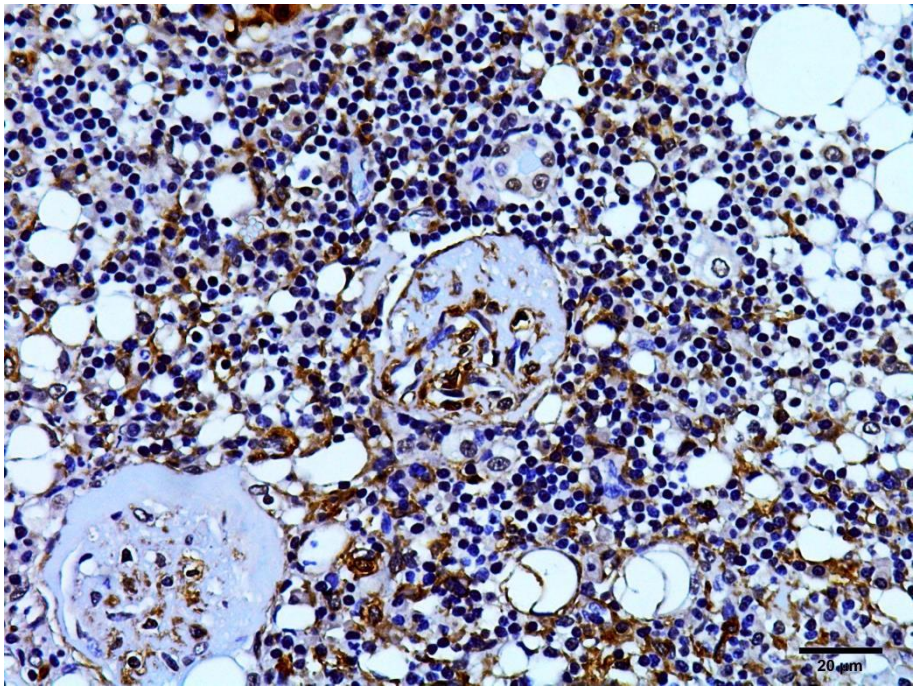


Fig 11. Immunohistochemical labelling for α-SMA. CTIN, cat. Glomerular α-SMA expression. Positive spindle cells inside glomeruli undergoing obsolescence. Bar, 20mm.

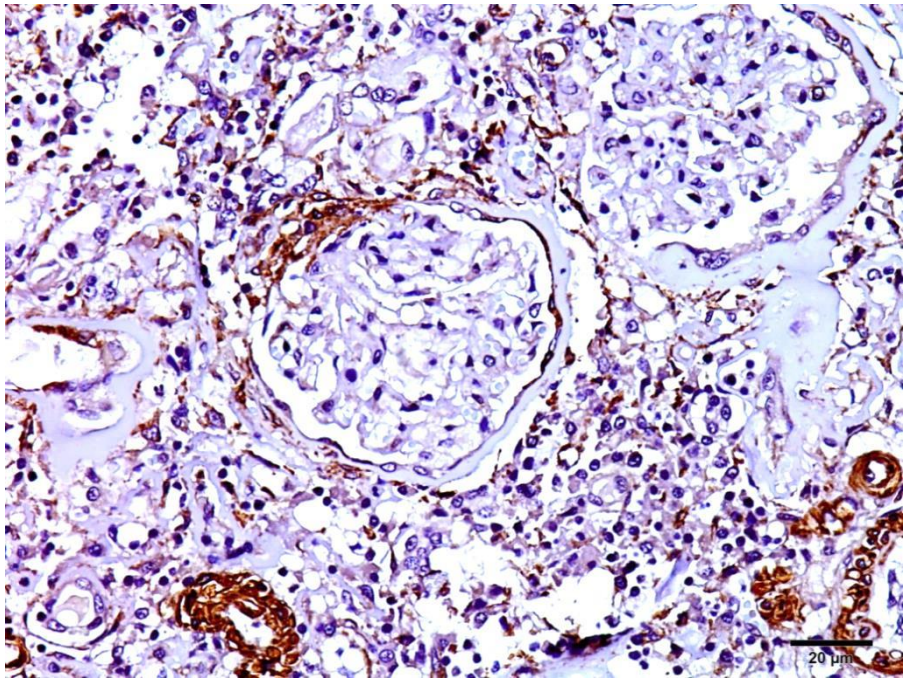


Fig. 12. Immunohistochemical labelling for α-SMA. CG, cat. Fusiform to slightly stellate positive cells in periglomerular lymphoplasmacytic inflammation. Bar, 20mm.

**Table 1**

**Age, sex and breed of 25 cats with CKD**

| *Morphological diagnosis* | *Number of Cases* | *Average ages (interval)* | *Sex* | *Breed* |
|---|---|---|---|---|
| **CTIN** | 20 | 11.4 (3 to 20 years) | 11 female<br>9 male | 15 MB*<br>5 Persian<br>1 Siamese |
| **CG** | 5 | 11 (8 to 14 years) | 3 female<br>2 male | 4 MB*<br>1 Siamese |

*mixed-breed cats

**Table 2**

**Interstitial fibrosis and inflammation scores, gross changes and thickness of cortical layer from 25 cats with CTIN.**

| *Fibrosis (*)* | | *Inflammation(*)* | | *Macroscopic shrinkage (*)* | *Rounding (*)* | *Cortical layer thickness average in mm (interval)* |
|---|---|---|---|---|---|---|
| **VI** | (5) | **VI** | (1) | | | 207.7 |
| | | **III** | (3) | (5) | (5) | (171.03-265.04) |
| | | **II** | (1) | | | |
| | | **I** | (0) | | | |
| **III** | (7) | **VI** | (0) | | | 290.6 |
| | | **III** | (5) | | (7) | (202.7-555.7) |
| | | **II** | (2) | (7) | | |
| | | **I** | (0) | | | |
| **II** | (10) | **VI** | | | | 361.3 |
| | | **III** | | (7) | (4) | (505.04-276.9) |
| | | **II** | (5) | | | |
| | | **I** | (5) | | | |
| **I** | (4) | **IV** | | | | 403.3 |
| | | **III** | | (2) | (1) | (330.3-541.6) |
| | | **II** | | | | |
| | | **I** | (4) | | | |

*number of occurences

**Table 3**

**Interstitial fibrosis and inflammation scores, gross changes and thickness of cortical layer from 5 cats with CG**

| *Fibrosis (*)* | | *Inflammation (*)* | | *Macroscopic shrinkage (*)* | *Rounding (*)* | *Cortical layer thickness average in mm (interval)* |
|---|---|---|---|---|---|---|
| **II** | (3) | **VI** | (0) | | | 303.9 |
| | | **III** | (0) | (3) | (0) | (127.4-401.5) |
| | | **II** | (3) | | | |
| | | **I** | (0) | | | |
| **I** | (2) | **IV** | (0) | | | 537.5 |
| | | **III** | (0) | (1) | (0) | (345.3-729.7) |
| | | **II** | (0) | | | |
| | | **I** | (2) | | | |

*number of occurences

**4 ARTIGO 2 –**

# Nonrenal lesions of uraemia in 78 domestic cats

**M. B. Ambrosio*, M. M. Hennig*, H. H. L. Nascimento, A. dos Santos, M. M. Flores*, R. A. Fighera*, L. F. Irigoyen*, , G. D. Kommers***

**Laboratório de Patologia Veterinária, Departamento de Patologia, Universidade Federal de Santa Maria, Av. Roraima 1000, Camobi, Santa Maria-RS, Brasil.*

Autor para correspondência: G. D. Kommers (e-mail: glaukommers@yahoo.com).

Artigo a ser submetido para a revista Jounal of Comparative Pathology,

## Summary

Uraemia is a clinical syndrome caused by the increase of uraemic toxins in blood stream as a consequence of intrinsic kidney or lower urinary tract diseases. Cats seem to be more affected by urinary diseases than dogs, especially considering chronic kidney disease (CKD) as one of the most important illness for the specie. Considering the lack of information regarding the systemic consequences of uraemia in cats, this study aims to investigate the prevalence, clinical and pathologic aspects of nonrenal lesions of uraemia in cats with urinary tract diseases, with special attention to the differences between cats and what is known for dogs. Cats necropsied between 2000 and 2019 were investigated for urinary tract diseases and nonrenal lesions of uraemia. The prevalence of cats with nonrenal uremic lesions was 5,8%, and there was a higher number of adults and elderly animals when compared to young cats, and more male cats were affected than female. Anorexia, apathy and vomiting were the most common clinical signs reported, and CKD was observed in the majority of cats with uraemia. Pulmonary oedema was the most frequent lesion observed, and there was a notable variation between the gastric lesions observed in this study and what has been reported in the literature. Haemorrhagic and ulcerative gastritis was frequently observed. Soft tissue mineralization and parathyroid hyperplasia were uncommon features of cats with uraemia and fibrous osteodystrophy was not observed. Cats with urinary tracts diseases of this study did not show the same variety of nonrenal uremic lesions usually present in dogs with uremic syndrome and multsisystemic presentation of uraemia was only observed in about 24% of the cases.


*Keywords: cat, chronic kidney disease, uraemia.*

## Introduction

Uraemia is a clinical syndrome associated with renal failure and lower urinary tract diseases (Cianciolo and Mohr, 2016), caused by the increase of metabolic compounds, which under normal conditions should be excreted by the healthy kidneys and involves clinical signs and systemic lesions (Vanholder *et al*., 2003; Serakides and Silva, 2016). It is a complex event that, besides the retention of toxins in the blood, involves derangements on hormonal balance and enzymatic processes (Cowgill, 2003).

Clinical manifestations and gross findings of uraemia are important evidences of renal failure. There are differences in the severity of uremic lesions between species, and they depend mostly upon the period of time that the animal was exposed to uremic toxins. Thus, in acute renal failure (ARF) these lesions can be less severe when compared to long term kidney diseases (Cianciolo and Mohr, 2016; Breshears and Confer, 2017).

According to previous studies developed in this same laboratory (LPV-UFSM) about uraemia in dogs, the syndrome prevalence ranged from 3,8% (Silveira *et al*., 2015) to 4,43% (Dantas and Kommers, 1997), considering the total number of dogs necropsied during a determined period of time. In another research aiming to determine the prevalence of renal failure in dogs in a veterinary routine, it was found to be around 11% from a universe of 935 dogs. From these patients, 76.6% died of renal failure (Sosnar *et al*., 2003).

The literature often uses the dog as the main model to describe nonrenal uremic lesions in domestic animals (Cianciolo and Mohr, 2016; Breshears and Confer, 2017), but the urinary tract diseases also play an important role in morbidity and mortality of cats. The prevalence of renal failure in cats according to Sosnar *et al*. (2003) has exceeded the prevalence in dogs. Togni *et al*. (2018) reported that urinary tract diseases are among the main causes of death and euthanasia in cats. Furthermore, CKD is the most common metabolic

disease in cats, and the prevalence is considered higher than in dogs (Polzin 2011; Brown *et al*., 2016). There are few researches specifically focused on the systemic consequences of urinary tract diseases in the feline specie, and the more accurate knowledge of the type and frequency of development of uremic lesions may lead to advances in the management of uremic patients.

Therefore, the aim of this study was to characterize the prevalence, clinical and pathological aspects of uraemia in cats, considering the anatomical distribution of lesions, attempting to the main differences between the development of the syndrome in dogs and cats.

## Material and Methods

Cases of cats with nonrenal lesions of uraemia submitted to necropsy were investigated in the archives of the LPV-UFSM between January 2000 and October 2019. Cases were included in the study when the necropsy reports showed urinary tract diseases as the cause of death concomitant with lesions considered nonrenal lesions of uraemia according the specific literature (Breshears and Confer, 2017). The total number of cats necropsied in the laboratory in this period was also recorded.

Information about age, sex and breed, clinical signs, laboratory findings (blood urea nitrogen, serum creatinine concentration and haematocrit value, according to [Fielder, 2020]), gross and histologic findings were taken from necropsy reports. Considering the age category, cats were divided in three groups, according to Togni *et al*. (2018): young (less than one year old), adults (from one to ten years old) and elderly (more than ten years).

The type of primary injury that led to azotaemia and, consequently, to the uremic syndrome was analysed and divided into prerenal, renal and postrenal causes. Nonrenal uremic lesions were described according to gross and histologic aspects and the anatomical

distribution for each case, according to specific literature (Breshears and Confer, 2017). Paraffin blocks of selected cases were sectioned in 3.0 µm thick and submitted to haematoxylin and eosin (HE) technique in order to illustrate this study.

## Results

During the studied period, 1.330 cats were necropsied in the LPV-UFSM and 78 cats (5.8%) presented nonrenal uremic lesions. Table 1 shows information referent to age, sex and breed of the studied cats. In 72 cases (72/78, 92%) cats had clinical signs related to uraemia (Table 2). Additional clinical signs reported were dehydration, hypothermia, weight loss, polyuria, dysuria, haematuria, anuria, polydipsia, salivation, abdominal pain, diarrhoea and dyspnoea.

In 27 cases, there was available data about the increase of urea and creatinine values, which characterized azotaemia, and reduction of haematocrit value was only mentioned in nine cases. Creatinine values, when reported, ranged from 3.2 mg/dl to 26.0 mg/dl (reference ranges 0.9-2.2 mg/dL) and urea values ranged from 300.0 mg/dl to 767.0 mg/dl (reference ranges 19-34 mg/dL). The minimum haematocrit value observed was 9% and the maximum, 21% (reference ranges 30-45%)

Considering the origin of azotaemia, and consequently the origin of nonrenal uraemic lesions (Table 3), 58 cases (58/78; 74.3%) were attributed to renal causes. In two cases, azotaemia was caused by concomitant renal and post renal lesions (acute tubular injury with necrohemorrhagic cystitis and chronic interstitial nephritis with traumatic ureteral rupture). In 18 (18/78; 23%) it was a consequence of a postrenal injury. The frequency and distribution of uremic lesions are demonstrated in Table 4 and 5.

The most prevalent uremic lesion was pulmonary oedema which affected 40 cats (40/78; 51.2%). Usually, the lungs were wet, heavy and slightly reddened. The cut surface of

the parenchyma drained variable amounts of fluid and the trachea contained a foamy fluid. Histology showed light eosinophilic amorphous material inside alveolar lumens (Fig. 1). In five cases, beyond pulmonary oedema, lesion of uremic pneumopathy was also observed. They were characterized by a firm lung parenchyma with multiple slightly white and firm areas in the pleural surface. On the histology, there were granular basophilic deposits in alveolar septa, interpreted as multifocal mineralization, with variable amounts of inflammatory infiltrate of neutrophils and foamy alveolar macrophages with deposition of strongly eosinophilic fibrillar material (fibrin).

The second most common uremic lesion observed was ulcerative or erosive and haemorrhagic gastritis (28/78; 35.8%). Gastric mucosa showed multiple ulcers and/or erosions with haemorrhagic content in most of the cases (Fig. 2). In 22/28 cases the stomach had a strong ammoniac odour when opened, in three cases there was mineralization of gastric mucosa and in two cases there was also oedema of the submucosal layer. In two cases gastritis was associated with thrombosis and fibrinoid necrosis of the vascular wall.

Ulcerative glossitis and stomatitis occurred in 22 cases (22/78; 28.2%) and was characterized by bilateral focal ulceration in the tongue ventral surface (Fig. 3) or multiple small ulcers and erosions in different sites of oral cavity mucosa and lips. Histologically, there was epithelial necrosis and ulceration with marked neutrophilic and histiocytic inflammatory infiltrate. There was necrosis and haemorrhage of the tongue muscle. In some cases, there was secondary bacterial infection.

Soft tissue mineralization was present in 11 cases (11/78; 14.1%). The organs involved in this condition were, in descending order of frequency, aorta (6), lungs (5), stomach (3), intercostal muscles (3), heart (endocardium; 3), spleen (1), tongue (1) and adrenal glands (1). Grossly, it was observed white and firm focal areas with irregular cut surface with crumbly texture with variable intensity and distribution between different organs.

In the histology, mineralization was identified as depositions of coarse and strongly basophilic material on the tissue surface.

Anaemia was observed in nine cats (9/78; 11.5%) in which haematocrit values were below the reference value for the specie. These cats also presented marked pale visible mucous membranes, watery blood, pale aspect of blood marrow on gross examination, rarefaction of erythroid lineage cells on bone marrow microscopic examination.

Haemorrhagic enteritis was observed in four cases (4/78; 5.1%) in which the serosa presented numerous petechiae and the mucosa surface was markedly red, filled with haemorrhagic content. In one of these cases there were multiple ulcers in enteric mucosa. In one case it was observed a focally extensive necrotic area in the mucosal layer. On histology, the epithelium was multifocally ulcerated; the remaining epithelium was necrotic and there was an adjacent neutrophilic inflammatory infiltrate with fibrin deposition.

In only three cases (3/78; 3.8%) bilateral parathyroid hyperplasia was observed. There was a notable increased in size of these endocrine glands. In one case (1/78; 1.8%) an ulcerative oesophagitis was present. There was evident necrosis of the oesophageal mucosa and multiple focus of erosion of the lining epithelium with fibrin deposition.

Cavities effusions were a common gross finding in this study. They were observed in 23 (23/78; 29.4%) cases affecting pleural cavity, in 13 cases (13/78; 16.6%) in the abdominal (peritoneal) cavity and in five cases (5/78; 6.4 %) there was fluid accumulation in the pericardial sac. Three cats with chronic glomerulonephritis had a combination of pleural effusion, ascites, pulmonary oedema and subcutaneous oedema, findings that were considered suggestive of nephrotic syndrome in the necropsy reports.

## Discussion

This study made possible to picture the profile of 78 cats with urinary tract diseases that had developed systemic lesions considered nonrenal uraemic lesions. There were more adults and elderly cats with uraemia in comparison to young cats, and about two thirds of the cats were male. This data was considered noteworthy, although it does not allow deducing age or sexing predispositions, since the total population of necropsied cats was not analysed regarding these parameters. The main disease that leads to uraemia is chronic kidney disease (CKD), mainly because of the prolonged exposure to uremic toxins (Ross, 2011; Bartges, 2012). Moreover, many studies point out a higher prevalence of CKD considering a geriatric population of cats (DiBartola *et al*., 1987; Lawler *et al*., 2006; Chakrabarti *et al*., 2013; Sparkes *et al*., 2016; Togni *et al*., 2018). Furthermore, among urinary tract diseases, feline lower urinary tract disease (FLUTD) is the name attributed to a group of conditions related to disturb of urine elimination in cats (Little, 2012) and castrated males have increased risk for all the specific causes of FLUTD (Lekcharoensuk *et al*, 2001).

As observed in dogs (Dantas and Kommers, 1997; Silveira *et al*., 2015), the majority of cats developed uraemic syndrome as a consequence of primary kidney diseases. Intrinsic renal failure in cats can be originated from many types of diseases, such as polycystic kidney disease (PKD), obstructive urolithiasis and pyelonephritis (Syme *et al*., 2006), as observed in the present study. However, the most frequent histopathological diagnosis attributed to cats with renal failure was chronic tubulointerstitial nephritis in this study, which is the most common recognized form of CKD in cats (Lucke, 1968; Dibartola *et al*., 1987; Chakrabarti *et al*., 2013; Brown *et al*., 2016).

The most frequent clinical sign observed in this study was anorexia. Anorexia is an unspecific sign present in both ARF and CRF (Elliot, 2011, Polzin, 2011; DiBartola and

Westropp, 2015; Quimby, 2016). It can be attributed to changes in taste and olfaction, to the nausea and vomiting caused by increased uremic toxins in the blood stream (Elliot, 2011), to ulcerative lesions in the oral cavity, and to gastritis (DiBartola and Westropp, 2015). Vomiting was observed in 30% of the cases of uremic cats and this value represented a half of the results obtained in studies with dogs (Dantas and Kommers, 1997; Silveira *et al*. 2015). Indeed, vomiting is considered a more common clinical manifestation in uremic dogs than in cats (DiBartola and Westropp, 2015). In uremic dogs, vomiting is a consequence of stimulation of chemoreceptors in a trigger zone in the stomach by uremic toxins (Borison and Hebertson, 1959) and it is possible that the same mechanism would be involved in vomiting in uremic cats (Quimby, 2016; Sparkes *et al*., 2016).

Pulmonary oedema was the most prevalent uraemic lesion observed in this study. The percentage of affected cats (51,2%) was similar to what was previously observed in dogs (Silveira *et al*., 2015). In these uremic dogs pulmonary oedema was also among the most common uremic lesions, being only overcome by ulcerative gastritis and mineralization of soft tissues. Uraemic pulmonary oedema is a consequence of the increased vascular permeability caused by the caustic effect of uremic toxins (Bass, 1952; Rackow et al., 1978; Cianciolo and Mohr, 2016; Breshears and Confer, 2017). It is important to emphasize that pulmonary oedema was the only uraemic lesion observed in 11 cats without available blood urea and creatinine values available in the necropsy reports. In these cats, it was not observed any other pathological process rather than primary urinary disease that could explain the pulmonary oedema or justify the cat's natural death (ten cases) or euthanasia (one case). However, it is not possible to determine certainly if the oedema was caused by uraemia in these cases.

The combination of pulmonary oedema with transudation of fibrin, mineralization and inflammation, characterizing uremic pneumopathy (Breshears and Confer, 2017) was rarely

observed in the cats of this study and it is commonly observed in uremic dogs (Cianciolo and Mohr, 2016; Dantas and Kommers, 1997; Boedec *et al*, 2012, Silveira *et al*., 2015).

Uremic gastropathy is a well described condition in uremic dogs, characterized by gastric oedema and congestion, haemorrhagic gastric content, mineralization and vascular lesions (Uzal *et al*, 2016). Among the available studies, gastric histopathologic findings in uremic dogs are variable. According to Peters *et al*. (2005), the histopathologic changes observed in the stomach of 22 out of 28 dogs (78.5%) with renal failure was oedema, mineralization and vasculopathy, whereas ulceration and necrosis of gastric wall were uncommon features. Recently, Cardoso *et al*. (2019) found uremic gastropathy in 15 of the 16 necropsied uremic dogs (93.15%). Erosive and/or ulcerative gastritis, oedema, hyperaemia, ammoniac odour and necrosis of gastric mucosa were commonly observed, while mineralization was only observed in four cases. According to Dantas and Kommers (1997) and Silveira *el al*. (2015), uremic gastropathy was the most prevalent lesion in uremic dogs, comprising 79,1 % and 56.5% of the cases, respectively. Haemorrhagic and ulcerative gastritis with oedema, vascular lesions and mineralization were frequently observed in these studies.

The most common gastric lesions observed in the cats of this study were ulcerative/ erosive and haemorrhagic gastritis. These changes were different from the results obtained in a study of uremic gastropathy in 37 cats with CKD. Gastric ulceration, oedema and vascular fibrinoid changes were not observed in gastric histopathologic analysis. Otherwise, the most important gastric lesions observed were fibrosis and mineralization (McLeland *et al*., 2014). This result can suggest that gastric lesion in uremic cats may show individual variations.

Ulcerative stomatitis and glossitis are common features of canine uraemia, characterized by dark and fetid ulcers in the tongue, lips and cheeks (Uzal *et al*, 2016). It was a frequent gross finding in the present study and also reported by Lucke (1968) in uremic cats.

The development of ulcerative lesions in oral cavity probably is a combination of ammonia caustic effect on oral mucous membranes and vascular ischemic injury. Ammonia is originated from the degradation of urea in salivary content by local urease-producing bacteria (DiBartola and Westropp, 2015; Uzal *et* al, 2016).

Anaemia ranges between 32% and 65% of cats with CKD (Dibartola *et al*., 1987; Elliot and Barber, 1998). Considering only cats with CKD (48 cases) in this study, the percentage of anaemia was around 18%. Impairment of renal endocrine function, results in reduced haematopoiesis by reducing erythropoietin production (Chalhoub *et al.*,2011) and it is considered the main source of anaemia in CKD, but a multifactorial character has been attributed to the pathogenesis of this type of anaemia (Chalhoub *et al*, 2011; Cianciolo and Mohr, 2016). Factors like inflammation (Stenvinkel, 2001), uremic toxins causing reduction in red blood cells survival and blood loss in gastrointestinal tract are believed to be involved (Chalhoub *et al.*, 2011).

There was a notable difference between the findings related to calcium metabolism between the cats of this study and what is reported in uremic dogs. Multiple soft tissue mineralization were frequently observed in uremic dogs, ranging from 55.9% (Silveira *et al*., 2015) to 93.7% (Cardoso *et al*., 2019) of the cases. Parathyroid hyperplasia was observed in 9,3% of uremic dogs (Silveira *et al*., 2015) and fibrous osteodystrophy ranged from 6.2% (Cardoso *et al*., 2019) to 8% of the cases (Silveira *et al*., 2015). In the present study, soft tissue mineralization rate was about 13%.

Similarly, parathyroid hyperplasia was an uncommon feature of this study and fibrous osteodystrophy was not reported in clinical history or observed during necropsies. In a previous study that investigated some aspects of systemic consequences of CKD in cats, parathyroid hyperplasia was observed only in six cats with long term kidney injury. In these cases, there was no evidence of pathological fractures or softening of the bones but a detailed

microscopic analysis of the bones revealed mild osteoclastic bone resorption, with deposition of fibrous tissue in the resorption site. These mild bone lesions in cats did not show a predilection for the skull bones (mandible and maxillae), which are the most severally affected bones in the canine renal secondary hyperparathyroidism (Lucke, 1968).

Additional lesions, frequently observed in uraemic syndrome in dogs, such as mural endocarditis, fibrinous pericarditis, ulcerative laryngitis and multiple soft tissue mineralization affecting heart, aorta, larynx, diaphragm and intestines (Dantas and Kommers, 1997; Silveira *et al*. 2015) were not observed in the uraemic cats analysed herein. In addition, the present study showed multisystemic presentation of uraemia in only 24% of the cases, suggesting that the consequences of urinary tract disease are less severe in cats in comparison to dogs.

The classic concept about the progression of CKD is based on a slow and progressive decline in kidney function, with continuous functional loss during the animal's life, until the development of renal failure and uraemia. However, many cats with CKD show stable renal function for a long period of time, sometimes dying from other diseases rather than renal processes (Polzin, 2011). There is a hypothesis that the feline CKD caused by tubulointerstitial lesion might not be merely a degenerative disease that causes mortality. One study trying to determine the relationship between kidney injury, cause of death, and age in a population of 676 adult cats detected longer mean life span in cats with kidney disease in comparison to animals with different causes of death. The authors therefore suggested that CKD in cats could represent an adaptive survival process of the feline specie (Lawler et al., 2006). Additionally, it is important to emphasize that the most common feature usually observed in the CKD of cats in the form of a tubulointerstitial lesion is a multifocal to segmental distribution, with remaining areas of preserved parenchyma (Brown et al., 2016) what could provide maintenance of the renal function. Such facts may help to understand, at

least considering the cases of CKD in this study, the smallest variety of nonrenal lesions of uraemia and the lower occurrence of multiple organs injury in uraemic cats.

## Acknowledgements

This research was financially supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil. The student M. B. Ambrosio has a Master's scholarship from CAPES.

## Conflict of Interest Statement

The authors declare no conflicts of interest with respect to the research, authorship or publication of this article.

## References


Bass HE, Greenberg MD, Singer E, Miller MA (1952) Pulmonary changes in uremia. *Journal of the American Medical Association*, **148**, 724-726.

Boedec LK, Heng HG, Snyder PW, Pressler BM (2012) Pulmonary Abnormalities in Dogs with Renal Azotemia. *Journal of Veterinary Internal Medicine***, 26**, 1099-106.

Borison HL, Hebertson LM. Role of medullary emetic chemoreceptor trigger zone (CT zone) in postnephrectomy vomiting in dogs. *American Journal of Physiology***, 197**, 850-852.

Breshears, MA, Confer AW (2017) The urinary system**.** In: *Pathologic Basis of Veterinary Disease.* 6th. Edit, JF Zachary, Elsevier, St. Louis, pp. 617-681.

Brown CA, Elliot J, Schmiedt CW, Brown SA (2016) Chronic kidney disease in aged cats: clinical features, morphology, and proposed pathogeneses. *Veterinary Pathology*, **53**,309-326.

Cardoso PGS, Pinto MPR, Moroz LR, Fontes TN, Santos RS et al. (2019) Dystrophic mineralization in uremic dogs: an update. *Pesquisa Veterinária Brasileira***, 39**, 889–899.

Chakrabarti S, Syme HM, Brown CA, Elliot J (2013) Histomorphometry of feline chronic kidney disease and correlation with markers of renal dysfunction. *Veterinary Pathology*, 50, 147–155.

Chalhoub S, Langston C, Eatroff A (2011) Anemia of renal disease: What it is, what to do and what's new. *Journal of Feline Medicine and Surgery*, **13**, 629–640.

Cianciolo RE, Mohr FC (2016) Urinary sistem. In: *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*, 6th Edit, MG Maxie, Elsevier Ltd, St Louis, pp. 377-463.

Cowgill LD (2003) Advanced therapeutic approaches for the management of uraemia – the met and unmet needs. *Journal of Feline Medicine and Surgery*, **5**, 57–67.

Dantas AF, Kommers GD (1997) Lesões extrarenais de uremia em 72 cães. *Ciência Rural*, **27**, 301-306.

DiBartola SP, Rutgers HC, Zack PM (1987) Clinicopathologic findings associated with chronic renal disease in cats: 74 cases (1973 -1984). *Journal of the American Veterinary Medical Association*, **190**, 1196–202.

Dibartola SP, Westropp JL (2015) Doenças do Trato Urinário. In: *Medicina Interna de Pequenos Animais.*5th Edit. RW Nelson, CG Couto, Elsevier, Rio de Janeiro, pp. 698-703.

Elliott DA (2011) Nutritional considerations for the dialytic patient. *Veterinary Clinics of North America: Small Animal Practice*, **41**, 239–250.

Elliot J, Barber PJ (1998) Feline chronic renal failure: clinical findings in 80 cases diagnosed between 1992 and 1995. *Journal of Small Animal Practice,* **39**, 78-85.

Lawler DF, Evans RH, Chase K, Ellersiek M, Qinghong L et al. (2006). The aging feline kidney: a model mortality antagonist? *Journal of Feline Medicine and Surgery*, **8**, 363-371.

Lekcharoensuk C, Osborne CA, Lulich JP. Epidemiologic study of rick factor for lower urinary tract diseases in cats. *JAVMA*, **218**, 219-239.

Little SE (2015) Distúrbios do trato urinário. In: *O gato: medicina interna.* 1st. Edit. SE Little, Roca, Rio de Janeiro, pp. 1406-1913.

Lucke VM (1968) Renal disease in the domestic cat. The *Journal of Pathology and Bacteriology*, **95** ,67–91.

Maxie MG, Newman SJ (2007) The urinary system. In: *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals.* 5th. Edit. MG Maxie, Elsevier, Saint Louis, pp.425-522.

McLeland SM, Lunn KF, Duncan CG, Refsal, KF, Quimby JM (2014) Relationship among Serum Creatinine, Serum Gastrin, Calciumphosphorus Product, and Uremic Gastropathy in Cats with Chronic Kidney Disease. *Journal of Veterinary Internal Medicine,* **28**, 827-837.

Peters RM, Goldstein RE, Erb HN, Njaa BL (2005) Histopathologic Features of Canine Uremic Gastropathy: A Retrospective Study. *Journal of Veterinary Internal Medicine***, 19**, 315-320.

Polzin, DJ (2011) Chronic kidney diseases in small animals. *Veterinary Clinics: Small Animal practice*, **41**, 15–30.

Polzin DJ (2011) Chronic kidney disease. In: *Nephrology and urology of small animals.* J Bartges, DJ Polzin, 1st Edit, Blackwell Publishing Ltd, Iowa, pp. 433-469.

Quimby JM (2016) Update on medical management of clinical manifestations of chronic Kidney disease. *Veterinary Clinics: Small Animal Practice*, **46**, 1163–1181.

Rackow EC, Fein A. Sprung C. Grodmsn RS (1978) Uremic pulmonary edema. *The American Journal of Medicine*, **64,** 1978.

Ross SJ (2011) Azotemia and uremia. In: *Nephrology and urology of small animals.* 8. Edit. J BARTGES, DJ POLZIN, Elsevier, Saint Louis, pp. 393-398.

Serakides R, Silva JF (2016) Sistema urinário. In: *Patologia Veterinária,* 2. Edit, R Santos, AC Alessi, Roca, Rio de Janeiro, pp. 518-527.

Silveira IP, Inkelmann MA, Tochetto C, Rosa FB, Fighera RA (2015) Epidemiologia e distribuição de lesões extrarenais e uremia em 161 cães. *Pesquisa Veterinária Brasileira*, **35**, 562-568.

Sosnar M, Kohout P, Ruzicka M, Vrbasová L (2003) Retrospective study of renal failure in dogs and cats admitted to university of veterinary and pharmaceutical sciences brno during 1999-2001. *Acta Veterinaria Brno*, **72**, 593–598.

Sparkes AH, Caney S, Chalhoub S, Elliot J, Finch N (2016) ISFM Consensus Guidelines on the Diagnosis and Management of Feline Chronic Kidney Disease. *Journal of Feline Medicine and Surgery*, **18**, 219–239.

Stenvinkel P. The role of inflammation in the anemia of end-stage kidney disease. *Nephrology Dialysis Transplantation*, **16**, 36-40.

Syme H, Markwell P, Pfeiffer D, Elliott J (2006) Survival of cats with naturally occurring chronic renal failure is related to severity of proteinuria. *Journal of Small Animal Practice*, **20**, 528-535.

Togni M, Curtis A, Vargas DP, Kommers GD, Irigoyen LF (2018) Causas de morte e razões para eutanásia em gatos na Região Central do Rio Grande do Sul (1964-2013). *Pesquisa Veterinária Brasileira***, 38**, 741–750.

Uzal FA, Plattner, BL, Hostetter JM (2016) Alimentary system. In:. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals.* 6th Edit, MG Maxie, Elsevier Ltd, St Louis, pp. 51-53.

Vanholder R, DeSmet R, Glorieux G, Argilés A, Baurmeister U. et al. (2003) Review on uremic toxins: classification, concentration, and interindividual variability. *Kidney International*, **63**, 1934-1943.

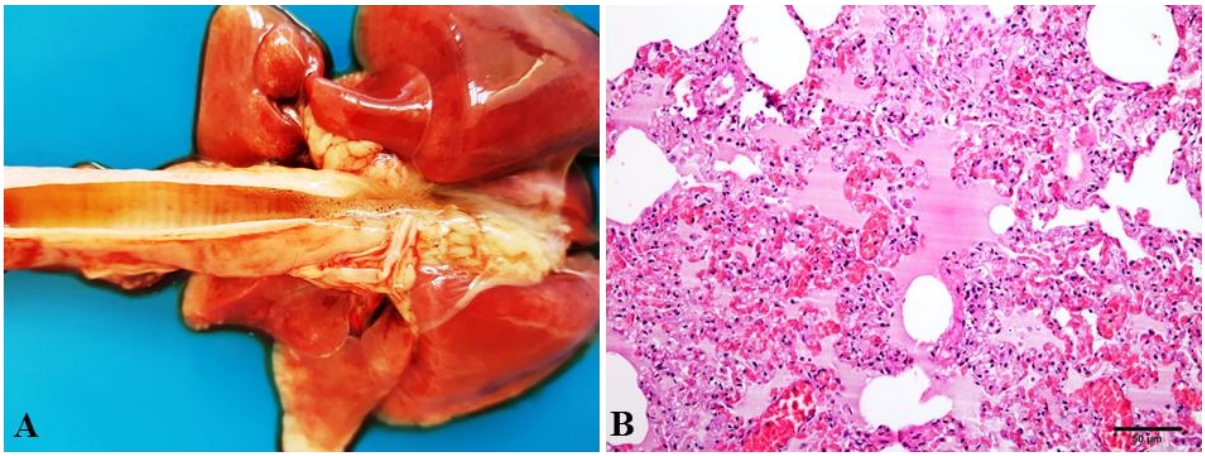


Fig. 1. Pulmonary oedema. (A) Presence of a considerable amount of foamy fluid in the trachea and uncollapsed lungs with wet appearance. (B) Alveoli filled with protein-rich amorphous eosinophilic fluid.

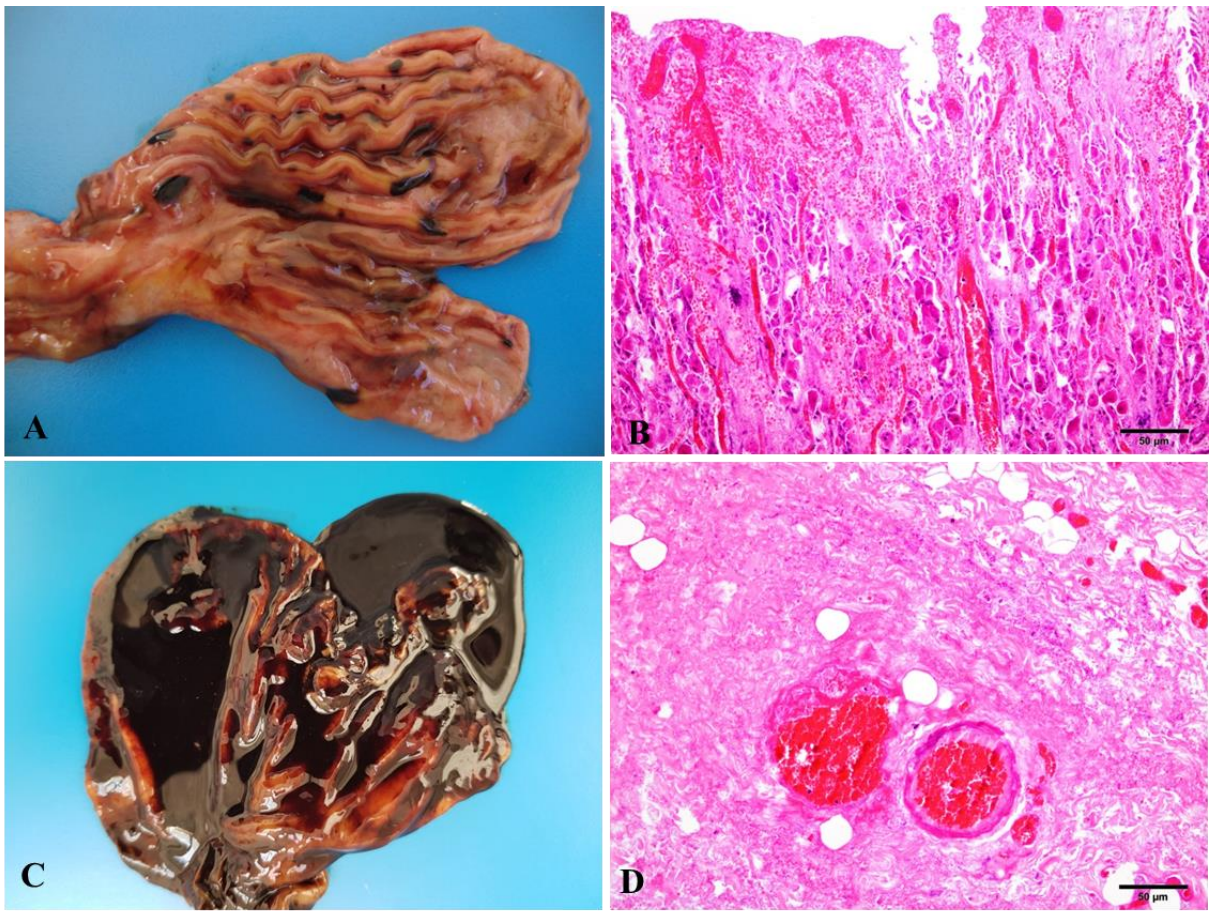


Fig. 2. Uraemic gastritis. (A) Multiple and variable size visible ulcers (from 0.5 to 2.0 cm diameter) in the gastric mucosal. (B) There is epithelium loss and parietal and principal mucosal cells necrosis. Throughout the mucosa, there are multiple areas of haemorrhage (C) Haemorrhagic gastric content. (D) Deposition of a bright eosinofilic material in the walls of small arteries of gastric submucosa layer (uremic vascular fibrinoid necrosis).

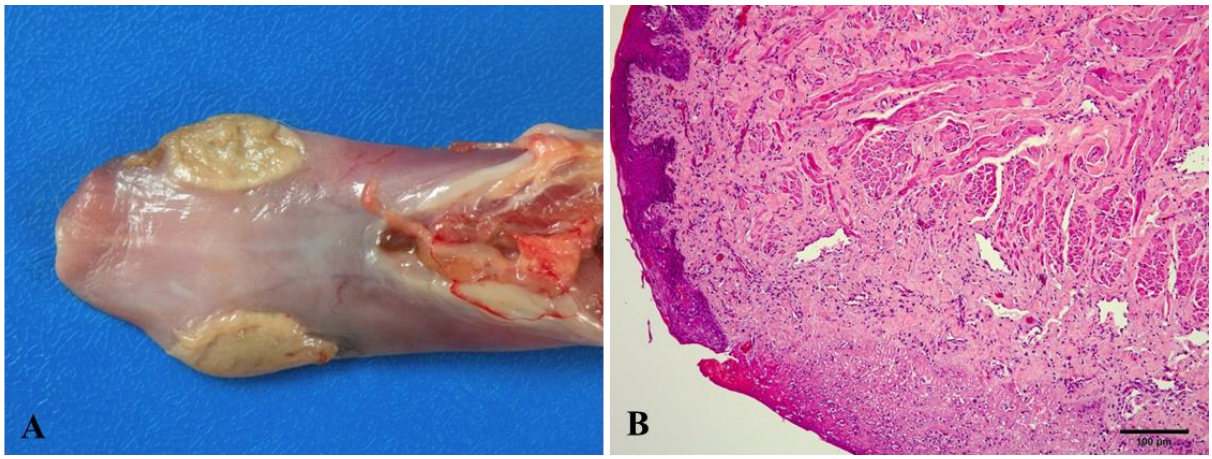


Fig. 3. Uraemic ulcerative glossitis. (A) Extensive bilateral symmetric ulcers on the ventral surface of the tongue. (B) Histologically, there is focally extensive ulceration of lining stratified squamous keratinized epithelium of the tongue, with erythrocyte and fibrin accumulation in the adjacent connective tissue.

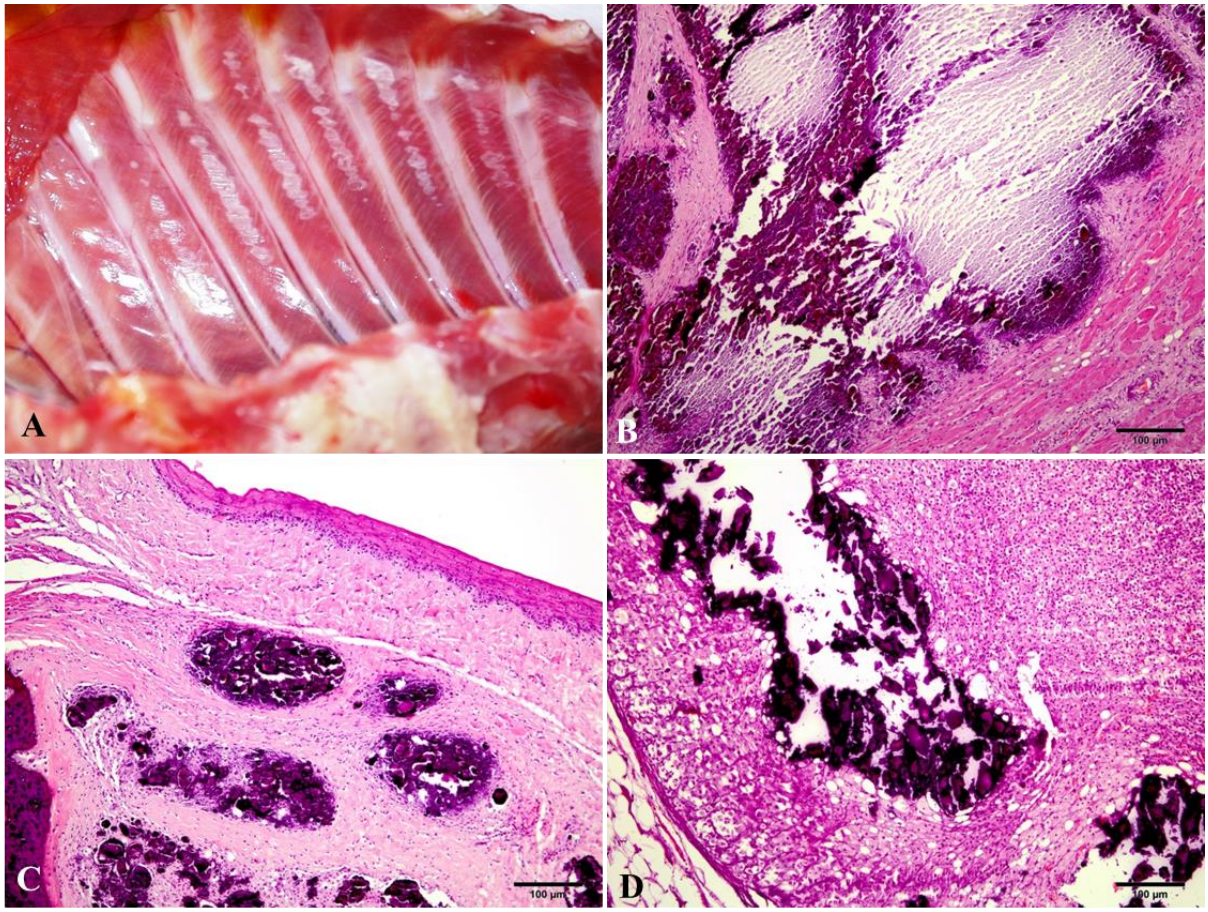


Fig. 4. Soft tissue mineralization. (A) Thoracic cavity (parietal pleura), streaks of white material in the subpleural surface, characterizing intercostal mineralization. (B) Deposition of basophilic mineral material between intercostal muscle fibers. (C) Multifocal mineralization of tongue skeletal muscle. (D) Focally extensive mineralization of the cortical zona fasciculata of the adrenal gland.

**Table 1**

**Epidemiological information about of the 78 cats with nonrenal uremic lesions**

| *Breed* | | *Sex* | | *Age category* | |
|---|---|---|---|---|---|
| Mixed-breed | 51 | Male | 56 | Adult | 54 |
| Siamese | 13 | Female | 22 | Elderly | 17 |
| Persian | 10 | | | Young | 7 |
| Burmese Sacred | 2 | | | | |
| Exotic Shorthair | 1 | | | | |
| Himalayan | 1 | | | | |

**Table 2**

**Clinical manifestations of uraemia in 78 cats.**

| *Clinical signs* | *Number of cases* | *Percentage (%)* |
|---|---|---|
| Anorexia | 39 | 48.75% |
| Apathy | 32 | 40% |
| Vomiting | 24 | 30% |
| Neurological signs | 18 | 22.5% |
| Oral ulceration | 8 | 10% |

**Table 3**

**Prevalence of primary urinary tract diseases affecting cats with uraemia**

| *Origin of azotaemia* | | *Total number of cases* |
|---|---|---|
| **Lesion** | **Occurrence*** | |
| **Renal** | | 58 |
| Chronic tubulointerstitial nephritis | 32 | |
| Chronic glomerulonephritis | 16 | |
| Acute tubular necrosis | 14 | |
| Bacterial pyelonephritis | 4 | |
| Polycystic kidney disease (PKD) | 3 | |
| Hydronephrosis | 3 | |
| Nephrolithiasis | 2 | |
| Granulomatous interstitial nephritis[a] | 1 | |
| Renal papillary necrosis | 1 | |
| Ischemic acute infarctions | 1 | |
| **Post renal** | | 18 |
| Necrotic/haemorrhagic/fibrinous cystitis | 16 | |
| Urethral obstruction | 8 | |
| Traumatic urinary bladder rupture | 1 | |
| Traumatic ureteral rupture | 1 | |

a: associated to feline infectious peritonitis

* total number of occurrences considering multiple lesions in the same animal

**Table 4**

**Type and frequency of nonrenal uremic lesions in 78 cats.**

| *Uremic lesion* | *Number of cases* | *Percentage (%)* |
|---|---|---|
| **Pulmonary oedema** | **40** | 51.2% |
| **Gastritis** | **28** | 35.8% |
| Ulcerative | 16 | |
| Ulcerative and haemorrhagic | 7 | |
| Erosive | 2 | |
| With oedema of gastric wall | 2 | |
| Thrombosis and vascular fibrinoid necrosis | 2 | |
| With ammonia odour | 22 | |
| **Oral cavity lesions** | **22** | 28.2% |
| Ulcerative glossitis | 14 | |
| Ulcerative stomatitis | 8 | |
| **Anaemia** | **12** | 11.5% |
| **Soft tissue mineralization** | **11** | 14.1% |
| Aorta | 6 | |
| Lungs | 5 | |
| Stomach | 3 | |
| Intercostal muscles | 3 | |
| Heart | 3 | |
| Spleen | 1 | |
| Tongue | 1 | |
| Adrenal gland | 1 | |
| **Enteritis** | **4** | 5.1% |
| Haemorrhagic | 4 | |
| Ulcerative | 1 | |
| **Parathyroid hyperplasia** | **3** | 3.8% |
| **Necrotic and erosive esophagitis** | **1** | 1.3% |

**Table 5**

**Distribution of nonrenal lesions in 78 cats**

| *Number of affected organs/anatomical sites* | *Number of cases* | *Percentage (%)* |
|---|---|---|
| One | 37 | 47.4% |
| Two | 22 | 28.2% |
| Three or more | 19 | 24.4% |

## 5 DISCUSSÃO

Esta dissertação de mestrado foi dividida em duas partes, o que resultou em dois artigos científicos. A fonte de inspiração para os temas centrais de ambos os artigos foi o próprio cotidiano da rotina diagnóstica do LPV-UFSM. A observação de características únicas da DRC na espécie felina, tanto macroscópicas (redução do tamanho e marcado arredondamento dos rins), quanto histológicas, possibilitaram a realização de um estudo de caracterização macroscópica, histológica e imuno-histoquímica de 25 gatos com DRC.

Apesar da relação entre a perda funcional e estrutural dos rins não ser necessariamente constante, na DRC há perda funcional dos néfrons (POLZIN, 2011b), resultando, geralmente, em redução do tamanho dos rins (CIANCIOLO; MOHR, 2016). A redução do tamanho dos rins foi observada em 22 dos 25 casos do estudo, variando de bilateral, bilateral assimétrica e unilateral. Os rins reduzidos de tamanho apresentaram maiores graus histológicos de fibrose intersticial ($p=0.021$) e a redução do tamanho renal foi correlacionada à severidade da inflamação crônica ($p=0.0039$) e da fibrose intersticial ($p<0,001$).

Os resultados da observação criteriosa da macroscopia dos rins dos 25 gatos deste estudo demonstraram que a diminuição do tamanho dos rins com arredondamento, foi uma apresentação exclusiva da NTIC. A causa para o arredondamento renal foi a combinação de redução da espessura da camada cortical, inflamação crônica, fibrose intersticial, atrofia tubular e esclerose glomerular. Todos esses caracteres histológicos apresentaram-se mais acentuados lateralmente ao hilo renal, se perpetuando em direção aos polos renais, levando ao achatamento, e posterior arredondamento dos rins.

Além da característica macroscópica única, a NTIC caracterizou-se por inflamação linfocítica com fibrose intersticial organizada em faixas, com orientação radial, com distribuição multifocal segmentar, com redução da espessura da cortical e obsolescência glomerular, muito semelhante ao descrito em estudos anteriores (CHAKRABARTI et al., 2013; DIBARTOLA et al., 1987; MCLELAND et al., 2019). Os componentes histológicos descritos sugerem que a etiologia da NTIC, principal forma de DRC observada na espécie felina, ainda não completamente compreendida, envolva eventos isquêmicos do tecido renal (BROWN et al., 2016)

A presença de miofibroblastos, células imunomarcadas positivamente para α-SMA, observada tanto na NTIC, quanto na GNC dos gatos deste estudo, sugere que essas células estejam envolvidas nos processo de fibrose intersticial e na obsolescência glomerular nas

DRCs, mesmo nos casos de lesão menos acentuada. A perda tecidual, observada principalmente nas NTIC, fortemente correlacionada à severidade da fibrose intersticial, pode ter envolvimento dos miofibroblastos tanto como produtores de MEC, quanto pela função contrátil destas células (STRUTZ; ZEISBERG, 2006, SUN et al., 2016).

Dada a importância das doenças do trato urinário em felinos (SOSNAR et al., 2003; TOGNI et al., 2018) e a escassez de informações sobre uremia na espécie felina, tanto nos trabalhos realizados no LPV-UFSM, quanto na literatura, foi realizado um estudo visando determinar a prevalência, e caracterizar os aspectos clínicos, patológicos e a distribuição anatômica das lesões extrarrenais de uremia em gatos.

Em um universo de 1330 gatos necropsiados no LPV-UFSM entre os anos de 2000 e outubro de 2019, 78 gatos apresentaram lesões extrarrenais de uremia (5,8%). Este estudo permitiu traçar o perfil dos gatos com uremia, além das principais manifestações clínicas observada nos animais. Lesões intrínsecas renais foram as principais doenças atribuídas à azotemia, e consequentemente à uremia, e as lesões crônicas foram as mais prevalentes, especialmente a NTIC. Esse predomínio de lesões renais crônicas pode ser explicado pelo seu caráter progressivo, resultando em exposição prolongada do animal às toxinas urêmicas (CLARKSON; THOMAS, 2011).

As lesões extrarenais de uremia comumente observadas nos gatos deste estudo, em ordem decrescente de frequência, foram: edema pulmonar, gastropatia urêmica, glossite e estomatite ulcerativas. Menos frequentemente, os animais apresentaram mineralização de tecidos moles, anemia, enterite hemorrágica ou ulcerativa e hiperplasia das paratireoides. Vale ressaltar, que o caráter multissistêmico da uremia normalmente relatado para a espécie canina (CARDOSO et al., 2019; DANTAS; KOMMERS, 2007; SILVEIRA et al., 2015;) foi observado em apenas 24% dos gatos deste estudo, os quais apresentaram ainda uma menor variedade de lesões relacionadas à uremia.

# 6 CONCLUSÃO

O estudo de caracterização dos aspectos morfológicos da DRC em gatos revelou que a NTIC foi o principal diagnóstico morfológico observado nos casos estudados. O formato arredondado dos rins, observado somente na NTIC, foi atribuído a maior severidade da fibrose intersticial e da inflamação crônica, junto à redução da espessura da camada cortical renal, atrofia tubular e esclerose glomerular, observadas de maneira mais acentuada principalmente nas regiões laterais ao hilo renal. A técnica de imuno-histoquímica utilizando o anticorpo anti-α-SMA foi uma ferramenta útil para a detecção de miofibroblastos no tecido renal de gatos com diferentes graus de intensidade de DRC. Houve maior intensidade da imunomarcação para α-SMA nos casos em que o comprometimento tecidual pela lesão histológica era mais acentuado, reforçando o papel dos miofibroblastos no desenvolvimento da fibrose intersticial presente na DRC dos felinos.

Lesões consideradas extrarrenais de uremia foram observadas em 78 gatos com lesões do trato urinário, entre os anos de 2000 e 2019. As principais causas de uremia nesses gatos foram doenças renais crônicas, principalmente na forma da NTIC. Edema pulmonar e gastrite ulcerativa e/ou hemorrágica e foram as lesões mais frequentemente observadas, em 51,2% e 35,8% dos casos, respectivamente. Lesões relacionadas às falhas no metabolismo do cálcio e fósforo (mineralização de tecidos moles, hiperplasia das paratireoides e osteodistrofia fibrosa) foram achados incomuns. A apresentação multissistêmica não foi a forma mais comum da uremia na espécie felina e os gatos apresentaram menor variedade de lesões extrarrenais de uremia. Isso pode ser reflexo da estabilidade da função renal comumente observada em gatos com DRC e ao comprometimento segmentar do tecido renal no caso das NTIC, que pode conferir manutenção prolongada da função renal ao longo da vida do animal.

## 7 REFERÊNCIAS


ALPERS, C. E.; FOGO, A. B. O Rim e seu sistema coletor. In: KUMAR, V.; ABBAS, A. K.; ASTER, J. C. **Robbins Patologia Básica.** 9. ed. São Paulo: Elsevier, 2013. cap. 13, p. 517-550.

ARESU, L. et al. Epithelial-mesenchymal transition (EMT) of renal tubular cells in canine glomerulonephritis. **Virchows Archiv**, v. 451, n. 5, p. 937–942, 2007.

BARTGES, J. W. Chronic kidney disease in dogs and cats. **Veterinary Clinics of North America - Small Animal Practice,** v. 42, p. 669–692, 2012.

BOEDEK, L. K. Pulmonary Abnormalities in Dogs with Renal Azotemia. **Journal of Veterinary Internal Medicine**, v. 26, n. 5, sep-oct. 2012.

BRESHEARS, M. A.; CONFER, A. W. The Urinary System**.** In: ZACHARY. J. F. **Pathologic Basis of Veterinary Disease.** 6. ed. St. Louis: Elsevier, 2017. cap. 11, p. 617-681.

BROWN, S. A. Pathophysiology and Management of Progressive Renal Disease. **The veterinary journal**, v. 154, p. 93-109, 1997.

BROWN, C. A. et al. Chronic kidney disease in aged cats: clinical features, morphology, and proposed pathogenesis. **Veterinary Pathology,** v. 53, n. 2, p. 309-326, 2016.

CARDOSO, P. G. S. et al. Dystrophic mineralization in uremic dogs: an update. **Pesquisa Veterinária Brasileira,** v. 39, n. 11, p. 889–899, nov. 2019.

CHALHOUB, S.; LANGSTON, C.; EATROFF, A. Anemia of renal disease: what it is, what to do and what's new. **Journal of Feline Medicine and Surgery**, v. 13, p. 629-640, 2011.

CHAKRABARTI, S. et al. Histomorphometry of feline chronic kidney disease and correlation with markers of renal dysfunction. **Veterinary Pathology**, v. 50, n. 1, p. 147–155, 2013.

CIANCIOLO, R. E., et al. World small animal veterinary association renal pathology initiative: classification of glomerular diseases in dogs. **Veterinary Pathology**, v. 53, n. 1, p. 113-135, 2016.

CIANCIOLO, R. E.; MOHR, F. C. Urinary System. In: MAXIE, M. G. **Jubb, Kennedy, and Palmer's Pathology of Domestic Animals.** 6ª ed. St. Louis: Elsevier, 2016. v. 2, cap. 4, p. 377-463.

CLARKSON, C. E.; FLETCHER, T. F. Anatomy of the kidney and proximal ureter. In: BARTGES, J.; POLZIN, D. J. **Nephrology and urology of small animals.** 1st ed. Iowa: Blackwell Publishing Ltd, 2011. cap. 1, p. 21-27.

DANTAS, A. F. M.; KOMMERS, G. D. Lesões extrarenais de uremia em 72 cães. **Ciência Rural,** v. 27, n. 2, p. 301-306, 1997.

DIBARTOLA, S. P.; RUTGERS, H. C.; ZACK, P. M. Clinicopathologic findings associated with chronic renal disease in cats: 74 cases (1973-1984). **Journal American Veterinary Medical Association**, v. 190, p. 1196–202, 1987.

DIBARTOLA, S. P.; WESTROPP, J. L. Manifestações Clínicas das Doenças do Trato Urinário. In: NELSON, R. W.; COUTO, C. G. **Medicina interna de pequenos animais.** 5 ed. [S.l.]: Elsevier, 2015. Cap. 41, p. 1838-1857.

GEDDES, R. F. et al. The role of phosphorus in the pathophysiology of chronic kidney disease. **Journal of Veterinary Emergency and Critical Care**, v. 23, n. 2, p. 122-133, 2013.

GOLDSTEIN, R.E. et al., Gastrin concentrations in plasma of cats with chronic renal failure. **Journal American Veterinary Medical Association**, v. 213, p. 826–828, 1998.

GRANT, D. C.; FORRESTER, S. D. Glomerulonephritis in dogs and cats: diagnosis and treatment. **Compendium in continuing education for the practising veterinarian: north American edition**, v. 23, n. 9, p. 798-804, 2011.

HAMILTON, J. B.; HAMILTON, R. S.; MESTLER, G. E. Duration of life and causes of death in domestic cats: influence of sex, gonadectomy, and inbreeding. **Journal of Gerontology,** v. 24, n. 4, p. 427-437, 1969.

JEPSON, R. E. Current understanding of the pathogenesis of progressive chronic kidney disease in cats. **Veterinary Clinics of North America: Small Animal Practice**, v. 46, n. 6, p. 1015-1048, 2016.

KHAN, T. M.; KHAN, K. N. Acute Kidney Injury and Chronic Kidney Disease. **Veterinary Pathology**, v. 52, n. 3, p. 441-444, 2015.

KING, L. G. et al. Anemia of chronic renal failure in dogs. **Journal of Veterinary Internal Medicine**, v. 6, n. 5, p. 264–70, 1992.

KUMAR, V.; ABBAS, A. K.; ASTER, J. C. Inflamação e Reparo. In: ___. **Robbins Patologia Básica.** 9. ed. São Paulo: Elsevier, 2013. cap. 2, p. 29-72.

LAWSON, J. et al. Renal fibrosis in feline chronic kidney disease: Known mediators and mechanisms of injury. **Veterinary Journal**, v. 203, n. 1, p. 18–26, 2015.

LIU, Y. Renal fibrosis: new insights into the pathogenesis and therapeutics. **Kidney International**, vol. 69, n. 2, p. 213- 217, jan. 2006.

LUND, E. M. et al. Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. **Journal American Veterinary Medical Association**, v. 214, n. 9, p.1336–1341, 1999.

MACDOUGALL, D. F. et al. Canine chronic renal disease: Prevalence and types of glomerulonephritis in the dog. **Kidney International**, v. 29, n. 6, p. 1144–1151, 1986.

MACK, M. YANAGITA, M. Origin of myofibroblasts and cellular events triggering fibrosis. Kidney international, v. 87, n. 2, p. 297-307, aug. 2014.

MARINO, C. L. et al. Prevalence and classification of chronic kidney disease in cats randomly selected from four age groups and in cats recruited for degenerative joint disease studies. **Journal of Feline Medicine and Surgery**, v.16, n. 6, p. 1-8, 2014.

MCLELAND, S. M. et al. A comparison of biochemical and histopathologic staging in cats with chronic kidney disease. **Veterinary Pathology**, v. 52, n. 3, p. 524-534, 2015.

MINKUS G. et al. Evaluation of renal biopsies in cats and dogs — histopathology in comparison with clinical data. **Journal of Small Animal Practice**, v. 35, n. 9, p. 465–472, 1994.

MULLER-PEDDINGHAUS, R.; TRAUTWEIN, G. Spontaneous Glomerulonephritis in Dogs: I. Classification and Immunopathology. **Veterinary Pathology,** v. 14, n. 1, p. 1–13, 1977.

O'NEILL, D. G. et al. Prevalence of disorders recorded in cats attending primary-care veterinary practices in England. **The veterinary journal**, v. 202, n. 2, p. 286-291, nov. 2014.

PETERS, R. M. et al. Histopathologic Features of Canine Uremic Gastropathy: A Retrospective Study. **Journal of Veterinary Internal Medicine**, v. 19, p. 315-320, 2005.

PRESSLER, N. Nephrotic syndrome. In: BARTGES, J.; POLZIN, D. J. **Nephrology and urology of small animals.** 1st ed. Iowa: Blackwell Publishing Ltd, 2011. cap 44, p. 415-438.

POLZIN, D. J. Chronic kidney diseases in small animals. **Veterinary Clinics of North America: Small Animal Practice**, v. 41, n. 1, p. 15–30, 2011a.

POLZIN, D. J. Chronic kidney disease. In: BARTGES, J.; POLZIN, D. J. **Nephrology and urology of small animals.** 1st ed. Iowa: Blackwell Publishing Ltd, 2011b. cap.48, p. 433-469.

POLZIN, D. J. Chronic kidney disease. In: ETTINGER, S. J.; FELDMAN, E. C.; CÔTÉ, E. **Textbook of Veterinary Internal Medicine**. 8th.ed. Saint. Louis: Elsevier, 2017. cap. 324, p. 4693-4734.

ROSS, S. J. Azotemia and uremia. In: BARTGES, J.; POLZIN, D. J. **Nephrology and urology of small animals.** 1st ed. Iowa: Blackwell Publishing Ltd, 2011. cap. 41, p. 393-398.

SAWASHIMA, K. et al. Expression of α-smooth muscle actin and fibronectin in tubulointerstitial lesions of cats with chronic renal failure. **American Journal of Veterinary Research**, v. 61, n. 9, p. 1080–1086, 2000.

SILVEIRA, I. P. et al. **Epidemiologia e distribuição de lesões extrarenais e uremia em 161 cães.** Pesquisa Veterinária Brasileira, vol. 35, n. 4, p. 562-568, jun. 2015.

SERAKIDES, R.; SILVA, J. F. Sistema Urinário. In: SANTOS, R. de L.; ALESSI, A.C. (Org.). **Patologia veterinária.** 2. ed. Rio de Janeiro: Roca, 2016. cap. 5, p. 460-532.

SHERK, M. Trato urinário superior. In: LITTLE, S. E. **O gato: medicina interna.** 1. ed. Rio de Janeiro: Roca, 2015. cap. 32, p. 1378-1386.

SLAUSON, D. O.; LEWIS, R. M. Comparative pathology of glomerulonephritis in animals. **Veterinary Pathology**, v. 16, n. 2, p. 135–164, 1979.

SOSNAR, M. et al. Retrospective study of renal failure in dogs and cats admitted to university of veterinary and pharmaceutical sciences brno during 1999-2001. **Acta Veterinaria Brno**, v. 72, n. 4, p. 593–598, 2003.

STRUTZ, F.; ZEISBERG, M. Renal Fibroblasts and Myofibroblasts in Chronic Kidney Disease. Journal of the american society of nephrology, v. 17, n. 11, p. 2992-2998, nov, 2006.

SUMNU, A.; GURSU, M.; OZTURK, S. Primary glomerular diseases in the elderly. **World Journal Nephrology**, v. 4, n. 2, p. 263–270, 2015.

SUN, Y. B. et al. The origin of renal fibroblasts/myofibroblasts and the signals that trigger fibrosis. Differentiation, v. 92, n. 3, p. 102-107, sep. 2016.

TOGNI, M. et al. Causas de morte e razões para eutanásia em gatos na Região Central do Rio Grande do Sul (1964-2013). **Pesquisa Veterinária Brasileira,** v. 38, n. 4, p. 741–750, abr. 2018.

VADEN, S. L.; GRAUER, G. F. Glomerular disease. In: BARTGES, J.; POLZIN, D. J. **Nephrology and urology of small animals.** 1st ed. Iowa: Blackwell Publishing Ltd, 2011. cap. 53, p. 556-563.

VADEN, S. L. Glomerular Diseases. In: ETTINGER, S. J.; FELDMAN, E. C.; CÔTÉ, E. **Textbook of Veterinary Internal Medicine**. 8. ed. St. Louis: Elsevier, 2017. cap. 325, p. 4735- 4764.

WHITE, J. D. Persistent haematuria and proteinuria due to glomerular disease in related Abyssinian cats. **Journal of Feline Medicine and Surgery**, v. 10, n. 3, p. 219-229, 2008.

YABUKI, A. et al. Comparative study of chronic kidney disease in dogs and cats: induction of myofibroblasts. **Research in Veterinary Science**, v. 88, n. 2, p. 294-299, 2010.

.