UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

CARACTERIZAÇÃO DO ÍNDICE DE RESISTIVIDADE RENAL E PADRONIZAÇÃO ULTRASSONOGRÁFICA E DOPPLERFLUXOMÉTRICA RENAL DE MUARES

MARIA CRISTINA REIS CASTIGLIONI

Botucatu, SP
Fevereiro 2018

UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

CARACTERIZAÇÃO DO ÍNDICE DE RESISTIVIDADE RENAL E PADRONIZAÇÃO ULTRASSONOGRÁFICA E DOPPLERFLUXOMÉTRICA RENAL DE MUARES

MARIA CRISTINA REIS CASTIGLIONI

Dissertação apresentada junto ao Programa de Pós-Graduação em Biotecnologia Animal para obtenção do título de Mestre.

Orientadora: Profª Drª Vânia Maria de Vasconcelos Machado
Co-Orientador: Prof. Dr. José Nicolau Próspero Puoli Filho

Botucatu, SP
Fevereiro 2018

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSANGELA APARECIDA LOBO-CRB 8/7500

Castiglioni, Maria Cristina Reis.
Caracterização do índice de resistividade renal e padronização ultrassonográfica e dopplerfluxométrica renal de muares / Maria Cristina Reis Castiglioni. - Botucatu, 2018

Dissertação (mestrado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Medicina Veterinária e Zootecnia
Orientador: Vânia Maria de Vasconcelos Machado
Coorientador: José Nicolau Próspero Puoli Filho
Capes: 50501038

1. Muar. 2. Equino - Doenças. 3. Cavalo - Doenças. 4.Ultrassonografia veterinária. 5. Rins - Doenças.

Palavras-chave: Cavalo; Equino; Histograma; Rim; Ultrasonografia trans-abdominal.

Nome do Autor: Maria Cristina Reis Castiglioni

Título: CARACTERIZAÇÃO DO ÍNDICE DE RESISTIVIDADE RENAL E PADRONIZAÇÃO ULTRASSONOGRÁFICA E DOPPLERFLUXOMÉTRICA RENAL DE MUARES

Data da defesa: 16 de fevereiro de 2018.

COMISSÃO EXAMINADORA

Profª. Drª. Vânia Maria de Vasconcelos Machado
Presidente e Orientadora
Departamento de Reprodução Animal e Radiologia Veterinária, FMVZ – UNESP, campus Botucatu

---

Prof. Dr. Alexandre Secorun Borges
Membro Titular
Departamento de Clínica Veterinária, FMVZ – UNESP, campus Botucatu

---

Prof. Dr. Stefano Carlo Filippo Hagen
Membro Titular
Departamento de Cirurgia, FMVZ – USP, campus Cidade Universitária

"Algo só é impossível até que alguém duvide e resolva provar ao contrário".

(Albert Einstein)

DEDICATÓRIA

Dedico este trabalho à minha família, por sempre apoiarem e incentivarem a minha busca por conhecimento:

Meus pais, Antônio Carlos e Maria Fátima, meus modelos de ética e de vida, que nunca duvidaram da minha capacidade e sempre me incentivaram a querer mais, a aprender mais.

À minha falecida avó Mabília, que sempre esteve presente durante meu crescimento e que me apresentou a grande paixão da minha vida: os animais. Obrigada por ter incentivado o meu amor a tudo que é vivo, seja planta ou animal.

AGRADECIMENTOS

Primeiramente agradeço aos meus amigos, Jeana Pereira da Silva, Jéssica Leite Fogaça e Michel de Campos Vettorato, que permitiram que este trabalho fosse realizado. Agradeço a colaboração dos meus colegas do setor de imagem.

À minha orientadora, professora Vânia Maria de Vasconcelos Machado, que me orientou e apoio neste longo caminho. Obrigada pela paciência, disponibilidade e ensinamentos.

À paciência e atenção do meu co-orientador, professor José Nicolau Próspero Puoli Filho, que permitiu que meu trabalho fosse realizado. Agradeço em especial todos os ensinamentos sobre a etologia equina, um aprendizado que sempre carregarei comigo.

Agradeço à pós-graduação da Faculdade de Medicina Veterinária e Zootecnia de Botucatu (FMVZ – UNESP Botucatu), por disponibilizar todos os recursos necessários para a realização desta pesquisa, e em especial ao Setor de Radiologia Animal, Laboratório Clínico Veterinário e Setor de Equideocultura da Fazenda Experimental da Edgárdia.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pelo auxílio financeiro através da Bolsa de Mestrado concedida.

Por último, mas não menos importante, gostaria de agradecer a Imagem S.A. pelo apoio fornecido. Sem sua ajuda não seria possível realizar este trabalho.

LISTA DE TABELAS

LISTA DE FIGURAS

LISTA DE ABREVIAÇÕES

%	Porcento
cm	Centímetros
g	Gramas
kg	Quilogramas
m/s	Metros por segundo

SUMÁRIO

PÁGINA

CASTIGLIONI, M.C.R. **Caracterização do índice de resistividade renal e padronização ultrassonográfica e dopplerfluxométrica renal de muares.** Botucatu, 2017. 82p. Dissertação (Mestrado) – Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, Universidade Estadual Paulista.

RESUMO

Por ser um híbrido entre égua e jumento, o muar apresenta características anatômicas e fisiológicas intermediárias. Existem poucos estudos sobre esse híbrido e mesmo com a extensa literatura sobre cavalos domésticos não está claro se esta serve como referência para os muares. O propósito deste estudo foi caracterizar os rins dos muares por meio do exame ultrassonográfico modo Bidimensional e Doppler (colorido e pulsado). Foram avaliados os rins de 12 muares sendo os achados formam comparados com rins de 12 cavalos e dados pré-existentes. Foram observados os seguintes achados: 1) topografia renal semelhante, salvo maior distância do rim direito à coluna lombar nos muares; 2) dimensões renais semelhantes, salvo a zona medular mais espessas nos muares; 3) ecogenicidade renal menor no muar; 4) menor captação do sinal Doppler renal nos muares; e 5) índices de resistividade e de pulsatividade menores nos muares, indicando menor resistência vascular e maior perfusão sanguínea. Embora os rins de muares possuam diversas semelhanças com os dos cavalos, existem particularidades anatomo-fisiológicas que resultam em diferenças ultrassonográficas significantes.

Palavras-chave: Equino; Cavalo; Ultrassom trans-abdominal; Histograma; Rim; Características sonográficas.

CASTIGLIONI, M.C.R. **Renal resistive index characterization and renal ultrasonographic and dopplefluxometric padronization of mules.** Botucatu, 2017. 82p. Dissertação (Mestrado) – Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, Universidade Estadual Paulista.

ABSTRACT

As hybrids of mare and jack donkey, mules are expected to present intermediary anatomical and physiological characteristics. There are few studies and even with the extensive literature about horses, it is not clear if it serves as reference for mules. The purpose of this study is characterize the kidneys of mules using ultrasonographic examination on Bidimensional and Doppler (color and spectral) modes. Kidneys from 12 mules were evaluated and compared to kidneys of12 horses and pre-existent data. The following results were obtained: 1) similar renal topography with the exception of a higher distance between the right kidney and lumbar column in mules; 2) similar renal dimensions with the exception of a thicker renal medulla in mules; 3) lower renal echogenicity in mules; 4) worse Doppler signal reception in mules; and 5) smaller resistive and pulsatile indexes in mules, inferring a lower vascular resistance and higher blood perfusion. Although the kidneys of mules are similar to that of horses there are anatomical and physiological particularities that results in significant ultrasonographic differences.

Keywords: Equine; Horse; Trans abdominal ultrasound; Histogram; Kidney; Sonographic characteristics

CAPÍTULO 1

Introdução e Justificativa

Revisão de Literatura

Referências

1. INTRODUÇÃO E JUSTIFICATIVA

Os rins são responsáveis pela filtração do sangue, eliminando substâncias indesejadas e mantendo o equilíbrio ácido-base, eletrolítico e hídrico do organismo. O comprometimento deste órgão pode desencadear na perda total ou parcial dessas funções e, consequentemente, na perda da homeostase corpórea e origem de distúrbios metabólicos e tóxicos.

Nos estágios iniciais, o dano ao parênquima renal pode ser revertido, no entanto as doenças renais produzem sinais clínicos inespecíficos, tornando difícil a detecção de injúrias agudas, resultando no comprometimento do tecido renal (WILSON, 2007; MCLELAND, 2015). Atualmente o diagnóstico das doenças renais deriva da combinação dos achados obtidos pela anamnese, exame físico, hemograma, função renal, urinálise, exame ultrassonográfico e biópsia renal (GEOR, 2007; MCLELAND, 2015).

A avaliação ultrassonográfica do trato urinário tornou-se um procedimento padrão no auxílio do diagnóstico de afecções renais em humanos e pequenos animais, Apesar disso, ainda são escassos estudos sobre esse assunto na espécie equina. Atualmente a anatomia ultrassonográfica dos rins dos cavalos domésticos (*Equus caballus*) apresenta-se consolidada na Medicina Veterinária e recentemente, foi definida a variação de normalidade do índice de resistividade renal de cavalos não sedados (MACRI et al., 2015). Entretanto, outros membros do gênero *Equus*, como os jumentos, muares, bartodos e bardotas ainda são pouco estudados, resultando em uma deficiência de informações sobre esses equinos.

Oriundos do cruzamento entre éguas e jumentos, os muares possuem características morfológicas, fisiológicas e comportamentais intermediárias a essas duas espécies. As mulas e os burros são considerados mais fortes e calmos do que os cavalos e, embora sejam distinguidos por essas características, os muares são propensos às mesmas disfunções orgânicas e processos lesivos que acometem os demais animais (THE DONKEY SANCTUARY, 2017).

Mesmo com a extensa literatura sobre cavalos domésticos, não é possível utilizar os mesmos parâmetros para as mulas e os burros, dado que por serem híbridos torna-se esperado que possuam características intermediárias à ambos os progenitores. Entretanto, até o presente momento, não foi possível encontrar estudos caracterizando os rins dos muares pelo exame ultrassonográfico. Adicionalmente, não existem informações concretas referindo que os muares possuam aspecto sonográfico semelhante ao dos cavalos domésticos (*Equus caballus).*

Diante do exposto, o presente trabalho propõe inferir parâmetros de análise ultrassonográfica e dopplerfluxométrica dos rins de muares pela ultrassonografia trans-abdominal, determinando o grau de semelhança ultrassonográfica com cavalo doméstico. Contribuindo dessa forma com a manutenção e aumento da expectativa de vida dos muares, ao inferir valores de referência para auxiliar no diagnóstico de alterações renais e acompanhar o manejo terapêutico.

.

2. OBJETIVOS

2.1 GERAIS:

- Estabelecer parâmetros renais de análise ultrassonográfica e dopplerfluxométrica de muares através da ultrassonografia transabdominal, comparando-os com cavalos domésticos (*Equus caballus*).

2.2 ESPECÍFICOS:

- Definir parâmetros de referência de localização, tamanho, contornos, espessura, ecogenicidade e definição do parênquima renal nos muares por meio da ultrassonografia em modo bidimensional.
- Definir parâmetros de referência para ecogenicidade e definição do parênquima renal nos muares por meio do histograma em escala de cinza.
- Delinear a extensão e as características da arquitetura vascular renal nos muareas pela ultrassonografia com Doppler colorido e pulsado, analisando o espectro e morfologia das ondas das artérias renais, interlobulares e arqueadas, padronizando valores de referência para os índices de resistividade e pulsatividade.

3. REVISÃO DE LITERATURA

3.1. OS MUARES

3.1.1. DEFINIÇÃO

Possuindo 63 cromossomos, os muares são animais híbridos oriundos do cruzamento entre uma égua, que possui 64 cromossomos, e um jumento, com 62 cromossomos (BURNHAN, 2002; SMITH, 2009; THE DONKEY SANCTUARY, 2017). Embora sejam híbridos possuem estabilidade genética, apresentando características esperadas, como porte e conformação corporal semelhante aos cavalos (*Equus ferus caballus*), e extremidades (orelhas e membros) semelhantes aos asininos (*Equus asinus)* (BURNHAN, 2002; SMITH, 2009; THE DONKEY SANCTUARY, 2017). As fêmeas são conhecidas como mula e os machos, burros, sendo que ambos apresentam características físicas, fisiológicas e comportamentais intermediárias a essas espécies de equinos.

3.1.2. INFLUÊNCIAS DE ORIGEM PATERNA

Ao contrário dos cavalos domésticos, os jumentos são animais nativos dos ambientes áridos e semiáridos africanos, possuindo deste modo diferenças anatomo-fisiológicas significantes que lhe conferem maior força e resistência, permitindo sua adaptação a condições extremas (BURNHAN, 2002; SMITH, 2009; BURDEN & THIEMANN, 2015; THE DONKEY SANCTUARY, 2017). Devido ao vigor híbrido os muares podem herdar parte dessas características, sendo assim considerados mais fortes e resistentes do que os cavalos.

Devido à influência paterna, tanto as mulas quanto os burros possuem orelhas longas e pelagem densa, além de possuírem conformação craniana e pélvica diferentes de sua progenitora (BURNHAN, 2002; SMITH, 2009; MATTHEWS; VAN LOON, 2013; BURDEN; THIEMANN, 2015; THE DONKEY SANCTUARY, 2017). Também são menos propensos à fuga, mais calmos e resistentes à dor do que os cavalos, mas são mais predispostos a se defenderem de ameaças ou estímulos negativos (MATTHEWS et al., 2005;

PROOPS et al., 2009; SCHIMT, 2009; BURDEN; THIEMANN, 2015; THE DONKEY SANCTUARY, 2017;).

A influência paterna também é observada no metabolismo e fisiologia dos muares, sendo possível observar variações renais, cardiovasculares e eletrolíticas que conferem aos muares maior resistência à desidratação e capacidade de sobrevivência em ambientes extremos do que cavalos domésticos (MALOIY, 1970; IZRAWLY et al., 1989; KASIRER-IZRAELI et al., 2001; MATTHEWS et al., 2005; MATTHEWS; VAN LOON, 2013). Atualmente existem documentados neste híbrido, parâmetros vitais e hematológicos distintos, além de uma distribuição e metabolização diferenciada de certos medicamentos como anestésicos e anti-inflamatórios (BURNHAN, 2002; MATTHEWS et al., 2005; MATTHEWS; VAN LOON, 2013).

Independente destas características, a manutenção dos muares é semelhante a dos asininos. Do ponto de vista nutricional, as mulas herdam a eficiência digestiva do seu progenitor, necessitando de uma menor quantidade de alimento quando comparados aos cavalos, sendo capazes de sobreviver em dietas de menor qualidade nutricional (SMITH, 2009; SMITH; BURDEN, 2013; BURDEN; THIEMANN, 2015). Por este motivo é recomendado seguir um protocolo nutricional semelhante aos jumentos, embora não possuam a mesma predisposição para obesidade ou laminite (SMITH; BURDEN, 2013). Além disso, embora seja mais resistente à sede, podendo ficar dias sem precisar de uma fonte de água, o muar possui o mesmo requerimento hídrico que os demais equídeos. (BURDEN; THIEMANN, 2015)

3.1.3. IMPORTÂNCIA

Em virtude de sua capacidade física e sua resistência natural, os muares foram animais de carga e trabalho importantes durante o desenvolvimento das civilizações (ELLENBERG, 2007; BURDEN; THIEMANN, 2015; THE DONKEY SANCTUARY, 2017). Achados arqueológicos indicam a sua presença em comunidades humanas há três mil anos, sendo que de acordo com a cultura esse híbrido era utilizado como animal de carga e transporte ou como meio de lazer (SMITH, 2009).

No continente americano, os muares desempenharam importantes funções na agricultura e na exploração colonizadora, servindo como meio para transporte de cargas, mantimentos e equipamentos (ELLENBERG, 2007; SMITH, 2009; THE DONKEY SANCTUARY, 2017). Atualmente os muares ainda são utilizados como animais de trabalho e carga em países subdesenvolvidos, mas possuem propósito quase que exclusivamente de lazer e esporte em países do 1º mundo, como a Inglaterra e os Estados Unidos da América (THE DONKEY SANCTUARY, 2017).

3.2. O SISTEMA RENAL DOS MAMÍFEROS

Nos mamíferos os rins são responsáveis pela manutenção do equilíbrio hídrico, eletrolítico e ácido-básico, possuindo importante papel na metabolização de substâncias endógenas e exógenas. Os rins realizam a filtração do sangue, excretando toxinas, metabólitos ou substâncias não desejadas pela produção da urina e reabsorvendo as necessárias, como água, glicose, íons orgânicos e sais inorgânicos (KÖNIG et al., 2004; TORIBIO, 2007; NEWMAN et al., 2009; VERLANDER, 2013). Ademais, também são responsáveis pela conversão da vitamina D em calcitrol e na produção hormonal de renina e a eritropoietina, que atuam respectivamente na manutenção da pressão sanguínea sistêmica e na indução da eritropoiese (KÖNIG et al., 2004; TORIBIO, 2007; NEWMAN et al., 2009; VERLANDER, 2013).

Cada rim é composto por parênquima, pelve e uma fina cápsula fibrosa lisa que recobre a superfície externa e forma as paredes da pelve. O parênquima renal macroscopicamente dividido em duas zonas distinta: a córtex, mais periférica e a medular, mais interna, sendo estas separadas pela junção córtico-medular (KÖNIG et al., 2004; TORIBIO, 2007; REED et al., 2010).

Microscopicamente o parênquima renal é composto por milhões de unidades funcionais conhecidas como néfrons, cada formada por um corpúsculo renal (glomérulo) e um sistema tubular (túbulos contorcidos proximais, alça de Henle, túbulos contorcidos distais e túbulos coletores) (Figura 1) (NEWMAN et al., 2009; VERLANDER, 2013). Os glomérulos renais são responsáveis pela filtração do plasma enquanto que o sistema de tubos é responsável pela reabsorção de solutos e substâncias desejadas pelo organismo (VERLANDER, 2013).

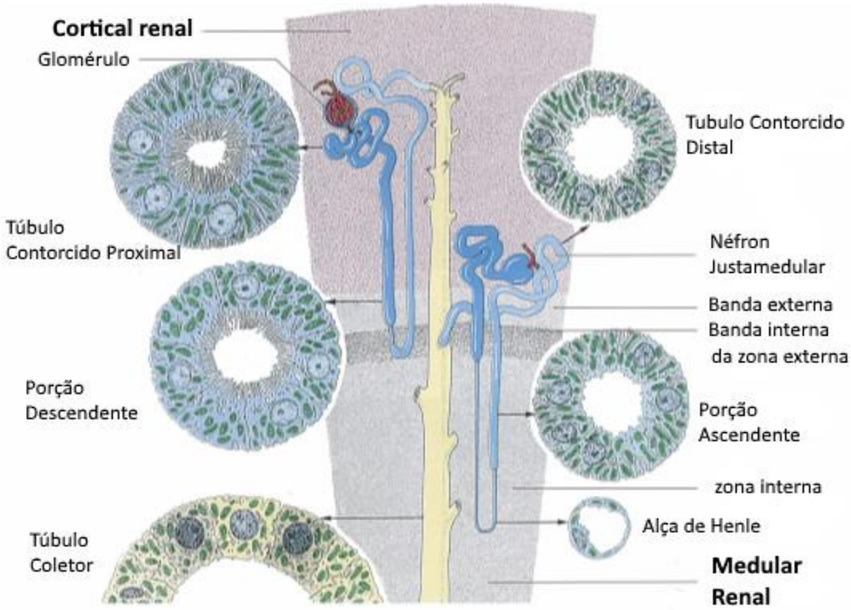

Figura 1 - Esquema representativo das estruturas funcionais do rim (néfrons). Adaptado de KÖNIG et al., 2004.

Independente da espécie, o número de néfrons não aumenta após o nascimento, embora essa unidade funcional aumente de tamanho durante o primeiro ano de vida (SCHOTT II; WOODIE, 2012).

O suprimento vascular de cada rim é proveniente da artéria renal, dividindo-se em diversas artérias interlobares ao adentrar o hilo renal. Estas percorrem a medular até a junção corticomedular, onde originam as artérias arqueadas e em seguida as artérias interlobulares (Figura 2). Percorrendo a córtex renal, as artérias interlobulares formam as arteríolas e os capilares responsáveis pela formação dos glomérulos renais e pela irrigação dos néfrons (KÖNIG et al., 2004). Uma vez filtrado, o sangue é drenado pelas veias interlobulares, arqueadas e interlobares até a veia renal, que sofre anastomose com a veia cava caudal (KÖNIG et al., 2004).

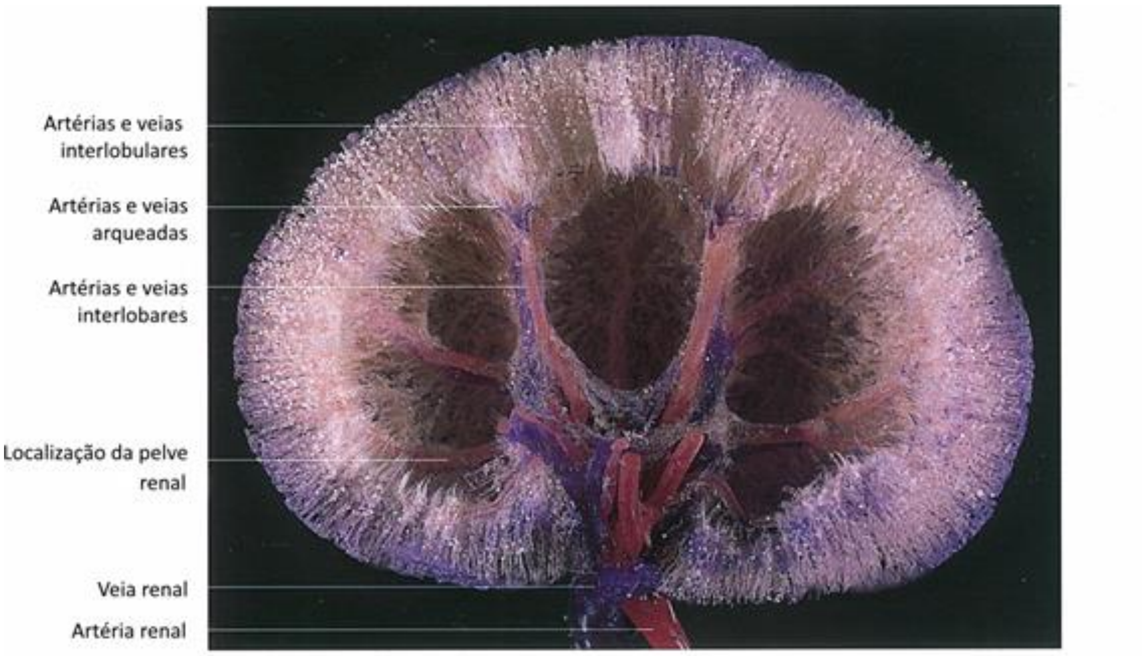

Figura 2 - Vasos sanguíneos profundos do rim esquerdo de um gato, peça de corrosão. Adaptado de KÖNIG et al., 2004.

Em condições de repouso os rins recebem de 15 a 20% do débito cardíaco, apresentando um consumo de oxigênio que é secundário apenas ao coração (NEWMAN et al., 2009; VERLANDER, 2013). Mesmo com esse alto fluxo de sangue, apenas 20% dele chega a irrigar a medular renal, sendo que 80% do sangue fica retido na córtex renal para melhorar o processo de filtração e reabsorção. Desta maneira, os rins são susceptíveis à hipóxia e, portanto, qualquer processo que diminuía a perfusão sanguínea renal poderia predispor lesões, particularmente na zona medular (VERLANDER, 2013; TORIBIO, 2007).

O sistema renal pode ser alvo de diversos estímulos e agentes nocivos através de contaminação ascendente ou via hematógena. O mesmo fluxo sanguíneo que supre o rim pode se tornar uma porta de entrada para a penetração de agentes infecciosos ou trazer substâncias que resultem em danos durante o processo de metabolização, filtração ou excreção (NEWMAN et al., 2009).

Qualquer lesão que venha a ocorrer em qualquer um dos componentes do néfron resulta na diminuição da função e progressiva injúria do rim (NEWMAN et al., 2009). Nos estágios mais precoces das doenças, o insulto primário se restringe a apenas em um dos componentes, no entanto, caso o insulto seja grave ou persista, o parênquima renal começa sofrer alterações crônicas que podem comprometer a integridade do néfron ou levar a sua perda funcional (NEWMAN et al., 2009). Os néfrons remanescentes sofrem hipertrofia compensatória em uma tentativa de preservar a função renal (TORIBIO, 2007; NEWMAN et al., 2009).

A diminuição ou bloqueio da função renal resultam em efeitos sistêmicos maiores, como uremia, azotemia, perda de proteína plasmática, desequilíbrio hídrico, eletrolítico e ácido-base, retenção de fármacos, entre outros (NEWMAN et al., 2009). Nos estágios iniciais, os danos ao parênquima renal podem ser revertidos, no entanto existe uma grande dificuldade em detectar precocemente as injúrias e doenças renais.

3.2.1. CARACTERÍSTICAS RENAIS EQUINAS

Atualmente as informações disponíveis sobre os rins dos equinos são oriundas dos estudos realizados nos cavalos domésticos (*Equus ferus caballus*). Suas características anatômicas e fisiológicas apresentam-se consolidadas na medicina veterinária, no entanto, outros membros do gênero *Equus*, como os jumentos, muares, bartodos e bardotas ainda são pouco estudados, resultando em uma deficiência de literatura sobre esses equinos. Portanto, os dados oriundos dos cavalos domésticos são utilizados como parâmetros de referência para os demais membros do gênero *Equus,* embora não esteja comprovado semelhança ou descartada sua diferença.

Como nos demais mamíferos, os rins dos equinos localizam-se dentro do espaço retroperitoneal, recobertos por uma camada de gordura que os protege contra choques mecânicos (SERTICH; TURNER, 1998; KÖNIG et al., 2004). O rim direito encontra-se imediatamente ventral às extensões dorsais das últimas costelas e das primeiras vértebras lombares, apresentando íntima relação com o fígado por estar localizado na fossa renal (SERTICH; TURNER, 1998; KÖNIG et al., 2004; SCHOTT II; WOODIE, 2012). Por sua vez, o rim esquerdo apresenta-se mais caudal e medial que o direito, no nível das vértebras lombares e medial ao parênquima esplênico (SERTICH; TURNER, 1998; KÖNIG et al., 2004; SCHOTT II; WOODIE, 2012).

Ambos os rins possuem íntimo contato com as alças intestinais, podendo sofrer pequenas movimentações devido à motilidade intestinal. O rim direito possui contato com o duodeno e o ceco, este primeiro ventral ao rim e o secundo ventrocaudal (SERTICH; TURNER, 1998). O cólon dorsal esquerdo (CDE), cólon ventral esquerdo (CVE) e cólon transverso (CT) encontram-se adjacentes ao rim esquerdo, sendo o CDE ventrolateral, o CVE ventral e o TC medial ao rim (SERTICH; TURNER, 1998).

Diferentemente dos outros animais doméstico, existe uma diferença marcante entre os rins em relação ao formato, sendo que o direito apresenta formato triangular, assemelhando-se a uma ferradura ou à um coração (*valentine heart*) e o esquerdo é alongado, podendo formar “U” ou um “J” invertido com a curvatura do seu polo caudal (Figura 3) (KÖNIG et al., 2004; REED et al., 2010; SCHOTT II; WOODIE, 2012). O rim direito pode mensurar entre 15 a 18 cm de comprimento, até 15 cm de espessura e entre 5 a 6 cm de altura (REED et al., 2010; SCHOTT II; WOODIE, 2012). Por sua vez o pode possuir até 18 cm de comprimento, entre 10 a 12 cm de espessura e de 5 a 6 cm de altura (REED et al., 2010; SCHOTT II; WOODIE, 2012).

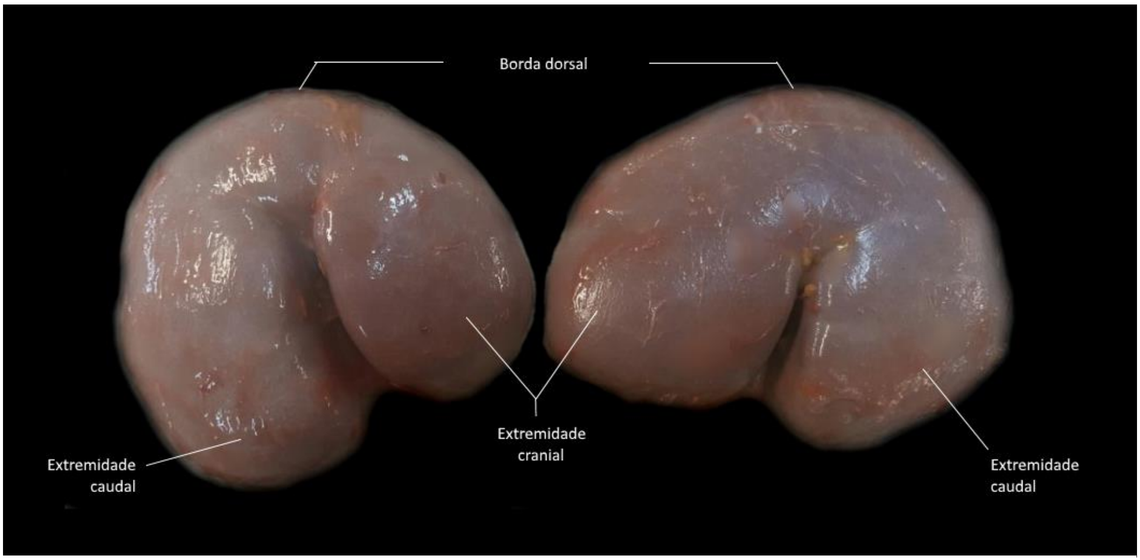

Figura 3 – Rim direto (à esquerda) e rim esquerdo (à direita) de uma mula com cápsula renal preservada, aspecto lateral**.** Arquivo pessoal.

Em relação ao tamanho e peso, o rim direto é discretamente maior do que o esquerdo. Os rins podem pesar de 600 a 1000 g em cavalos adultos com peso médio entre 400 a 500 kg, com uma discreta diferença de gramas entre os rins (REED et al., 2010; SCHOTT II; WOODIE, 2012).

Nos equinos, os rins são unilobares e como nos demais mamíferos, cada um é composto pelo parênquima renal, pelve renal e cápsula fibrosa. A pelve renal equina é composta por uma única cavitação central e dois amplos recessos, localizados em cada polo renal. A córtex renal equina é discretamente menos espessa do que a medular, sendo que a junção corticomedular menos distinta do que nos outros mamíferos (Figura 4) (KÖNIG et al., 2004; TORIBIO, 2007; SCHOTT II; WOODIE, 2012).

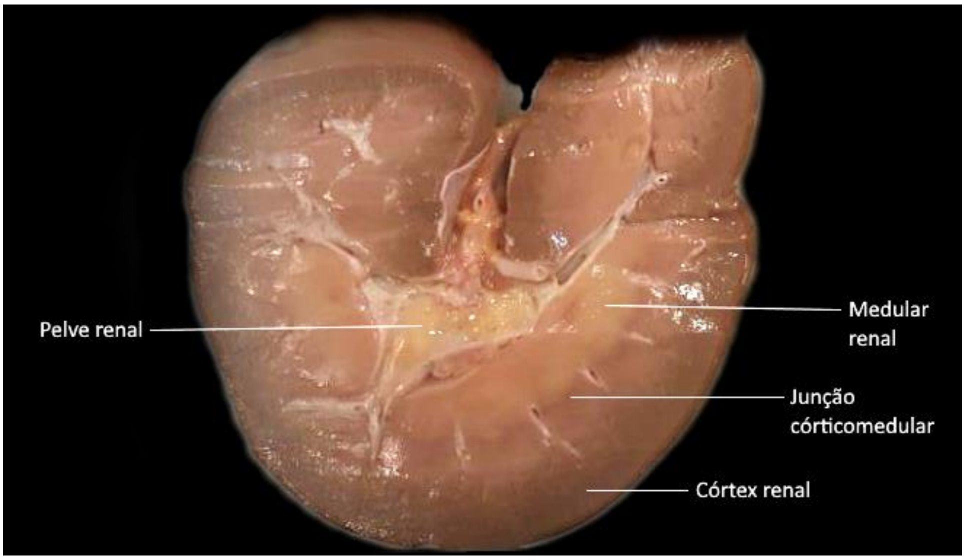

Figura 4 - Corte seccional do rim direito de uma mula. Arquivo pessoal

Cada rim equino possui aproximadamente dez milhões de néfrons, e embora os néfrons sejam histologicamente semelhantes aos dos demais mamíferos, eles possuem ductos coletores maiores e glomérulos mais proeminentes do que nas demais espécies de mamíferos (KÖNIG et al., 2004; REED et al., 2004; SCHOTT II; WOODIE, 2012). Nos equinos, o suprimento sanguíneo renal é realizado por mais do que uma artéria renal (Figura 5, podendo estar presentes artérias renais acessórias, permitindo na captação de 7,5 litros a 10 litros por minuto em um cavalo de tamanho médio (KÖNIG et al., 2004; REED et al., 2010; SCHOTT II; WOODIE, 2012).

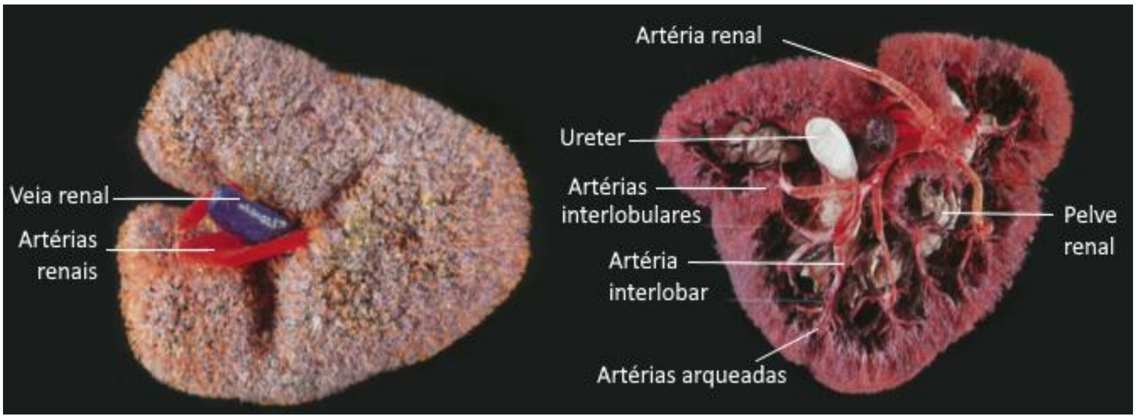

Figura 5 - À esquerda: vasos sanguíneos superficiais do rim direito de um cavalo (peça de corrosão). À direita: vasos sanguíneos profundos do rim esquerdo de um cavalo (peça de corrosão). Adaptado de KÖNIG et al., 2004

Embora as doenças renais primárias em equinos não sejam comuns, as doenças adquiridas, são consideradas complicações que podem levar a perda do animal caso não sejam diagnosticas e tratadas corretamente. Dentre elas, a mais comum é a falência renal aguda por necrose tubular aguda, uma complicação severa e comum de processos nefrotóxicos ou que resultem em neuropatia vasomotora (ORSINI; DIVERS, 2014; VAN METRE; SOTO, 2015). Dentre as causas mais comuns encontram-se a hipoperfusão renal seguida de isquemia tecidual, possíveis consequências da síndrome cólica, desidratação severa, sepse, coagulopatias, ou a lesões renais oriundas da administração inadequada de aminoglicosídeos, tetraciclinas, anti-inflamatórios não esteroidais, intoxicação por metais pesados, liberação de mioglobina secundária a miopatias como a rabdomiólise, entre outros (GEOR, 2007; ORSINI; DIVERS, 2014; VAN METRE; SOTO, 2015).

3.3. AVALIAÇÃO ULTRASSONOGRÁFICA

Na Medicina e Medicina Veterinária, principalmente cães e gatos, a avaliação ultrassonográfica do trato urinário se tornou um procedimento padrão no auxílio do diagnóstico de afecções renais (PLATT, 1997; TUBLIN et al. 2003; MATOON; NYLAND, 2015; PENNINCK; D'ANJOU, 2015). Através do exame ultrassonográfico bidimensional é possível avaliar o tamanho, contornos e características do parênquima além de detectar alguns tipos de anormalidades como edema perirrenal, cistos renais, nefrolitíase ou neoformações; auxiliando deste modo na categorização da doença renal e na determinação do prognóstico (GEOR, 2007; MATOON; NYLAND, 2015; PENNINCK; D'ANJOU, 2015).

No entanto, assim como nos demais exames complementares, este exame pode levar a achados inconclusivos por apenas permitir informações anatômicas básicas (TUBLIN et al., 2003; GEOR, 2007; MATOON; NYLAND, 2015). A ausência de alterações ultrassonográficas no modo bidimensional também não descarta a possibilidade da presença de injúrias agudas.

O modo Doppler permite avaliar a vascularização renal de modo qualitativo (modo colorido) e quantitativo (modo espectral) a perfusão renal, ajudando a determinar a alterações do fluxo sanguíneo renal, que podem indicar lesões renais agudas (aumento da resistência vascular), início de processos obstrutivos renais ou até mesmo estarem relacionados a alterações sistêmicas (hipovolemia, doenças cardiovasculares, entre outros) (CARVALHO, 2009; MATOON; NYLAND, 2015; PENNINCK; D'ANJOU, 2015).

Dentro dos principais parâmetros dopplerfluxométricos para a avaliação do fluxo arterial encontram-se o índice de resistividade (IR), índice de pulsatividade (IP), pico da velocidade sistólica (pVS), velocidade diastólica final (VDf), velocidade média (VM) e razão pVS:VDf. Adicionalmente, alterações no traçado da onda espectral das artérias podem ser encontraram em diversas alterações renais, como obstrução urinária, doença vascular, desordens do parênquima, entre outros (TAYLOR, 1990; TUBLIN et al., 2003)

Atualmente a ultrassonografia Doppler é utilizada para auxiliar na detecção precoce da insuficiência renal aguda, uma vez que é conhecido na medicina que a perfusão renal pode diminuir em até 50% antes dos níveis de creatinina e ureia aumentarem (PLATT, 1997; MARTIN et al., 2015; MATOON; NYLAND, 2015). Um dos valores mais utilizados para auxiliar no diagnóstico de doença renal aguda em humanos e cães é o índice de resistividade (IR). Obtido pelo exame de Doppler espectral, o IR calcula a resistência vascular de um determinado vaso através da subtração do pVS pela VDf, dividindo o resultado por pVS (Figura 6) (PLATT, 1997; MATOON; NYLAND, 2015).

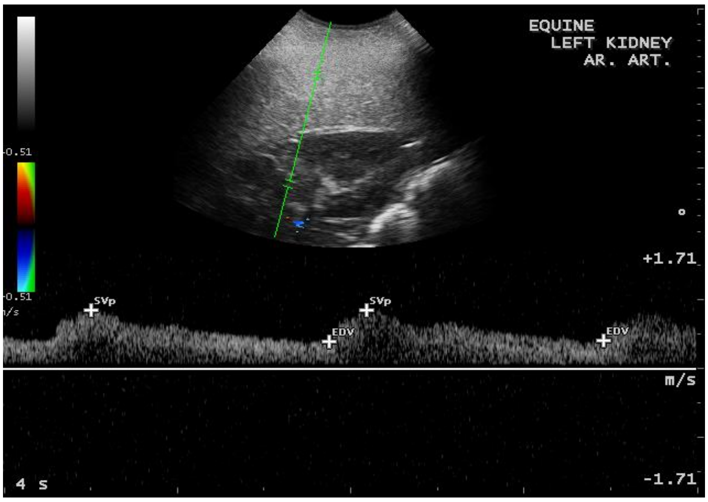

Figura 4 - Mensuração do índice de resistividade (IR) no traçado espectral da artéria arqueada de um animal da espécie equina, adulto e saudável. Sendo: SVp = pico da velocidade sistólica; EDV = velocidade diastólica final. Arquivo pessoal.

O valor do IR depende da interação entre complacência e resistência da artéria em questão, inferindo o nível da perfusão do tecido (TUBLIN et al., 2003; GINTHER, 2007). Esse índice se tornou um importante parâmetro de avaliação do fluxo sanguíneo arterial, sendo que de sua alteração pode ser inferidas doenças parenquimatosas ou vasculares (PLATT, 1997; TUBLIN et al., 2003; GEOR, 2007; MATOON; NYLAND, 2015). O aumento do IR renal indica um aumento da resistividade vascular, inferindo na diminuição da perfusão tecidual que pode ser causada pela presença de lesões agudas tubulointersticiais, vasculites, pielonefrites ou processos obstrutivos (CARVALHO, 2009). Por sua vez, um IR renal normal ou diminuído pode indicar normalidade ou até mesmo ocorrer em doenças renais que não resultem em alteração da resistência vascular (PLATT, 1997; CARVALHO, 2009). Em humanos observa-se a diminuição do IR com a progressão da doença renal crônica (PETERSON et al., 1997).

Entretanto, o aumento do IR renal já esta relacionado a diversas alterações sistêmicas em homens e animais, como a hipotensão sistêmica grave, bradicardia, presença de líquido perirrenal ou subcapsular (PLATT, 1997; TUBLIN et al., 2003; GEOR, 2007;CARVALHO, 2009; MATOON; NYLAND, 2015). Da mesma maneira, agentes anestésicos que levam a alterações hemodinâmicas também resultam na alteração deste índice (CARVALO, 2009; MACRI et al., 2015). Em humanos, a variação do IR renal relacionada à idade encontra-se bem estudada sendo relacionada com alterações da complacência vascular ou alterações dos pequenos vasos devido ao envelhecimento, sendo que o IR de crianças e idosos significantemente maior do que nos adultos (PLATT, 1997; TUBLIN et al., 2003).

O índice de pulsatividade (IP) é obtido através da subtração do pVS pelo VDf, dividindo em seguida pela VM (GINTHER, 2007; CARVALHO et al., 2008). Atualmente este índice é automaticamente calculado pelo software após a delimitação de um ciclo cardíaco completo da onda espectral (Figura 7). Semelhante ao IR, o IP auxilia na detecção de alterações da resistividade vascular, inferindo a presença de doenças parenquimatosas, início do processo de rejeição (transplantes) e até mesmo ajudando na caracterização da malignidade de certas doenças (GINTHER, 2007; CARVALHO et al., 2008). No entanto, esse índice é considerado mais fidedigno em fluxos arteriais com padrão de alta resistividade, que possuem fase negativa no traçado espectral, sendo pouco utilizado para a avaliação do fluxo arterial renal (GINTHER, 2007; CARVALHO et al., 2008).

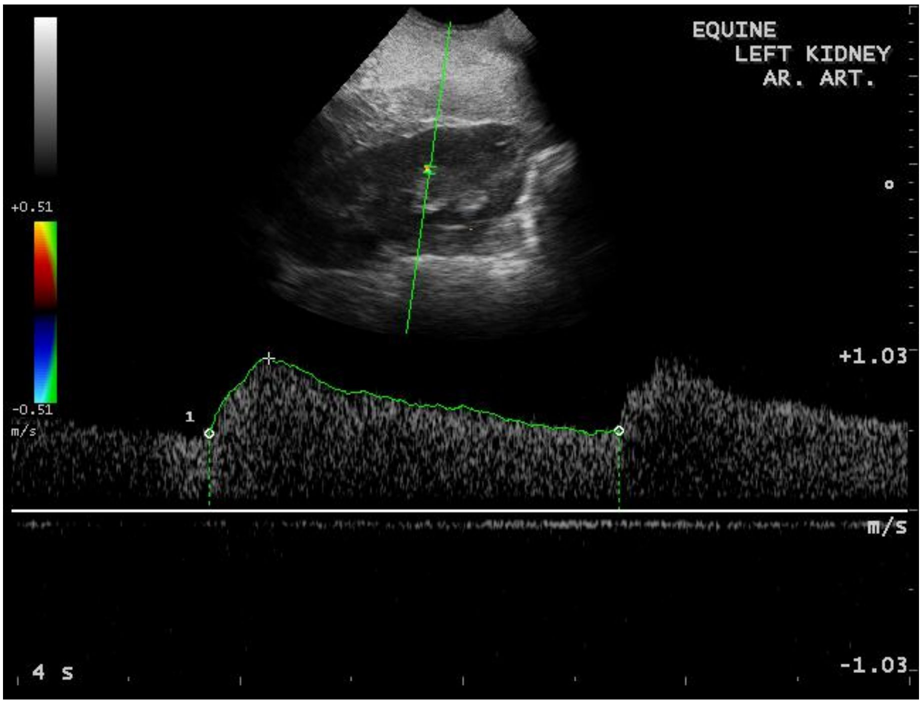

Figura 5 - Mensuração do índice de pulsatividade (IP) no traçado espectral da artéria arqueada de um animal da espécie equina, adulto, saudável. Arquivo pessoal.

Embora possua essa diferença, o IR e o IP são índices que possuem correlação forte, com um coeficiente de correlação maior do que 0,9, logo o aumento de um indica o aumento do outro (GINTHER, 2007; THOMPSON et al., 1988). Por este motivo apenas a mensuração de um deles é suficiente para a avaliação da resistência vascular, embora o IR ser recomendado para fluxos contínuos (artérias renais) e o IP para artérias que apresentam fluxo negativo ou ausente em um dos momentos do ciclo cardíaco (carótidas e aorta) (GINTHER, 2007).

3.3.1. CAVALO DOMÉSTICO

O exame ultrassonográfico abdominal nos equinos é um procedimento simples, rápido e prático de ser executado, podendo manter o animal em estação, não sendo necessário na maioria das vezes o emprego da contenção química (PENNINCK et al., 1986; REEF et al., 1998). Existem duas abordagens disponíveis descritas para a avaliação renal nos equinos: a transabdominal e a transretal, sendo esta última menos realizada e apenas quando é necessário obter mais informações sobre o rim esquerdo (PENNINCK et al., 1986; MATTHEWS; TOAL, 1996).

A abordagem transabdominal, também conhecida como transcutânea é rotineiramente utilizada para a avaliação de ambos os rins nos cavalos domésticos. Previamente ao exame é recomendada a realização da tricotomia da região de interesse, normalmente com lâmina número 40 ou tricótomo, seguida da sua limpeza e aplicação de gel acústico (PENNINCK et al., 1986; REEF et al., 1998). Caso não seja possível realizar a tosa, é recomendado utilizar abundante quantidade de álcool para deste modo obter o maior contato do transdutor com a pele (PENNINCK et al., 1986; MATTHEWS; TOAL, 1996; REEF et al., 1998). Deve-se sempre levar em consideração o tipo do material do transdutor quando for empregar um meio de contato, seja ele gel ou álcool, para que não ocorra danificação do equipamento (REEF et al., 1998).

Em cavalos adultos é recomendado utilizar transdutores de baixa frequência (3.5 - 5 MHz), que permitam a avaliação de regiões com 15 a 20 cm de profundidade (PENNINCK et al., 1986; MATTHEWS; TOAL, 1996; REEF et al., 1998). Na abordagem transcutânea o acesso dos rins é limitado, sendo apenas possível ser realizado pelo aspecto laterodorsal da parede abdominal (PENNINCK et al., 1986). Na maioria dos indivíduos não é possível realizar abordagem dos rins pelo aspecto ventral ou dorsal devido, respectivamente, à sobreposição das alças intestinais e aos processos transversos das vértebras lombares e da musculatura paravertebral (PENNINCK et al., 1986).

Em 2014 Habershon-Butcher e Hallowell demonstraram que é possível avaliar os rins de Puro Sangue Inglês através dos processos transversos das vertebras lombares (abordagem dorsal). Nesse trabalho foram obtidas mensurações renais mais acuradas do que pela abordagem lateral (HABERSHON-BUTCHER; HALLOWELL, 2014). Neste caso é recomendada a utilização de transdutor ultrassonográfico microconvexo de baixa frequência (3.5 MHz), para melhor encaixe entre os processos vertebrais (HABERSHON-BUTCHER; HALLOWELL, 2014). Como citato anteriormente, uma abordagem alternativa de avaliação do rim esquerdo a via transrretal (MATTHEWS; TOAL, 1996). Entretanto, devido a delicadeza da parede do reto é recomendado a utilização dessa abordagem a apenas operadores experientes, utilizando sonda linear de média frequência (5 - 7.5 MHz), movimentos gentis e a abundante quantidade de gel lubrificante (MATTHEWS; TOAL, 1996). Como limitação dessa abordagem temos a experiência de palpação do operador e também o comprimento do seu braço, sendo que caso este seja curto, talvez não seja possível avaliar o rim esquerdo em equinos de maior porte (MATTHEWS; TOAL, 1996). Uma possível e severa complicação dessa abordagem é laceração ou ruptura do reto, caso o operador não seja cuidadoso ou ocorra movimentos abruptos por parte do animal (MATTHEWS; TOAL, 1996).

Independente da via utilizada a avaliação ultrassonográfica permite a avaliação das características morfológicas e vasculares do parênquima renal. O rim direito localiza-se diretamente abaixo da parede abdominal, do 14º ao 17º espaço intercostal direito, ao nível ou ligeiramente acima da linha traçada do ílio ao ombro (Figura 8 e 9) (PENNINCK et al., 1986; REEF et al., 1998; DRAPER et al., 2012). O rim esquerdo apresenta-se do 16º espaço intercostal esquerdo até o vazio do flanco, ao nível da linha ílio-ombro, e íntimo contato com o baço, que se localiza superficial ao polo renal cranial (Figura 8 e 9) (PENNINCK et al., 1986; REEF et al., 1998; DRAPER et al., 2012).

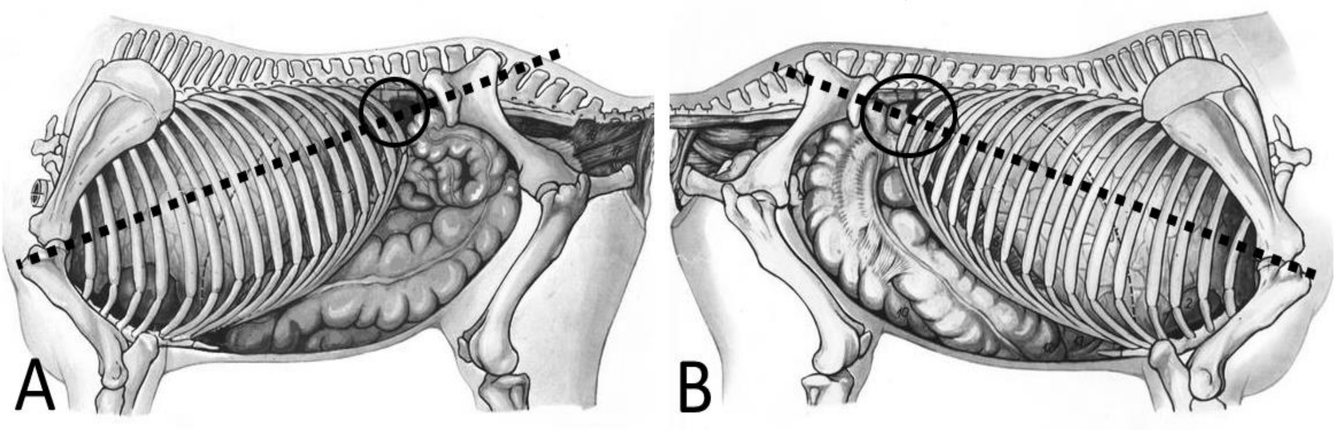

Figura 6 – Topografia do rim esquerdo (A) e direito (B) no abdômen equino demarcada pelo círculo preto. Linha tracejada conectando a tuberosidade ilíaca a articulação escapulo-umeral. Adaptado de POPESKO, 2011.

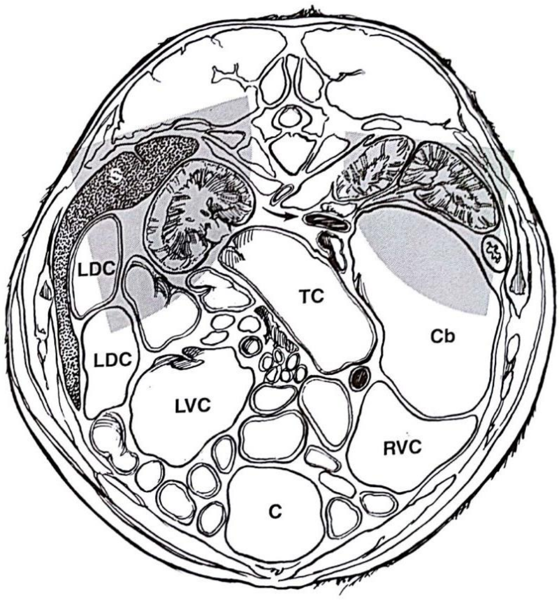

Figura 7 – Corte seccional do abdômen equino ilustrando a abordagem transbdominal na avaliação dos rins, ao nível da 17ª vertebra torácica. Áreas sombreadas representam os planos de escaneamento para examinar os rins. A seta aponta o duodeno. S = baço; Cb = base do ceco; C = corpo do ceco; RVC = cólon ventral direito; TC = cólon transverso; LDC = cólon dorsal esquerdo; LVC = cólon ventral esquerdo. Adaptado de SERTICH; TURNER, 1998.

O rim direito apresenta uma posição mais superficial e dorsal quando comparado com o esquerdo, com uma distância de 2,4 a 6,2 cm do plano cutâneo e entre 2 cm dorsal a 12 cm ventral da tuberosidade coxal (Figura 9) (DRAPER et al., 2012; SLOVIS, 2014). Por sua vez o rim esquerdo encontra-se entre 6,4 a 14,6 cm da pele e entre 2 cm dorsal a 15 cm ventral da tuberosidade (DRAPER et al., 2012; SLOVIS, 2014).

A avaliação renal pode sofrer interferência do trato intestinal, devido a movimentação do duodeno, ceco, alças de intestino delgado, cólon dorsal direito ou cólon menor (Figura 9) (MATTHEWS; TOAL, 1996; PENNINCK et al., 1986; REEF et al., 1998). Nos casos de interferência o ideal seria tentar avaliar o parênquima renal de outra forma, entretanto a limitação da abordagem impossibilita essa mudança, logo o recomendado é realizar posterior repetição do exame (MATTHEWS; TOAL, 1996; PENNINCK et al., 1986; REEF et al., 1998).

Nos equinos, devido as grandes dimensões dos rins, é considerado impossível mensurar de modo exato esses órgãos, embora seja possível estimar o comprimento (10 – 16 cm), largura (5 – 11 cm) e espessura/altura (8

– 9 cm) (DRAPER et al., 2012; MATTHEWS; TOAL, 1996; PENNINCK et al., 1986; SLOVIS et al., 2014). Draper e colaboradores (2012) observaram a presença de diferença das dimensões renais de acordo com a localização ou corte ultrassonográfico utilizado. Habershon-Butcher e Hallowell (2014) mostram que a abordagem dorsal permite uma avaliação mais acurada das dimensões renais.

A avaliação da arquitetura renal no modo bidimensional sofre influência do estado corporal, hidratação e, como mencionado anteriormente, do trato intestinal. Animais com sobrepeso, obeso, desidratado ou com excesso de gás nas alças intestinais adjacentes podem dificultar ou comprometer a avaliação ultrassonográfica (PENNINCK et al., 1986; MATTHEWS; TOAL, 1996). De modo geral a arquitetura renal é bem definida, diferenciando a cápsula, córtex, medula, gordura perirenal e vasos intrarenais (interlobares e arqueados) (PENNINCK et al., 1986; MATTHEWS; TOAL, 1996). A cápsula renal apresenta-se como uma estrutura fina iso a hiperecogênica envolvendo a córtex renal (PENNINCK et al., 1986; MATTHEWS; TOAL, 1996; REEF et al., 1998).

Em ambos os rins é possível definir a junção corticomedular, sendo esta mais evidente no rim direito que o esquerdo devido à diferença de profundidade e, portanto, atenuação do feixe sonoro, embora o rim equino possua uma junção menos evidente do que cães e gatos (PENNINCK et al., 1986; MATTHEWS; TOAL, 1996; SERTICH; TURNER, 1998; KÖNIG et al., 2004; TORIBIO, 2007; DRAPER et al., 2012; SCHOTT II; WOODIE, 2012). A córtex renal apresenta-se como uma região homogênea de 1 a 2 cm de espessura, sendo hipoecogênica quando comparada aos tecidos adjacentes como o parênquima hepático e esplênico (Figura 10) (PENNINCK et al., 1986; REEF et al., 1998; SERTICH; TURNER, 1998; SLOVIS et al., 2014). Em animais idosos a córtex pode ser iso ou até ligeiramente hiperecogênica ao fígado (REEF et al., 1998).

A medula renal possui um aspecto irregular, hipo a isoecogênica a córtex renal (Figura 10) (PENNINCK et al., 1986; REEF et al., 1998). Em alguns casos é possível ser observado aumento da ecogenicidade da córtex e medula na região de polos renais devido a formação do artefato de anisotropia (SERTICH; TURNER, 1998; SLOVIS et al., 2014).

Os recessos pélvicos apresentam-se como áreas circunscritas hipoecogênicas e a pelve renal como uma banda ou linha hiperecogênica na região central da medular renal (Figura 9) (PENNINCK et al., 1986; REEF et al., 1998). Esta região é considerada a região mais ecogênica do rim, sendo assim devido a gordura e tecido conjuntivo regional (PENNINCK et al., 1986; MATTHEWS; TOAL, 1996; REEF et al., 1998; SERTICH; TURNER, 1998).

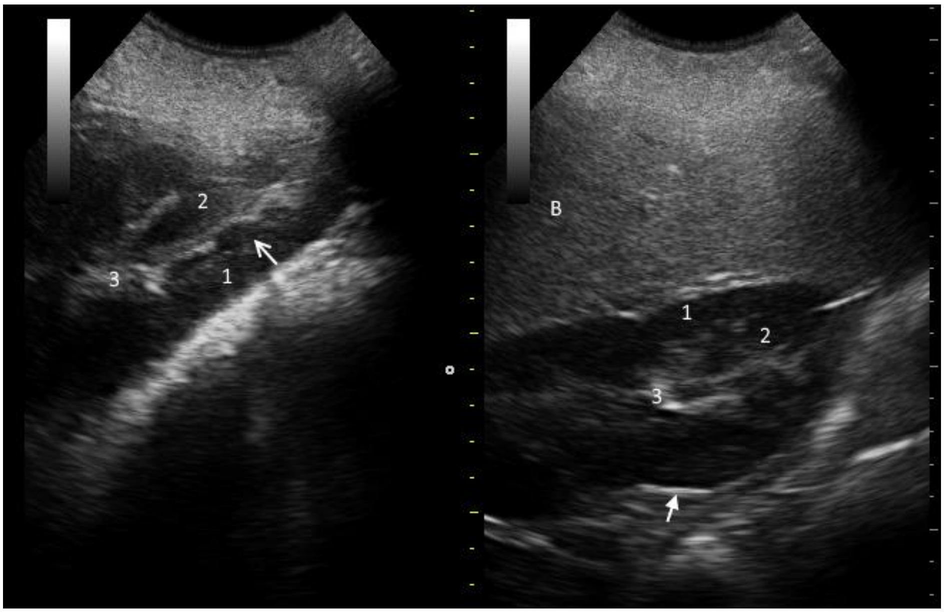

Figura 8 – Imagem ultrassonográfica bidimensional do rim direito (à esquerda) e rim esquerdo (à direita). À esquerda pode-se observar a córtex (1), medular (2), pelve renal (3) e junção corticomedular (seta aberta) do rim direito. À direita pode-se observar o baço (B) superficial ao rim, córtex (1), medula (2), pelve (3) e a cápsula renal (seta fechada) do rim esquerdo. Arquivo pessoal.

Os vasos interlobares e arqueados apresentam-se como estruturas tubulares anecogênicas, de pequeno calibre (menor que 3 mm), com bordas ecogênicas por permeio da medula renal, apresentando fluxo sanguíneo ao exame de Doppler colorido ou de amplitude (PENNINCK et al., 1986; REEF et al., 1998; SERTICH; TURNER, 1998). Já a artéria e a veia renal são normalmente visibilizados na região de hilo renal, com uma artéria ou vários ramos da artéria renal adjacentes a uma única veia renal (REEF et al., 1998; SERTICH; TURNER, 1998). A veia renal encontra-se cranial a porção proximal do ureter (SERTICH; TURNER, 1998).

Em cavalos domésticos a avaliação dopplerfluxométrica pelo Doppler pulsado é mais fácil de ser realizada no rim direito do que no esquerdo (MATTHEWS; TOAL, 1996). Atualmente existem poucos artigos quantificando o pVS, VDf, IR e IP, sendo que esses poucos produzem resultados diferentes entre si. HOFFMAN e colaboradores (1997) avaliaram as artérias arqueadas de 5 cavalos sedados, obtendo-se um pVS de 0,406 ± 0,116 m/s, VDf de 0,184 ± 0,057 m/s e IR de 0,549 ± 0,44. No mesmo trabalho HOFFMAN e colaboradores (1997) avaliaram as artérias pielorenais de outros 7 cavalos não sedados, obtendo-se um pVS de 1,047 ± 0,009 m/s, VDf de 0,549 ± 0,006 m/s e IR de 0,512 ± 0,004. Em 2015 MACRI e colaboradores avaliaram as artérias interlobares e arqueadas de 13 cavalos da raça Puro Sangue Inglês não sedados, obtendo-se valores de IR que diferenciavam entre os rins de machos não castrados, fêmeas, treinados ou não, variando de 0,48 a 0,58.

3.4. ULTRASSONOGRAFIA QUANTITATIVA – HISTOGRAMA EM ESCALA DE CINZA

A ultrassonografia modo bidimensional (B) permite a avaliação morfológica não invasiva dos tecidos e conteúdos através da criação de uma imagem bidimensional em tons de cinza (MATOON; NYLAND, 2015; PENNINCK; D'ANJOU, 2015). Neste modo, cada tom de cinza na imagem é proporcional à intensidade dos ecos gerados pela interação onda ultrassonográfica e tecido, e depende fortemente de certas características acústicas teciduais, como a impedância acústica (MATOON; NYLAND, 2015; PENNINCK; D'ANJOU, 2015). Devido à diferença desta característica entre os tecidos, a interação onda-tecido possui grande variação, resultando numa imagem com diferentes níveis de ecogenicidade (MATOON; NYLAND, 2015; PENNINCK; D'ANJOU, 2015).

Embora permita detecção de alterações morfológicas, a caracterização da ecogenicidade e ecotextura tecidual pelo modo B acaba por ser considerada subjetiva, uma vez que depende da percepção visual operador e da integridade dos órgãos ou tecidos a serem comparados (LEE et al., 2006; IVANCIC; MAI, 2008). Devido a esta subjetividade, sutis variações de ecogenicidade ou de ecotextura podem passar despercebidas, tornando necessária a utilização de técnicas complementares.

O histograma em escala de cinza (HEC) é um método acessível, prático e eficiente para a avaliação quantitativa da imagem ultrassonográfica bidimensional e deste modo uma caracterização objetiva da ecogenicidade e da ecotextura tecidual (LEE et al., 2006; IVANCIC; MAI, 2008; MAEDA et al., 2013). O HEC consiste na mensuração dos tons de cinza de uma região de interesse (ROI), fornecendo sua distribuição e informações quantitativas (Figura 11) (LEE et al., 2006; IVANCIC; MAI, 2008; MAEDA et al., 2013). As imagens ultrassonográficas são formadas por pixels que são representados numericamente em escalas de tons de cinza de acordo com a intensidade de brilho (LEE et al., 2006; IVANCIC; MAI, 2008). A escala de cinza varia de acordo com o equipamento ou software utilizado, porem normalmente é utilizado uma escala de 255 tons de cinza, sendo 0 equivalente ao preto e 255 o branco.

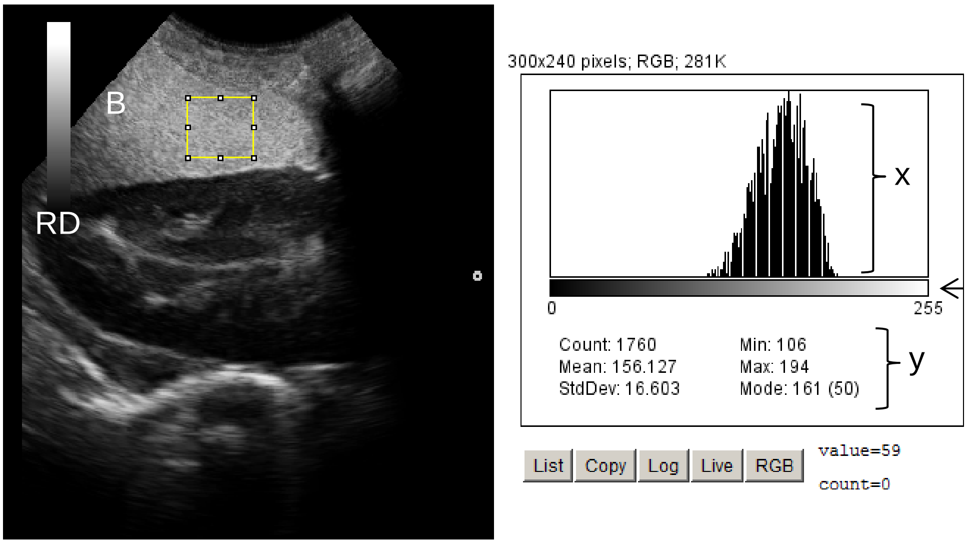

Figura 9 – Histograma em escalas de cinza (HEC) do parênquima esplênico de um equino. À esquerda imagem ultrassonográfica bidimensional mostrando o baço (B) e o rim esquerdo (RE), com a região de interesse (ROI) delimitada pelo retângulo. À direita o HEC em forma de gráfico (x) e seus valores descritivos (y). Escala de tons de cinza utilizada demonstrada pela seta aberta. Arquivo pessoal.

Na medicina humana, o HEC é utilizado com frequência em diferentes campos, principalmente nas áreas da obstetrícia e ginecologia, auxiliando na avaliação da maturidade fetal e detecção de patologias (HERSHKOVITZ et al., 2011; MAEDA et al., 2013). Já na medicina veterinária essa ferramenta de avaliação quantitativa ainda encontra-se restrita ao meio acadêmico, sendo estudos realizados caracterizando de modo objetivo a ecogenicidade e ecotextura tecidual dos órgãos de cães saudáveis e doentes (IVANCIC; MAI, 2008; LAM et al., 2014).

4. REFERÊNCIAS

BURDEN, F.; THIEMANN, A. Donkeys are different. *J Eq Vet Science*, v. 35, n. 5, p. 376-382, 2015.

BURNHAN, S.L. Anatomical differences of the donkey and mule. *AAEP PROCEEDINGS,* Orlando, n. 48, p. 102-109, 2002.

CARVALHO, C.F. *Ultrassonografia Doppler em pequenos animais*. 1ª edição. São Paulo: Roca Ltda, 2009.

DRAPER, A.C.E.; BOWEN, I.M.; HALLOWELL, G.D. Reference ranges and reliability of transabdominal ultrasonographic renal dimensions in throughbred horses. *Vet Radiol Ultrasound*, v. 5, n.3, p. 336-341, 2012.

ELLENBERG, G.B. *Mule south to tractor south: mules, machines and the transformation of the cotton south*. Tuscaloosa: University of Alabama Press, 2007.

GEOR, R.J. Acute renal failure in horses. *Vet Clinics of North America: Equine Practice*, v. 23, n.3, p. 577-579, 2007.

GINTHER, O.J. *Ultrasonic imaging and animal reproduction: Color-Doppler ultrasonography.* 1st edition. [local desconhecido]:Cross Plains Equiservices, 2007.

HABERSHON-BUTCHER, J.; BOWEN, M.; HALLOWEL, G. Validation of a novel translumbar ultrasound technique for measuring renal dimensions in horses. *Vet Radiol Ultrasound*, v. 55, n. 3, p. 323-330, 2014.

HERSHKOVITZ, R.; AMICHAY, K.; STEIN, G.Y.; TEPPER, R. The echogenicity of the normal fetal kidneys during different stages of pregnancy determined objectively. *Arch Ginecol Obstet*. v. 284, n. 4, p. 807-811, 2011.

HOFFMANN, K.L.; WOOD, A.K.; KIRBY, A.C. Use of Doppler ultrasonography to evaluate renal arterial blood flow in horses. *Am J Vet Res*, v.58, n.7, p.697-701, 1997

IVANCIĆ, M.; MAI, W. Qualitative and quantitative comparison of renal vs. Hepatic ultrasonographic intensity in healthy dogs. *Vet Radiol Ultrasound*, v. 49, n. 4, p. 368-373, 2008.

IZRAELY, H.; CHOSHNIAK, I.; STEVENS, C.E.; DEMMENT, M.W.; SHKOLNIK, A. Factors determining the digestive efficiency of the domestic donkey (*Equus asinus asinus*). *Am J Physiol*, v. 74, p. 1-6, 1989.

KASIRER-IZRAELI, H.; SHKOLNIK, A.; CHOSHNIAK, I. Kidney response to rapid rehydration in the donkey. *Isr J Zool*, v.47, n.1, p. 29-39, 2001.

KÖNIG, H.E.; MAIERL, J.; LIEBICH, H.G. Chapter 9: urinary system (organa urinaria). In: KÖNIG, H.E; LIEBICH, H.G. (Org.) *Veterinary anatomy of domestic mammals: textbook and colour atlas*. 1st edition. Germany: SchattauerGmbh, 2004. p. 365-380.

LAM, R.; NIESSEN, S.J.; LAMB, C.R. X-ray attenuation of the liver and kidney in cats considered at varying risk of hepatic lipidosis. *Vet Radiol Ultrasound*, v. 55, p. 141-146, 2014.

LEE, C.H.; CHOI, J.W.; KIM, K.A.; SEO, T.S.; LEE, J.M.; PARK, C.M. Usefulness of standard deviation on the histogram of ultrasound as a quantitative value for hepatic parenchymal echo texture: preliminary study. *Ultrasound Med Biol*, v. 32, n. 12, p. 1817-1826, 2006.

MACRI, F.; PUGLIESE, M.; PIETRO, S.D.; COCO, L.L.; NIUTTA, P.P.; NARDI, S.; QUARTUCCIO, M.; LANTERI, G.; PICCIONELLO, A.P. Doppler ultrasonographic estimation of renal resistive index in horse: comparison between left and right kidneys. *J Equine Vet Science*, v. 35, n. 2, p. 111-115, 2015.

MAEDA, K.; UTSU, M.; YAMAMOTO, N.; SERIZAWA, M.; ITO, T. Chapter 4: ultrasonic tissue characteriation with gray level histogram width. In: KURJAK, A.; ARENAS, J.B. (Ed.). *Donald school textbook of transvaginal sonograph*. 2nd edition. New Delhi: Jaypee Brothers Medical Publisher Ltd, 2013. p. 17-20.

MALOIY, G.M.O. Water economy of the Somali donkey. *Am J Physiol*. v. 219, n. 5, p.1522-1527, 1970.

MARTIN, A.; MACDONALD, J.; MOORE, J. Renal failure and its treatment. *Anest Intensive Care Med*, v. 16, n. 6, p. 267-274, 2015.

MATOON, J.S.; NYLAND, T.G. *Small animal diagnostic ultrasound*. 3th edition. Philadelphia: Elsevier Saunders, 2015.

MATTHEWS, H.K.; TOAL, R.L. A review of equine renal imaging techniques. *Vet Radiol Ultrasound*, v. 37, n. 3, p. 163-173, 1996.

MATTHEWS, N.S,; TAYLOR, T.S.; HARTSFIELD, S.M. Anaesthesia of donkeys and mules. *Equine Vet Educ*, v. 15, p. 102-107, 2005.

MATTHEWS, N.; VAN LOON, J.P.A.M. Anaesthesia and analgesia of the donkey and the mule. *Equine Vet Educ*, v. 25, n. 1, p. 47-51, 2013.

MCLELAND, S. Diseases of the equine urinary system. *Vet Clin Equine*, v. 31, p. 377-387, 2015.

NEWMAN, S.J.; CONFER, A.W.; PANCIERA, R.J. Sistema urinário. In: MCGAVIN, M.D.; ZACHARY, J.F. (Org). *Bases da patologia em veterinária*. 4ª edição. Rio de Janeiro: Elsevier, 2009. p. 613-692.

ORSINI, J.A.; DIVERS, T.J. *Equine emergencies: treatment and procedures.* 4th edition. St Louis: Elsevier Mosby, 2014.

PENNINCK, D.G.; EISENBERG, H.M.; TEUSCHER, E.E.; VRINS, A. Equine renal ultrasonography: normal and abnormal. *Vet Rad Ultrasound*, v. 27, n. 3, p. 81-84, 1986.

PENNINCK, D.; D'ANJOU, M.C. *Atlas of small animal ultrasonography*. 2nd edition. Iowa: Wiley Blackwell, 2015.

PETERSEN, L.J.; PETERSEN, J.R.; TALLERUPHUUS, U.; LADEFOGED, S.D.; MEHLSEN, J.; JENSEN, H.A. The pulsatility index and the resistive index in renal arteries: associations with long-term progression in chronic renal failure. *Nephrol Dial Transplant*, v.12, n. 7, p. 1376-1380, 1997.

PLATT, J.F. Doppler ultrasound of the kidney. *Seminars in Ultrasound, CT and MRI*, v. 18, n. 1, p. 22-32, 1997.

POPESKO, P. *Atlas de Anatomia Topográfica dos Animais Domésticos*. 5ª edição. [local desconhecido]: Manole, 2011.

PROOPS, L.; BURDEN, F.; OSTHAUS, B. Mule cognition: a case of hybrid vigour? *Anim Cogn*, v. 12, n. 1, p. 75-84, 2009.

PROOPS, L.; BURDEN, F.; OSTHAUS, B. Social relations in a mixed group of mules, ponies and donkeys reflect differences in equid type. *Behavioral Process*, v. 90, n. 3, p. 337-342, 2012.

REED, S.M.; BAYLY, W.M.; SELLON, D.C. *Equine Internal Medicine*. 3th edition. St Louis: Saunders Elsevier, 2010.

REEF, V.B. *Equine diagnostic ultrasound*. 1stedition. Philadenphia: Saunders, 1998.

SAVAGE, C.J. Urinary clinical pathologic findings and glomerular filtration rate in the horse. *Vet Clin Equine*, v. 24, p. 387-404, 2008.

SCHOTT II, H.C.; WOODIE, J.B. Chapter 64: kidneys and ureters. In: AUER, J.A.; STICK, J.A. (Ed.). *Equine surgery*. 4th edition. St Louis: Elsevier Saunders; 2012.

SERTICH, P.L.; TURNER, R.M. *Equine diagnostic ultrasound*. 1st edition. Philadelphia: W.B. Saunders Compay, 1998.

SLOVIS, N. Chapter 23: ultrasonography of the liver, spleen, kidney, bladder, and peritoneal Cavity. In: KIDD, J.; LU, K.G.; FRAZER, M.L. (Ed.). *Atlas of equine ultrasonography*. West Sussex: John Wiley & Sons, 2014. p. 409–27.

SMITH, D.C. *The book of mules: selecting, breeding and caring for equine hybrids*. Conecticut: The Lyons Press, 2009.

SMITH, D.G.; BURDEN, F.A. Chapter 16: pratical donkey and mule nutrition. In: GEOR, R.J.; HARRIS, P.A.; COENEN, M. (Ed). Equine applied and clinical nutrition: importance of nutrition for health, welfare and performance. London: Elsevier Saunders; 2013. p. 304-318.

TAYLOR, K.J.; HOLLAND, S. DOPPLER US Part I. Basic principles, instrumentation and pitfalls. *Radiology,* v. 174, n. 2, p. 297-307, 1990.

The Donkey Sanctuary [Internet]. 2017. Disponível em: <https://www.thedonkeysanctuary.org.uk>. Acesso em: 07 jul. 2017.

THOMPSON, R.S.; TRUDINGER, B.J.; COOK, C.M. Doppler ultrasound waveform indices: A/B ratio, pulsatility index and Pourcelot ratio. *Br J Obstet Gynaecol*, v. 95, n. 5, p. 581-588, 1988.

TORIBIO, R.E. Essentials of equine renal and urinary tract physiology. *Vet Clin North Am Equine Pract*. v. 23, n. 3, p. 533-561, 2007.

TUBLIN, M.E.; BUDE, R.O.; PLATT, J.F. The resistive index in renal Doppler sonography: where do we stand? *Am J Roetgen*, v. 180, n. 4, p. 885-892, 2003.

VAN METRE, D.C.; SOTO, D.R.D. Chapter 34: diseases of the renal system. In: SMITH, B.P (Ed.). *Large animal internal medicine*. 5th edition. St. Louis: Elsevier Mosby, 2015. p. 873-895.

VERLANDER, J.W. Section VII renal physiology. In: KLEIN, B.G. (Ed.). *Cunningham's Textbook of Veterinary Physiology*. 5th edition. Missouri: Elsevier Saunders, 2013. p. 460-494.

WILSON, M.E. Examination of the urinary tract in the horse. *Vet Clinics of North America*, v. 23, n. 3, p. 563-575, 2007.

CAPÍTULO 2

Trabalho Científico

Dopplerfluxometria das Artérias Renais de Muares Saudáveis Não Sedados: Comparação com Cavalos Domésticos

Renal Dopplerfluxometry of Health Non-Sedated Mules: Comparison with Horses

Trabalho a ser enviado para a revista:
Veterinary Radiology & Ultrasound

Original investigation – Renal Dopplerfluxometry of Health Non-Sedated Mules: Comparison with Horses

Maria Cristina Reis Castiglioni[1]; Jeana Pereira da Silva[1], Roberta Martins Basso[1], José Nicolau Próspero Puoli Filho[1], Vânia Maria de Vasconcelos Machado[1]

[1]São Paulo State University (Unesp), School of Veterinary Medicine and Animal Science, Botucatu, Brazil

Correspondence: Maria Cristina Reis Castiglioni - Departamento de Reprodução Animal e Radiologia Veterinária, Faculdade de Medicina Veterinária e Zootecnia, Rua Prof. Dr. Walter Maurício Correa s/nº, 18.618-681, Botucatu, SP, Brasil.

Email: maria.castiglioni@hotmail.com

Instruções para os autores:
http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1740-8261/homepage/ForAuthors.html#div_id28

Abstract

Doppler ultrasonography is essential to assess renal hemodynamic condition in humans and small animals, and the resistive index (RI) is considerate an important indicator of renal health. Currently there are few studies about diagnostic images' of mules. This study proposes to establish normal renal Doppler fluxometric parameters in mules comparing the results with horses. Eleven non-sedated health mules and horses were submitted to ultrasonographic examination of the kidneys. Using color and spectral Doppler modes the extra- and intra-parenchymal renal vascularization was evaluated. Mules presented less renal vascularization on color Doppler mode than horses, with less definition of the intra-parenchymal arteries and definition of only one renal artery, different than horses with presented at least three extra-parenchymal arteries. On spectral Doppler mode the attainment of the spectral waves was more laborious in mules than horses. It was obtained a RI of 0.45 ± 0.04 and pulsatile index (PI) of 0.65 ± 0.08 in mules and RI: 0.54 ± 0.04 and PI: 0.84 ± 0.12 in horses. There wasn't difference of these variables between kidneys or arteries but only when mules were compared to horses.

KEYWORDS:
renal resistive index; equine; trans abdominal ultrasonography; kidney

1. INTRODUCTION

Derived from the breeding of a mare with a donkey, the mule is a hybrid with intermediary characteristics, that increase its resistance to survive in extreme environments, with lower water intake and diets of poor nutritional quality.[1-3] The mules were historically important working and transportation animals and are still important in agribusiness of subdeveloped countries.[3-5]

In resting conditions the kidneys receive 15% to 20% of the cardiac output, yet only 20% of this flow irrigates the renal medulla, increasing the chances of hypoxic lesions.[6-10] Early detection of renal damage increases the chances of reversion and improves the prognosis, however acute renal injuries are difficult to diagnose due to its nonspecific clinical signals.[11, 12] In small animals the renal ultrasonographic Doppler examination became a standard procedure that allows renal blood flow analysis and thus provides functional information and assist detection of acute injuries.[13, 14]

Currently there are few studies in the *Equus* genus that are not related with domestic horses and even less about renal Doppler examination. Recently the resistive index (RI) was determinated in horses, yet there aren't studies providing concrete information about this index in mules.[15] The present study proposes to define renal Doppler fluxometric parameters in mules using trans-abdominal ultrasonography, comparing the findings with horses.

2. METHODS

2.1. Sample population

Eleven adult mules and eleven crossbred adult horses of similar age and weight that did not possess history of renal disease, renal macroscopic lesions by ultrasound and were considered health by clinical examination, total blood count, biochemistry and urinalysis were selected. All animals were from the experimental farm of this faculty. The animals were contained only by physical means and no alimentary or hydric fasting was instituted. This study is in accord with the ethical principles adopted by the National Council of Animal Control and Experimentation (CONCEA) and by the Ethical Commission in Animal Use (CEUA) of this faculty.

2.2. Ultrasound examination

The ultrasonographic examinations were performed by the author (M.C.R.C) and one of the coauthors (J.P.S) in an open, covered and well ventilated environment, using mobile ultrasound equipment Mylab 30 (Esaote, Genova, Italy) with 3-9 MHz convex transducer. Each kidney was initially located with the assist of ultrasound equipment and 70% isopropyl alcohol to promote contact surface. The region of interest was clipped and the skin was soaked with ample quantity of acoustic gel. Transverse and longitudinal images of the kidneys were used to obtain the ultrasonography images in color and spectral Doppler mode.

The renal vascularization was initially evaluated on color Doppler mode, observing the extra- and intra-parenchymal blood flow definition and intensity. Once the arteries were defined, spectral imaging of each type of artery was obtained with at least two complete trios of spectral waves. From spectral Doppler images the resistive index (RI) and pulsatile index (PI) were obtained. Due the high velocity of the renal blood flow the ultrasonographic examination was performed in the equipment cardiac mode with pulsed repetition frequency (PRF) and Doppler gain of 1.2-8.3 kHz and 55%-64% in color Doppler mode and 5.6-11.1 kHz and 76%-100% in spectral Doppler mode, repectivily Arterial blood pressure was measured during the ultrasound exam using non-invasive Doppler in the ventral coccygeal artery.[16, 17] Only spectral Doppler images obtained with arterial blood pressure within the equine normal range were used to minimize cardiovascular influence over the RI and PI.[16]

2.3. Data analysis

Statistical analyses were performed using commercial software for Windows (GraphPad Prism® ver 7.03, GraphPad Software, USA). Statistical significance was set at $P < 0.05$. The variables were initially submitted to non-pared test T to confirm absence of difference between sonographers. The dopplefluxometric variables were submitted to the Shapiro-Wilk normality test and descriptive analyses, obtaining the mean, median, standard deviation, standard error, minimum, maximum and coefficient of variance. Kruskal-Waalis test, ANOVA and Dunn's multiple comparisons test were used to compare RI and PI between the animals (mule and horse), kidneys (right and left) and arteries (renal, interlobar and arcuate). For correlation analyses between RI and PI Spearman and Pearson tests were used, followed by linear regression analyses.

3. RESULTS

3.1. Doppler imaging

Extra- and intra-parenchymal blood flow was observed in all horses (11/11), with a well-defined visualization of the flow in color Doppler mode (Fig. 12) and distinct spectral wave formation in spectral Doppler mode. In two cases it was necessary to repeat the ultrasonographic examination due to inadequate color and spectral Doppler signal captation, with an interval of days between exams. In all horses more than one renal artery with at least one entering the kidney at the renal cranial pole, hilar region and caudal pole ere observed (Fig. 12). The right kidney exhibited higher quality of Doppler images than the contralateral.

In mules, only in one individual (1/11) it was not possible to observe renal blood flow even with PRF and Doppler gain adjustment and posterior reexamination. On another three (3/10) it was necessary to repeat the examination at least two times in a period of 2 to 24 hours to obtain adequate Doppler images. The color and spectral Doppler images were more difficult to obtain in mules, with lesser definition of the flow (Fig. 12) and spectral waves. This finding was more evident in the left kidney and in some animals the renal artery blood flow (2/10), intra-parenchymal blood flow (1/10) or both (1/10) could not be detected. In all kidneys that allowed extra-parenchymal blood flow characterization showed the renal artery entering at the hilar region (Fig. 12).

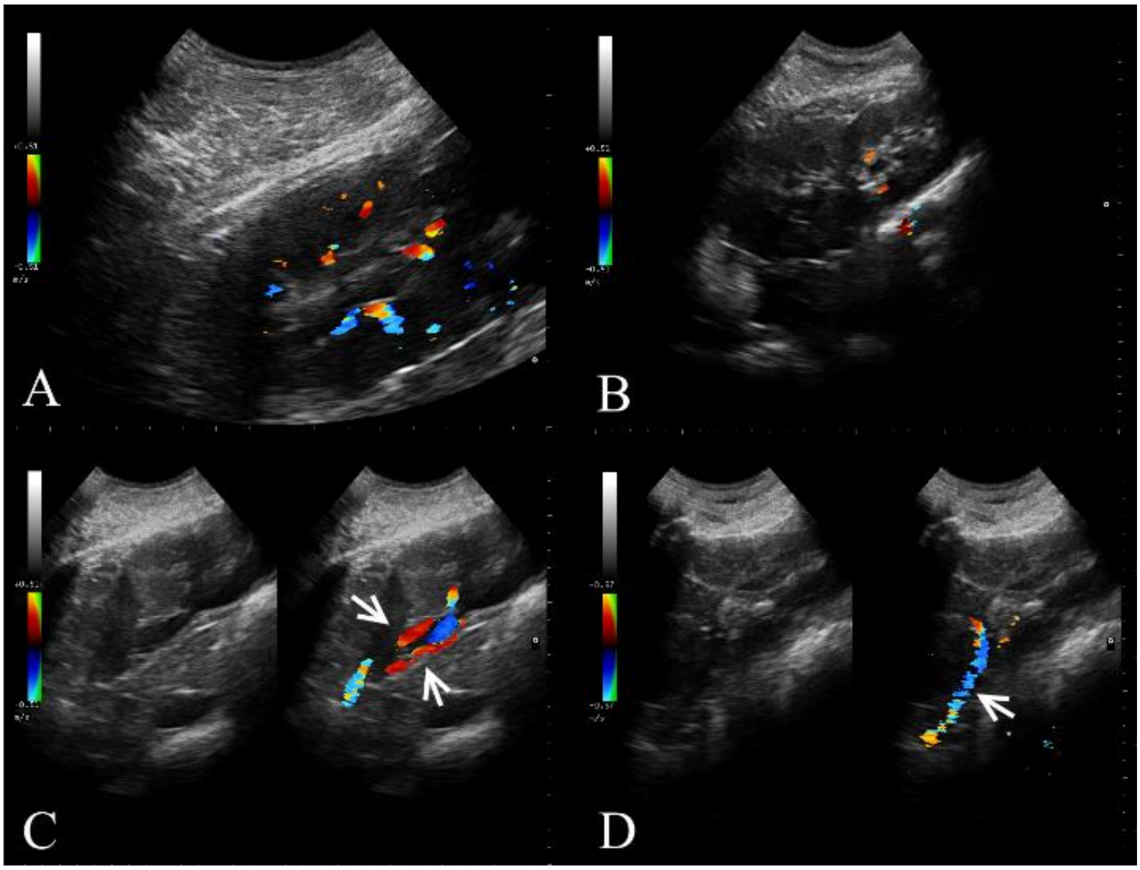

Fig. 10. Color Doppler images of renal vascularization in horse (A, C) and mule (B, D): higher quality of Doppler signal captation within renal parenchyma in horse (A) when comparing to mule (B); visualization of more than one renal artery (arrows) in horse (C); and of only one renal artery (arrow) in mule (D).

3.2. Arterial Spectral Wave

All spectral waves showed parabolic flow velocity profile of low resistance flow (Fig. 13). This aspect is observed in all renal, interlobar and arcuate arteries. Spectral enlargement, small spectral window and in some waves a small early systolic peak were observed (Fig. 13).

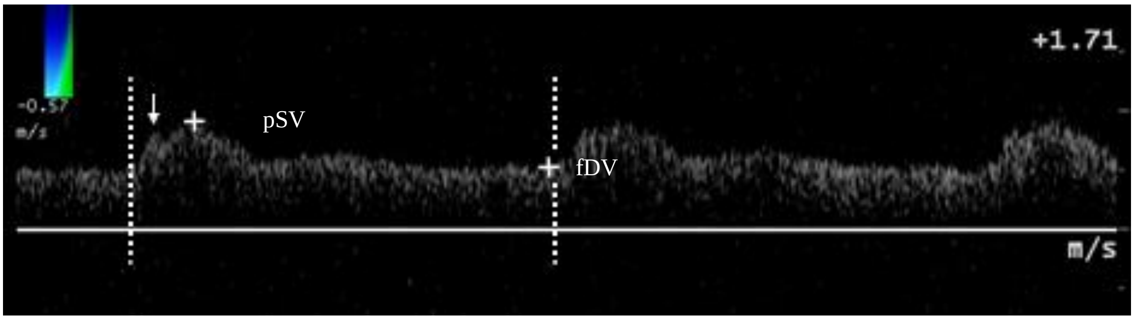

Fig. 11. Spectral wave of renal artery of mule: ample systolic peak, with gradual decrease of velocity during the diastolic phase, indicating low resistance flow of parabolic profile. Systolic-diastolic cycle delimitated by the dashed lines. Early systolic peak (ESP) represented by the arrow. pVS: peak of sistolic velocity, fDV: final diastolic velocity.

3.3. RI AND PI

A total of 639 RI and 305 PI were obtained in mules and 759 RI and 381 PI in horses. There was a significant difference of RI and PI between the mules and horses kidneys (P Value < 0.05), not observed between kidneys (P value > 0.05) or measurement sites (P value > 0.05). The descriptive analyses of these variables are found in Table 1.

Table 1. Descriptive analyses of the renal RI and PI obtained in 11 mules and 11 horses

	Mule		Horse	
	RI	**PI**	**RI**	**PI**
N	639	305	759	381
Mean	0.45	0.65	0.54	0.84
SD	0.04	0.08	0.04	0.12
Median	0.45	0.65	0.54	0.83
SE	0.001	0.005	0.001	0.006
CV	9.81	13.48	8.78	13,90
Minimun	0.30	0.39	0.43	0.58
Maximun	0.59	0.89	0.66	1.30

Abbreviations: RI, resistive index; PI, pulsatile index; N, number of variables analyzed; SD, standard deviation; SE, standard error.; CV, coefficient of variation.

Strong correlation between RI and PI was observed in mules (r value of 0.95; P value < 0.05) and moderate in horses (r value of 0.73, P value < 0.05), with the linear regression analyses confirming these findings (R^2: 0,91 in mules and R^2: 0,54 in horses) allowing the following regression equation: $IP = IR\ x\ 1{,}81 - 0{,}17$ for mules, and $IP = IR\ x\ 1{,}60 - 0{,}20$ for horses (Fig. 14).

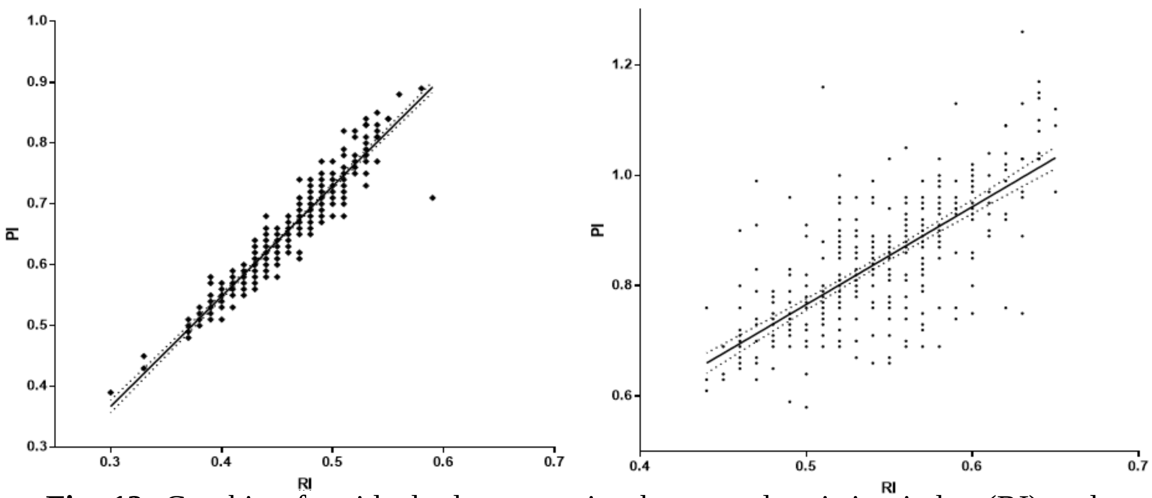

Fig. 12. Graphic of residuals demonstrating how renal resistive index (RI) and renal pulsatile index (PI) correlate in 11 mules (left) and 11 horses (right). Presence of regression line and 95% confidence interval in both graphics. Spearman Correlation.

4. DISCUSSION

Few studies are performed in mules, however even with the extensive literature on horses and the growing on donkeys it’s still not advisable to use their parameters as standard for mules’ evaluation. The mules receive characteristics from species which are physiologically and physically different.[1, 4, 18, 19] Given this the mules can be similar or have intermediary characteristics.

On color Doppler mode the mule kidneys displayed vascularization less apparent than the horses, with obvious visualization of only one the renal artery at hilar region (Figure 1). The members of the *Equus* genus have more than one renal artery, with similar number between equine species, so even with the detection of only one renal artery in this study, it does not excludes the existence of more in mules.[6, 8, 20] Perhaps this difference is correlated with anatomical variations between mules and horses.

Comparing donkeys and mules with horses, it’s noticed a different saddle and croup conformation due a more inclined pelvis.[2, 4, 18, 19] This difference, even small, could result in changes on the organs position, that even small, could chance the Doppler angulation and affect signal captation.[21-25] The same could be inferred about the difference between left and right kidney. Due to its more superficial position, the right kidney suffers less influence from bowel movement, presenting a more stable localization than the left one. Intestinal peristaltism influence results in changes of the renal position compromising the Doppler signal captation.[2, 22, 23, 26] In this study this observed in color and spectral Doppler modes.

To avoid this type of interference in the Bidimensional mode the sonographer should change its approach.[2, 8, 24, 26] However due the organ topography it is challenging to find a different access, as it was also observed in this study and in in these cases it is recommend posterior ultrasonographic repetition.[2, 8, 24, 26] In this study it was necessary to repeat the ultrasonographic examination of two horses and four mules with a period of wait longer in horses (days) than mules (2 - 24 hours). The repetition was successful in all horses and failed in one out four mules. We noticed in that the interval between examinations should be higher than a day even though we did not find it mentioned by the literature.[2, 8, 26]

As described by Matthews and Toal[26] it was easier to obtain spectral waves on the right kidney in mules and horses. Due to the small caliber of the renal vessels it was observed that mules possessed the same type of arterial blood flow found in horses:

parabolic flow velocity profile of low resistance flow (Figure 2).[22] This type of blood flow indicates low arterial resistance, RI and PI, and it's expected due high supply of blood by the cardiac output to the kidneys.[22, 27] Small early systolic peaks are considerate a normal finding of this type of flow (Figure 2), and has been described in humans and dogs.[22]

The spectral Doppler allows the quantitative evaluation of the blood flow via the measurement of hemodynamic indexes, allowing comparison of the blood flow between systolic and diastolic phases, and analysis of the vascular complacency and resistance. Nonetheless some of these indexes are dependent of the Doppler angle, such as the peak of systolic velocity (pSV) and final diastolic velocity (fDV).[21, 25, 28] The value of these variables depends on the sonographer experience and capacity of the ultrasound machine to obtain insonation angles below 60º, as angles higher than this can results in calculation errors and false values.[21, 25, 27, 28]

Other indexes such as RI and PI are independent, allowing evaluation without interference. [21, 22, 28] Due to the difficulties found during this experiment and to allow repeatability only the RI and PI values were considered. While it was not observed difference of these values between kidneys or arteries, it was observed between the equine species. The lack of difference between the measurement sites has already been reported in humans by Knapp et al. but has not yet been described in veterinary, even though some studies have already provide renal RI values of one or more arteries in domestic horses, but not of them all.[15, 29]

In this study it was noticed that the mules have smaller renal RI and PI (RI: 0.45 ± 0.04, PI 0.65 ± 0.08) values than horses (RI: 0.54 ± 0.04, PI: 0.84 ± 0.12) indicating that the mules have lower vascular complacency and resistance, suggesting a higher vascular perfusion when compared with horses.[30] Mules inherit various physiological characteristics from the donkeys such as higher resistance to dehydration and capacity to survival in semi-arid environments.[31, 32] When compared with horses, the mules are capable to concentrate more the urine, reducing its volume and maintaining the water and sodium levels during long periods of dehydration or even abrupt rehydration.[31, 32] Therefore the higher vascular perfusion could be related to higher renal metabolism and greater control over the water and ions loss.

Both indexes are considered sensitives indicators to changes in vascular resistance and complacency, however the use of RI is recommended in arteries with continuous blood flow, like the renal arteries, and PI is more reliable in high resistance arteries, with negative or absence flow in the spectral wave, such as the aorta. [24, 30, 33, 34] Nonetheless they possess a strong positive correlation in humans (higher than 0.9) and as seen in this study also strong in mules (0.95) but moderate in horses (0.73).[33,34] For this reason measurement of only one is enough to evaluate the arterial vascular resistance and to infer the value of the other with the regression equation.[33, 34]

Undeterred by the fact that there is a lack of renal dopplefluxometric values of mules in the literature for comparison, there are RI values of sedate and non-sedate horses to compare with the ones obtained in this study. While the animals in this study were not sedated the RI found are similar to the ones obtained by Hoffman et al. but within the interval found by Macri et al..[15] With Hoffman et al.[29] the RI of sedated horses was 0.549 ± 0.044 in arcuate arteries and 0,512 ± 0.004 in the pielorenal arteries of non-sedated horses. In the case of Macri et al.[15] the values of RI varied according with the kidneys (right: 0.50, left: 0.56) and degree of training (trained: 0.52 to 0.55, non-trained: 0.48 to 0.58).

In this study it was not possible to observe statistical significant difference of RI or PI between kidneys, fact that was also not reported by Hoffman et al.[29] The only obvious difference between the studies is the race of the horses, being in this study used only crossbreds, not stated in Hoffman et al.[29], and only Thoroughbred in Macri et al.[15] With these findings it become important to undertake new studies about renal dopplefluxometry in equines, more specifically with donkeys to observe if the mules possess similar or intermediary values; and also with different races of horses, to observe if there is a difference between them.

Among the complication found in this study only two of them were significant: failure to correct the insonation angle due limitations of the ultrasound equipment and intestinal motility. The first one made it difficult to obtain clear spectral Doppler images and reliable values of pSV and fDV. In this study the ultrasound machine only allowed to use PRF low enough for the evaluation of the renal arteries in the cardiac mode, that didn’t allowed angular correction. This limitation could be overcome through the use of equipment capable of using lower PRF values in the abdominal mode or that allow angular correction in the cardiac mode. The second complication can compromise the renal Bidimensional and Doppler evaluation through superposition of the kidney by adjacent intestinal content or due strong intestinal movements, as previously mentioned. Perhaps this interference could be reduced with fasting, something that is not usually used in horses for before abdominal ultrasonographic evaluation but it's obligatory in small animals.

This study shows significant renal dopplefluxometric differences between mules and horses, with lower values of renal RI and PI that indicate a higher renal blood flow in mules. It was also possible to observe that obtaining of Doppler images is more laborious in mules than horses, especially in the left kidney. Otherwise it was possible to observe similarities of the dopplefluxometry between mules and horses, and those differences indicate a necessity of undertaking similar studies with donkeys, to observe if the mules possess similar or intermediary RI values.

LIST OF AUTHOR CONTRIBUTIONS

Category 1

(a) Conception and Design: Castiglioni MCR, Machado VMV, Filho JNPP

(b) Acquisition of Data: Castiglioni MCR, Silva JP

(c) Analysis and Interpretation of Data: Castiglioni MCR, Basso RM, Machado VMV

Category 2

(a) Drafting the Article: : Castiglioni MCR, Machado VMV

(b) Revising Article of Intellectual Content: Castiglioni MCR, Silva JP, Machado VMV

Category 3

(a) Final Approval of the Completed Article: Castiglioni MCR, Silva JP, Basso RM, Filho JNPP, Machado VMV

ACKNOWLEDGEMENTS

The authors thank R.T. Jessica Leite Fogaça and R.T. Michel Vettorato Campos for the help with the collection of data. The authors also thank National Council for Scientific and Technological Development (CNPq) for financial support and Imagem S.A. for material support.

REFERENCES

[1] Matthews NS, Taylor TS, Hartsfield SM Anesthesia of donkeys and mules. Equine Vet Educ. 2005; 15:102-7.

[2] Matthews N, van Loon JPAM. Anesthesia and analgesia of the donkey and the mule. Equine Vet Educ. 2013; 25(1):47-51.

[3] The Donkey Sanctuary [Internet]. England. 2017 [updated in 2017 Jul 07; citation in 2017 Jan 10]. Available in: https://www.thedonkeysanctuary.org.uk

[4] Burden F, Thiemann A. Donkeys are different. J Eq Vet Science, 2015; 35(5): 376-82.
[5] Ellenberg GB. Mule south to tractor south: mules, machines and the transformation of the cotton south. Tuscaloosa: University of Alabama Press; 2007.
[6] König HE, Maierl J, Liebich HG. Chapter 9: urinary system (organa urinaria). In: König HE, Liebich HG. Veterinary anatomy of domestic mammals: textbook and color atlas. 1st edition. Germany: SchattauerGmbh; 2004. p. 365-80.
[7] Newman SJ, Confer AW, Panciera RJ. Urinary system. In: McGavin MD, Zachary JF. Pathologic basis of veterinary disease. 4th edition. St. Louis: Mosby; 2006.
[8] Reed SM, Bayly WM, Sellon DC. Equine Internal Medicine. 3th edition. St Louis: Saunders Elsevier; 2010.
[9] Verlander JW. Section VII renal physiology. In: Klein BG. Cunningham's Textbook of Veterinary Physiology. 5th edition. Missouri: Elsevier Saunders; 2013. p. 460-94.
[10] Toribio RE. Essentials of equine renal and urinary tract physiology. Vet Clin North Am Equine Pract. 2007;23(3); 533-61.
[11] McLeland S. Diseases of the equine urinary system. Vet Clin Equine, 2015; 31:377-87.
[12] Wilson ME. Examination of the urinary tract in the horse. Vet Clinics of North America. 2007; 23(3):563-75.
[13] Matoon JS, Nyland TG. Small animal diagnostic ultrasound. 3th edition. Philadelphia: Elsevier Saunders; 2015.
[14] Platt JF. Doppler ultrasound of the kidney. Seminars in Ultrasound, CT and MRI. 1997; 18(1):22-32.
[15] Macri F, Pugliese M, Pietro SD, Coco LL, Niutta PP, Nardi S, Quartuccio M, Lanteri G, Piccionello, AP. Doppler ultrasonographic estimation of renal resistive index in horse: comparison between left and right kidneys. J Equine Vet Science, 2015; 35(2):111-5.
[16] Robinson EN. Current therapy in equine medicine. 6th edition. Philadelphia: Saunders; 2006.
[17] Parry BW, McCarthy MA, Anderson GA, Survey of resting blood pressure values in clinically normal horses. Equine Vet J. 1984; 16(1): 53-8.
[18] Burnhan SL. Anatomical differences of the donkey and mule. AAEP PROCEEDINGS. 2002; 48:102-9.
[19] Smith DC. The book of mules: selecting, breeding and caring for equine hybrids. Conecticut: The Lyons Press; 2009.
[20] Schott HC 2nd, Woodie JB. Chapter 64: kidneys and ureters. In: Auer JA, Stick JA. Equine surgery.4th edition. St Louis: Elsevier Saunders; 2012.
[21] Nelson TR, Pretorius DH. The Doppler signal: where does it come from and what does it mean? Am J Roentgenol. 1988; 151(1): 439-47.
[22] Szatmári V, Sótonyi P, Vörös K. Normal duplex Doppler waveforms of major abdominal blood vessels in dogs: a review. Vet Radiol Ultrasound. 2001; 42(2): 93-107.
[23] Yanik L. The basics of Doppler ultrasonography. Vet Med. 2002; 3:388-400.
[24] Carvalho CF, Chammas MC, Cerrill, GG. Physical principles of Doppler ultrasonography. Cienc Rural. 2008; 38(3): 872-9.

[25] Merritt CRB. Doppler US: the basics. RadioGraphics. 1991; 11(1): 109-19.
[26] Penninck DG, Eisenberg HM, Teuscher EE, Vrins A. Equine renal ultrasonography: normal and abnormal. Vet Rad Ultrasound. 1986; 27(3):81-4.
[27] Wood MM, Romine LE, Lee YK, Richman KM, O'Boyle MK, Paz DA, Chu PK, Pretorius DH. Spectral Doppler signature waveforms in ultrasonography: a review of normal and abnormal waveforms. Ultrasound Quarterly. 2010; 26(2): 83-99.
[28] Kim SH, Kim WH, Choi BI, Kim CW. Duplex Doppler US in patients with medical renal disease: resistive index vs serum creatinine level. Clin Radiol. 1992; 45:85-7.
[29] Hoffmann KL, Wood AK, Kirby AC. Use of Doppler ultrasonography to evaluate renal arterial blood flow in horses. Am J Vet Res. 1997; 58(7):697-701.
[30] Bude RO, Rubin JM. Relationship between the resistive index and vascular compliance and resistance. Radiology. 1999; 211(2):411-7.
[31] Kasirer-Izraeli H, Shkolnik A, Choshniak I. Kidney response to rapid rehydration in the donkey. Isr J Zool; 2001; 47(1):29-39.
[32] Maloiy GMO. Water economy of the Somali donkey. Am J Physiol. 1970; 219(5):1522-7.
[33] Ginther OJ. Ultrasonic imaging and animal reproduction: Color-Doppler ultrasonography. [local desconhecido]:Cross Plains Equiservices; 2007.
[34] Thompson RS, Trudinger BJ, Cook CM. Doppler ultrasound waveform indices: A/B ratio, pulsatility index and Pourcelot ratio. Br J Obstet Gynaecol. 1988; 95(5):581-8.

CAPÍTULO 3

Trabalho Científico

Ultrassonografia quantitativa renal: comparação entre muares e cavalos domésticos

Renal quantitative ultrasound: comparison between mule and domestic horses

Trabalho a ser enviado para a revista:
Journal of Equine Veterinary Science

Original Research – Renal quantitative ultrasound: comparison between mule and horse

Maria Castiglioni, Michel Vettorato, Jeana Silva, Jéssica Fogaça, José Filho, Vânia Machado

São Paulo State University (Unesp), School of Veterinary Medicine and Animal Science, Botucatu, Brazil

Correspondence: Maria Cristina Reis Castiglioni - Departamento de Reprodução Animal e Radiologia Veterinária, Faculdade de Medicina Veterinária e Zootecnia, Rua Prof. Dr. Walter Maurício Correa s/nº, 18.618-681, Botucatu, SP, Brazil.: maria.castiglioni@hotmail.com

Instruções para os autores:
https://www.elsevier.com/wps/find/journaldescription.cws_home/636592?generatepdf=true

Abstract

Bidimensional ultrasonography is a simple and fast method to evaluate the abdominal organs through the analysis of the tissue's echogenicity, even though it's considerate a subjective method and liable to inter- and intra-sonographers variations. The gray-level histogram is a computation method that allows quantitative analysis of these variables, increasing the accuracy of the ultrasonographic findings. This study purpose is to quantify the renal echogenicity and echotexture of adult health horses and mules. Ultrasonographic examination was performed in 12 horses and 12 mules to obtain bidimensional images of the renal cortex, renal medulla, liver and spleen, maintaining same frequency, gain and image deepness. Posteriorly these images were analyzed using gray-level histogram, obtaining echogenicity quantitative values and proportions of each region, observing differences of echogenicity and echotexture between organs and animals. It was observed in mules that renal cortex is 6.59 times less echoic and 0.17 times more homogeneous than the spleen, different than horses. Comparing to the liver, the renal cortex is 1.46 times more hypoechoic and 0.81 times more homogeneous than the liver, similar to horses. The renal cortex is 3.49 more echoic and 0.45 more heterogeneous than medulla in mules, but in horses the renal cortex is 2.07 more echoic and 0.63 more heterogeneous. The most important difference between mules and horses was a more hypoechoic and homogeneous renal medulla in mules, indicating higher water content than horses.

Highlights

Quantitative values of renal, liver and spleen echogenicity and echotexture were defined.

Comparative ratios of renal, liver and spleen echogenicity and echotexture were obtained.

Observed difference of renal medulla between mules and horses.

Keywords: Gray-level Histogram; Equine; Kidney; Sonographic features.

1. Introduction

Renal ultrasonographic examination is a simple, non-invasive and fast method that can be performed in non-sedated standing horses.[1, 2] The B-mode permits the morphological characterization of tissues and detection of changes, assisting the diagnosis and prognosis.[3, 4, 5] Ultrasonographic characteristics are shaped by intrinsic (tissue integrity, body score, hydration level, gastrointestinal motility) and extrinsic factors (sonographer's experience and knowledge, ultrasound gains, frequency and deepness).[6, 7, 8, 9, 10, 11] The evaluation of renal characteristics is performed comparing the renal zones between each other and between other tissues, such as liver and spleen.[1, 2, 8] Some of these variables are subjective, other's susceptible to variations and errors, making it necessary to use complementary techniques in order to increase accuracy of the examination.

The gray-level histogram (GLH) is an accessible, easy and efficient method to evaluate ultrasonographic images, allowing objective characterization of the tissue's echogenicity and echotexture.[6, 7, 12] The GLH consists of the analysis and raking of grays tones in a region of interest (ROI) providing distribution, descriptive analysis and objective values of echogenicity and echotexture.[6, 7, 12] This complementary technique is underused in the veterinary science and there is a lack of studies about this technique in equines. This study aims to quantify the kidneys', liver's and spleen's echogenicity and echotexture in mules and horses, obtaining ratios between those regions for comparison, providing reference values for a more objective evaluation of equine renal parenchyma.

2. Materials and methods

2.2 Sample population

Twenty four equines were used for this study, twelve crossbred horses and twelve mules with similar weight (385-413 kg), body score (3-4/5) and age (3-25 years). Only clinically health animals with normal blood count, seric biochemistry and urinalysis were selected. This study is in accordance with the ethical principles adopted by the National Council of Animal Control and Experimentation (CONCEA) and by the Ethical Commission in Animal Use (CEUA) of this faculty.

2.2 Image acquisition

The ultrasound exam was performed in an open, covered and well ventilated environment, using mobile ultrasound equipment MyLab 30 (Esaote, Genova, Italy) and multifrequency convex transducer (3.0-9.0 MHz). Fasting was not performed and chemical restraint was not necessary. The screening windows of each kidney, head of spleen and the right dorsocaudal region of hepatic parenchyma were located with ultrasound, clipped and soaked with 70% isopropyl alcohol and acoustic gel.

Using longitudinal and transversal's scans the images were obtained using constant values of frequency and gain (3.5 MHz and 61%) (Figure 15). All sonographic images were posteriorly saved in the .JPEG format.

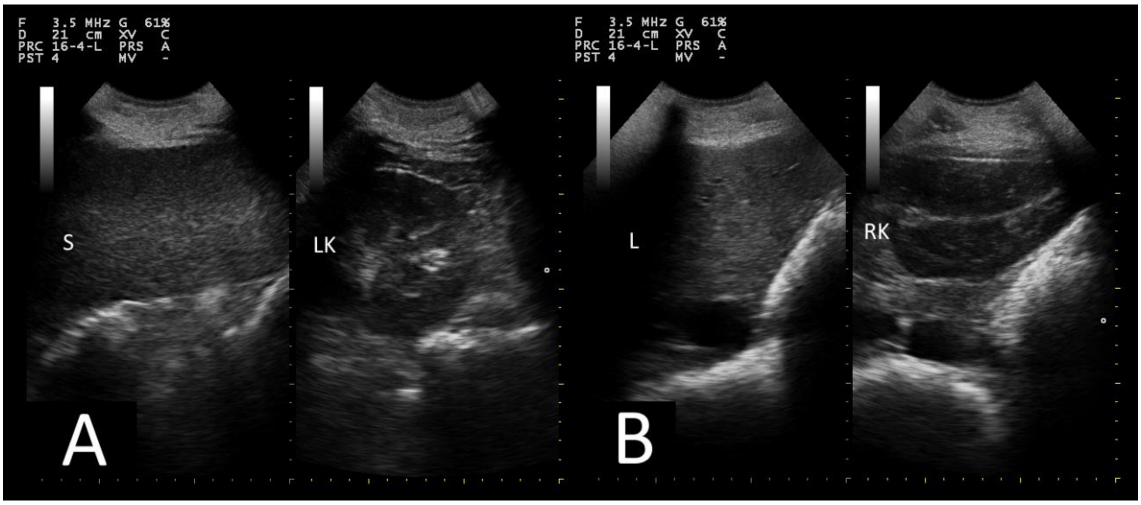

Figure 13. Bidimensional images divided in half allowing comparison of the echogenicity and echotexture between tissues. (A) Comparison between splenic parenchyma (S) and left kidney (LK). (B) Comparison between hepatic parenchyma (L) and right kidney (RK).

2.3 Gray-level histogram

All sonographic images were evaluated by the author (M.C.) and one of the coauthors (M.V.). Only images with adequate definition of the parenchyma and cortical-medullar junction (CMJ) were used for GLH. For the renal cortex and medulla evaluation a square ROI of 0.5 cm² (500 to 1000 pixels) was selected in each region, avoiding extremities or other renal structures, such as the renal capsule, the CMJ, diverticula or vessels (Fig. 16). For liver and spleen the ROIs had 1.0 cm² (1500 to 2000 pixels) (Fig. 16).

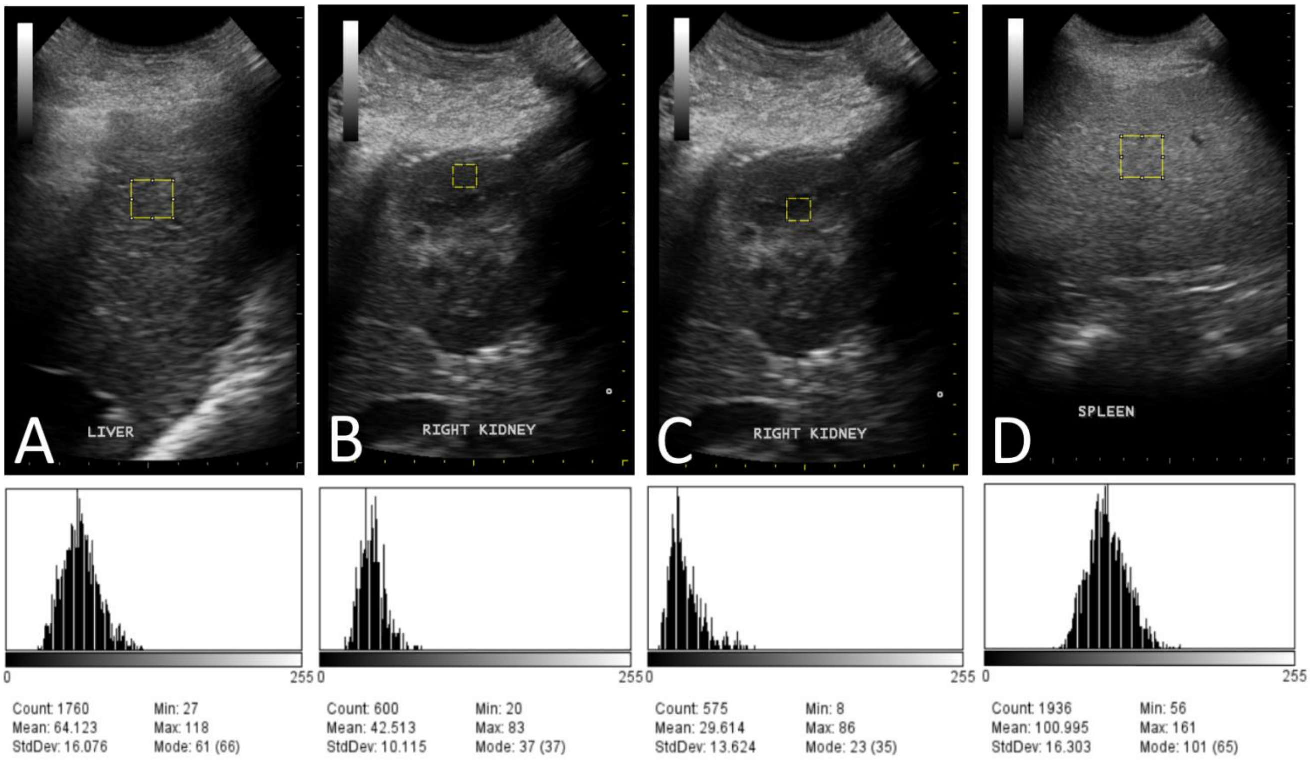

Figure 16. Bidimensional images and the respective gray-level histogram (down) of liver (A), renal cortex (B), renal medulla (C) and spleen (D). The region of interest (ROI) is marked by yellow doted lines.

The GLH analysis was performed using ImageJ software version 1.51k (National Institutes of Health, Bethesda, USA), with a gray scale of 255 shades. The following quantitative parameters were obtained from each ROI: number of pixels (COUNT), most frequent gray tonality (MODE), number of pixels with the MODE value (MODE COUNT), and pixel's mean (MEAN), standard deviation, minimum and maximum values (Fig. 16). The MEAN and MODE variables were used to analyze the ROI's echogenicity, and a MEAN ratio was created to compare the echogenicity between: liver (Li) and right kidney cortex (RKC), RKC and right kidney medulla (LKM), left kidney cortex (LKC) and left kidney medulla (LKM), and between spleen (Sp) and LKC. It was also calculated a ratio between MODE COUNT and COUNT (MC:C ratio) of each ROI to evaluate echotexture. Posteriorly it was also compared the MC:C ratio between the ROIs, comparing the echotexture.

2.4. Statistical analysis

Statistical analysis was performed GraphPad Prism program v. 7.00 for Windows (GraphPad Software, San Diego, CA) by the author (M.C.) with statistical significance set at $P < 0.05$. MEAN, MODE, MC:C ratio and MEAN ratio were submitted to normality test and descriptive analysis. Unpaired t test or Mann-Whitney test were used to observe difference between the organs (kidney, liver and spleen), renal zones (cortical and medulla) and animals (horse and mules).

3. Results

A total of 48 renal cortex, 48 renal medulla, 24 hepatic and 24 splenic regions were analyzed by GLH, obtaining at least 2 values of each ROI. The descriptive values of MEAN, MODE, MC:C ratio and MEAN ratio found in table 2 and table 3. It was not observed difference of MEAN and MODE value's of each ROI ($P < 0.05$), but it was noticed difference between regions ($P < 0.05$). Comparing horses to mules, it was only observed similarity of MEAN and MODE between the hepatic parenchyma, with difference between the other ROI. When comparing MC:C ratio and MEAN ratio between horses and mules it was possible to observe difference of RKC:RKM, LKC :LKM, Sp:LKC ratios but not of Li:RKC ratio. In mules the renal cortex is 3 times more echoic than medulla, about 6 times less than Sp and 1.5 times less than Li. In mares the renal cortex is about 2 times more echoic than medulla, 3 times less than the Sp and almost similar to the Li (ratio of 1.05). It is possible to observe a MC:C ratio of 0.81 between Li:RKC in mules, suggesting that the hepatic parenchyma is more heterogeneous. The same is observed between Sp:LKC due a ratio of 0.17, but when comparing RKC:RKM and LKC :LKM the ratio are, respectively, 0.45 and 0.28, indicating that the medulla is more homogeneous, unrelated to the kidney. The same types of results are observed with the mares only with higher ratio values, indicating higher differences between ROIs.

Table 2
Descriptive analysis of MEAN, MODE and MODE COUNT:COUNT ratio (%) of the renal cortex, renal medulla, liver and spleen from 12 mules and 12 horses

ROI			Mean	SD	Median	SE
Horse						
Right Kidney	Cortex	MEAN	50.97	16.92	48.07	2.78
		MODE	45.24	17.58	45.00	2.88
		rMC:C	4.85	1.38	4.74	0.23
	Medulla	MEAN	28.43	12.79	29.61	2.07
		MODE	25.24	12.79	27.50	1.96
		rMC:C	7.88	3.46	6.98	0.56
Left Kidney	Cortex	MEAN	24.41	12.46	25.31	2.23
		MODE	21.48	12.61	20.00	2.26
		rMC:C	9.24	7.29	1.33	1.33
	Medulla	MEAN	14.29	10.44	10.95	1.87
		MODE	11.52	11.52	8.00	1.82
		rMC:C	15.21	10.83	12.20	1.94
Spleen		MEAN	77.61	28.78	77.73	5.64
		MODE	77.73	31.31	69.50	6.14
		rMC:C	3.67	0.77	3.61	0.15
Liver		MEAN	54.04	23.89	48.00	5.09
		MODE	48.82	24.09	42.50	5.13
		rMC:C	3.99	0.97	3.86	0.22
Mule						
Right Kidney	Cortex	MEAN	31.17	13.58	26.58	2.47
		MODE	26.73	14.90	23.50	2.72
		rMC:C	6.23	1.89	5.61	0.33
	Medulla	MEAN	11.05	7.74	8.87	1.41
		MODE	7.30	6.75	6.50	1.23
		rMC:C	17.36	16.57	11.48	3.02
Left Kidney	Cortex	MEAN	7.65	7.25	6.47	1.66
		MODE	4.63	7.16	2.00	1.64
		rMC:C	23.16	19.44	16.65	4.31
	Medulla	MEAN	2.56	3.33	1.19	0.74
		MODE	1.20	2.96	0.00	0.66
		rMC:C	50.63	27.61	52.21	6.17
Spleen		MEAN	45.37	27.37	32.74	6.28
		MODE	38.47	27.37	28.00	6.27
		rMC:C	4.70	1.86	4.65	0.42
Liver		MEAN	40.82	17.29	41.73	4.19
		MODE	33.47	16.13	34.00	3.93
		rMC:C	4.45	2.07	3.71	0.50

Abbreviations: ROI, region of interest; rMC:C, ratio Mode Count:Count; SD, standard deviation; SE, standard error. *Shapiro-Wilk normality test.

Table 3
Descriptive analysis of MEAN and MODE COUNT:COUNT ratios from 12 mules and 12 horses

	Mean	SD	Median	SE	Mean	SD	Median	SE
	Horse							
	MEAN				MODE COUNT:COUNT			
Li:RKC	1.10	0.49	1.05	0.09	0.89	0.26	0.85	0.06
RKC:RKM	2.15	1.05	1.95	0.17	0.68	0.35	0.61	0.05
Sp:LKC	5.55	5.28	3.04	1.03	0.48	0.22	0.47	0.04
LKC:LKM	2.17	1.19	2.07	0.21	0.74	0.46	0.63	0.09
	Mule							
	MEAN				MODE COUNT:COUNT			
Li:RKC	0.95	0.97	0.69	0.23	0.86	0.57	0.81	0.11
RKC:RKM	4.46	3.70	3.16	0.67	0.47	0.23	0.45	0.05
Sp:LKC	9.34	7.38	6.59	1.69	0.27	0.24	0.17	0.24
LKC:LKM	6.70	6.94	3.49	1.59	0.59	0.57	0.28	0.14

Abbreviations: Li, liver; RKC, right kidney cortex; RCM, right kidney medulla; Sp, spleen; LKC, left kidney cortex; LKM, left kidney medulla; SE, standard error; SD, standard deviation.

4. Discussion

Even though diagnostic images can be generated in high resolution scales, of 4096 to 65536 grays tones, they are still evaluated in medical systems with lower resolution, of 256 to 1024 gray tones, hindering detection of subtle or initials lesions and compromising the diagnostic evaluation.[13] Still this interference is considerate insignificant due the fact that the human has a limited capacity to differentiate between grays tones, being capable to distinguish only 600 to 1000 gray tones, according to the environment luminosity conditions.[13, 14, 15] Additionally the tissue echogenicity and echotexture are the result of the interaction between the propagation velocity of the sound wave and the acoustic properties of the tissues, so according to the acoustic proprieties the interaction can result in different degrees of sound propagation, absorption, reflection and diffraction, defining the characteristics of the returning echo and therefore, the location and intensity of the gray tone in the ultrasound image.[15, 16] Therefore the sonographic echogenicity and echotexture evaluation is considerate a subjective and unsafe method to detect or control discrete changes.[1, 6, 7, 8, 9, 10, 11, 15]

Quantitative analysis provides more accurate values of the tissue characteristics such as echogenicity and echotexture, and can be performed during or posteriorly the sonographic examination.[6, 7, 12] In GLH the variables MEAN and MODE permits echogenicity analysis with higher values inferring a more echoic to hyperechoic region and lower values infer echoic to hypoechoic region. Differences between the values of MEAN and MODE indicate a non-parametric distribution of the grayscale that occur due high contrast, inadequate sample size or localization. In this study it was not observed difference between these variables inferring a low variability of gray tones in the ROI and adequate sampling. The arrangement of grays tones of an image determinates its echotexture. In GLH the ratio between MODE COUNT and COUNT (MC:C ratio) permits its analysis. The division of the number of MODE pixels (MODE COUNT) by the total number of pixels in a ROI (COUNT) with posterior multiplication by 100% allows evaluating of how much MODE (the most common tone) represents the ROI. In this case, higher ratios infer that MODE is representative of the ROI, and so a more homogeneous echotexture. Lower values indicate the opposite, less representative and more heterogeneous.

This study's findings partially coincide with to those described by the literature: the renal cortex is more hypoechoic and homogeneous than the hepatic or splenic parenchyma but more echoic and heterogeneous than the renal medulla.[1, 2, 10, 17] However while its described that Sp is more homogeneous than Li, the opposite was observed.[1, 2, 8]

Lower values of MEAN and MODE were found in mules even though the same sonographic parameters were used and this difference could be associated with the subcutaneous adipose tissue, that lower the tissue echogenicity due increasing sound beam attenuation.[16] Members of the *Equus* genus have similar sites of subcutaneous adipose tissue, but it is possible to observe inter- and intra-species variations about the sites with priority of deposition and mobilization due to metabolic and physiologic differences.[18, 19] Different than domestic horses, donkeys have higher accumulation in the base of tail, barrel, neck and abdominal adipose cover.[18, 19] Due to being a hybrid, mules can possess some of the same sites that the donkeys have, differentiating them from horses and explaining the different echogenicity and echotexture between horses and mules. According to Smith and Burden (2013)[19] the body score evaluation in mules must be a combination of the ones used in horses and donkeys.

Other discrepancy observed was the difference between the kidneys, being the right more echoic and heterogeneous than the left. Even though two structures are histological similar, such as the kidneys, the difference localization can result in significant differences between the structures.[15, 16, 20] With only the intercostal musculature and abdominal wall in the way of the sound wave the right kidney has a more superficial location, differently than the left one, that is located deeper due the presence of the spleen between him and the skin, increasing sound wave attenuation and reducing the renal echogenicity.[2, 10]

During ultrasonographic examination this type of interference is normally controlled with gain control and frequency, witch modifies the absolute echogenicity of the image and affecting GLH results.[20] In this study this variables were maintained constant to avoid interference with the GLH analysis but it resulted in difference of the echogenicity and echotexture between the kidneys in horses and mules.[15, 20] Currently there isn't a pre-existent value of gain or frequency to be used for the renal evaluation, and probably due to the fact that each animal, equipment or exam can result in different interaction between sound wave and tissue, there is a high chance of obtaining different gray tones of a same region. These are the most significant limitations of this study and due this high variability the MEAN, MODE and MC:C ratio values become unreliable reference parameters. However they can still be used in comparison, allowing the creation of a comparative method of evaluation that is less subjective than the human eye. Through this it was possible to observe in a more quantitative mode how the tissues correlate according with echogenicity and echotexture.

In this study the comparison of MEAN ratios between the ROI confirmed the previous results and allowed to compare the echogenicity between organs, horses and mules. With the exception of Li:RKC ratio, the other ratios were of higher value in mules, indicating significant difference that could be correlated to difference of sound wave attenuation or with the tissue's composition between horses and mules. Absence of echogenicity difference between liver and right renal cortical (Li:RKC ratio) indicate similar values of MEAN and MODE between mules and horses, inferring lack of significant composition difference. However, difference of RKC:RKM and LKC:LKM ratio between animals indicate difference of renal medulla echogenicity between mules and horses.

These findings were also observed during the ultrasound examination and could infer that the mules have a more hypoechoic renal medulla than horses, indicating lower tissue density and higher water content.[16] The renal parenchyma in mammals is histologic divided in two distinct zones, the cortical and medulla. The first one is composed by Bowman's capsules, convoluted tubules, initial portion of collecting ducts and corresponding vascularization, while the last by Henle loops, rest of the collecting ducts and corresponding vascularization.[21]

This difference between mules and horses could be correlated to anatomical and physiological characteristics that mules inherits from its progenitor. Different than horses, donkeys are originated from arid and semi-arid environments and are capable to survive longs periods without water and abrupt re-hydration.[21, 22, 23] Donkeys have thicker renal medulla and longer Henle loops, allowing higher concentration of sodium chloride and urea concentration, factors that increase the osmotic pressure, water reabsorption and urine concentration.[21] This way the donkeys and mules can have a renal medulla with higher water content and more hypoechoic than horses.[21]

5. Conclusion

In this study it was observed that in mules the renal cortex is 6.59 times less echoic and 0.17 times more homogeneous than the spleen (horses: 3.04 more echoic and 0.47 more homogeneous), but 1.46 times more hypoechoic and 0.81 times more homogeneous than the liver, similar to domestic horses. It was also observed that renal cortex is 3.49 more echoic and 0.45 more heterogeneous than medulla in mules, but in horses the renal cortex is 2.07 more echoic and 0.63 more heterogeneous, indicating a more hypoechoic and homogeneous renal medulla in mules, that could be related to a higher water content inherit from donkeys. However it becomes necessary of undertake new studies with donkeys and animals of different body score conditions, to observe if mules' renal medulla is similar to donkeys and if the proportion found in this study can be used with different weights and sizes.

Acknowledgements
We thank S.A. Image, Brazil, for providing the necessary equipment. This project had support by the National Council for Scientific and Technological Development (CNPq), Brazil.

References

[1] Penninck DG, Eisenberg HM, Teuscher EE, Vrinz A. Equine renal ultrasonography: normal and abnormal. Vet Rad Ultrasound. 1986; 27(3):81-4.
[2] Reef VB. Equine diagnostic ultrasound. 1st ed. Philadenphia: Saunders; 1998.

[3] Geor RJ. Acute renal failure in horses. Vet Clin North Am Equine Pract. 2007; 23(3):577-9.

[4] Matoon JS, Nyland TG. Small animal diagnostic ultrasound. 3rd ed. Philadelphia: Elsevier Saunders; 2015.

[5] Penninck D, D’Anjou MC. Atlas of small animal ultrasonography. 2nd ed. Iowa: Wiley Blackwell; 2015.

[6] Ivancić M, Mai W. Qualitative and quantitative comparison of renal vs. hepatic ultrasonographic intensity in healthy dogs. Vet Radiol Ultrasound. 2008; 49(4):368-73.

[7] Lee CH, Chol JW, Kim KA, et al. Usefulness of standard deviation on the histogram of ultrasound as a quantitative value for hepatic parenchymal echo texture: preliminary study. Ultrasound Med Biol. 2006; 32(12):1817-26.

[8] Matthews HK, Toal RL. A review of equine renal imaging techniques. Vet Radiol Ultrasound. 1996; 37(3):163-73.

[9] Schott II HC, Woodie JB. Chapter 64: kidneys and ureters. In: Auer JA, Stick JA (Eds.). Equine surgery. 4th ed. St Louis: Elsevier Saunders; 2012.

[10] Sertich PL, Turner RM. Equine diagnostic ultrasound. 1st ed. Philadelphia: W.B. Saunders Company; 1998.

[11] Toribio RE. Essentials of equine renal and urinary tract physiology. Vet Clin North Am Equine Pract. 2007; 23(3):533-61.

[12] Maeda K, Utsu M, Yamamoto N, et al. Chapter 4: ultrasonic tissue characteriation with gray level histogram width. In: Kurjak A, Arenas JB (Eds.). Donald school textbook of transvaginal sonograph. 2nd ed. New Delhi: Jaypee Brothers Medical Publisher Ltd; 2013. p.17-20.

[13] Kimpe T, Tuytschaever T. Increasing the number of gray shades in medical display systems: how much is enough? J Digit Imaging. 2007; 20(4):422-32.

[14] Allison JW, Barrm LL, Massoth RJ, et al. Understanding the process of quantitative ultrasonic tissue characterization. Radiographics. 1994; 14(5):1099-108.

Bifelow TA, Labyed Y. Chapter 4: attenuation compensation and estimation. In: Mamou J, Oelze ML (Eds.). Quantitative ultrasound in soft tissues, New York: Springer; 2014. p. 71-94.

[15] Smith-Levitin M, Blicstein I, Albrecht-Shach AA, et al. Quantitative assessment of gray-level perception: observers’ accuracy is dependent on density differences. Ultrasound Obstet Gynecol. 1997; 10(5):346-9.

[16] Ghoshal G, Oelze ML, O'Brien Jr WD. Chapter 2: quantitative ultrasound history and success. In: Mamou J, Oelze ML. (Eds.). Quantitative ultrasound in soft tissues, New York: Springer; 2014. p. 21-42.

[17] Slovis N. Chapter 23: ultrasonography of the liver, spleen, kidney, bladder, and peritoneal Cavity. In: Kidd J, Lu KG, Frazer ML. (Eds.). Atlas of equine ultrasonography. West Sussex: John Wiley & Sons; 2014. p.409–27.

[18] Quaresma M, Payan-Carreira R, Silva SR. Relationship between ultrasound measurements of body fat reserves and body condition score in female donkeys. Vet J. 2013; 197(2):329-34.

[19] Smith DG, Burden FA. Chapter 16: practical donkey and mule nutrition. In: Geor RJ, Harris PA, Coenen M. (Eds.). Equine applied and clinical nutrition: importance of nutrition for health, welfare and performance. London: Elsevier Saunders; 2013. p.304-18.

[20] Manley JA, O'Neill WC. How echogenic is echogenic? Quantitative acoustics of renal cortex. Am J Kidney Dis. 2001; 37(4):706-11.

[21] Hill RW, Wyse GA, Anderson M. Animal physiology. 3rd ed. Massachusetts:Sinauer Associates; 2012.

[22] Kasirer-Izraeli H, Shkolnik A, Choshniak I. Kidney response to rapid rehydration in the donkey. Isr J Zool. 2001; 47(1):29-39.

[23] Maloiy GMO. Water economy of the Somali donkey. Am J Physiol. 1970; 219(5):1522-7.

CAPÍTULO 4

Trabalho Científico

Topografia Renal e Características Ultrassonográficas Morfométricas Renais de Muares: Comparação com Cavalos Domésticos

Renal topography and ultrasonographic dimensions in mules: comparison with horses

Trabalho a ser enviado para a revista:
Anatomia, Histologia, Embryologia

ORIGINAL ARTICLE: Renal topography and ultrasonographic dimensions in mules: comparison with horses

M. Castiglioni[1]; J. Fogaça[1], J. Filho[1], V. Machado[1]

[1] São Paulo State University (Unesp), School of Veterinary Medicine and Animal Science, Botucatu, SP, Brazil.

Correspondence: Maria Cristina Reis Castiglioni - Departamento de Reprodução Animal e Radiologia Veterinária, Faculdade de Medicina Veterinária e Zootecnia, Rua Prof. Dr. Walter Maurício Correa s/nº, 18.618-681, Botucatu, SP, Brazil.: maria.castiglioni@hotmail.com

Instruções para os autores:
http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1439-0264/homepage/ForAuthors.html

Summary

Renal topography varies between species, changing clinical and surgical approaches. As a hybrid the mule has its own characteristics, but can also inherit characteristics from its progenitors, thus it's not recommended to use the extensive literature about horses as reference without further studies. This work proposes to characterize the renal topography and dimensions of mules, comparing 12 mules with 12 horses of similar age, weight and body score. In mules the right kidney (RK) was located between 16th intercostal space (ICS) and 3rd lumbar vertebra (LV) and the left one (LK) amid 17th ICS and 4th LV. Both kidneys have different distances from the lumbar spine (RK: 23 ± 2.3cm; LK: 25 ± 2.8cm), coxal tuberosity (RK: 23 ± 2.3cm; LK: 15 ± 1.5cm) and skin (RK: 3.1 ± 0.7cm to 11.4 ± 1.3cm; LK: 5.6 ± 1.1 to 13.9 ±1.5 cm). With the exception of length (RK: 14.62 ± 1.08cm; LK: 11.05 ± 1.67cm), the RK and LK have identical dimensions (height: 7.20 ± 0.89cm; width: 11.05 ± 1.67cm). There were differences of the thickness between cortical (1.19 ± 0.16 cm) and medular (1.99 ± 0.41 cm) zones but not between kidneys. Comparing to horses, mules have similar renal dimensions and topography, with the exception of a greater distance between kidneys and LSp and a thicker medulla zone. These findings could indicate a small paternal influence, indicating necessity of further studies with mules and donkeys.

Number of figures: 3
Number of tables: 3

1. INTRODUCTION

Knowledge of an organ's topography and morphology is fundamental for a successful clinical or surgical approach. The renal topography can vary between animals, changing the way certain procedures are performed such as physical examination, ultrasonographic examination or sampling method for histopathologic examination. While these changes occur in higher degree between classes and orders, it can also occur within them (König *et al.*, 2004). Other variables can also chance between species, such as dimension, shape, macroscopic and microscopic aspects

Currently most studies in the genus Equus are performed in domestic horses due to their availability and socioeconomic importance. However other members of this genus such as donkeys have anatomical and physiological peculiarities that are still not well studied. Among them we can observe physiological and physical differences providing greater strength, resistance and durability to arid and semi-arid conditions (Burnhan, 2002; Smith, 2009; Burden & Thiemann, 2015). The mules are hybrid animals derived from the crossing of a donkey with a horse, inheriting anatomical and physiological intermediary characteristics between these two species (Matthews *et al*., 2005; Smith, 2009; Matthews & Van Loon, 2013; Burden & Thiemann, 2015).

Thus even with the extensive literature on domestic horses, it is not possible to use the same parameters for mules, since they can inherit unique characteristics derived from donkeys. To the author's knowledge, until this moment it was not possible to find a study characterizing the mule's kidneys, or providing ultrasonographic information about the degree of similarity between mules and horses. Thus the present work proposes to determinate the localization and size of the mule's kidney, and to compare with horses to determine the degree of similarity.

2. MATERIALS AND METHODS

2.1. Animals

A total of twelve mules and twelve horses, of similar age, weight and body score were used for this study. Inclusion criteria in this study were: healthy at the physical examination, no history of renal disease and absence of significant findings at the blood count and serum biochemistry. This study is in accordance with the ethical principles adopted by the National Council of Animal Control and Experimentation (CONCEA) and by the Ethical Commission in Animal Use (CEUA) of this faculty.

2.2. Data and image acquisition

No fasting or chemical restrain was used for the image and data acquisition. The ultrasonographic examination was performed by the author (M. Castiglioni) in an open, covered and well ventilated environment using MyLab®30 mobile ultrasound (Esaote, Italy) and multifrequential convex transducer (3 to 9 MHz).

At the beginning of each evaluation the kidneys, spleen, liver and transverse processes of the lumbar vertebra were located using ultrasonography. Once located, each structure had its topography marked with the help of chalk or whiteboard pens. The location of the kidneys was evaluated according to the intercostal spaces and lumbar vertebrae, and the following measurements were obtained: center of the right kidney and lumbar spine (RKLSp), center of the right kidney and coxal tuberosity (RKCT), center of the left kidney and lumbar spine (LKLSp), and center of the left kidney and coxal tuberosity (LKCT). For posterior allometric analysis it was also measurement the weight (Wt), wither's height (WHt) and thoracic circumference (ThC) of each mule (Figure 18). These external measurements were obtained using weighing tape and self-retracting tape by the author (M. Castiglioni) and one of the co-authors (J. Fogaça).

Subsequently, shaving of the region corresponding to the kidneys was performed, applying abundant amount of acoustic gel. To measure the renal dimensions, the longitudinal sections were used to obtain length and height, and the transversal for the width (Figure 17). For kidney with "U" or "J" shape, only the largest width value was considered. The renal volume was automatically measured by the volume equation of

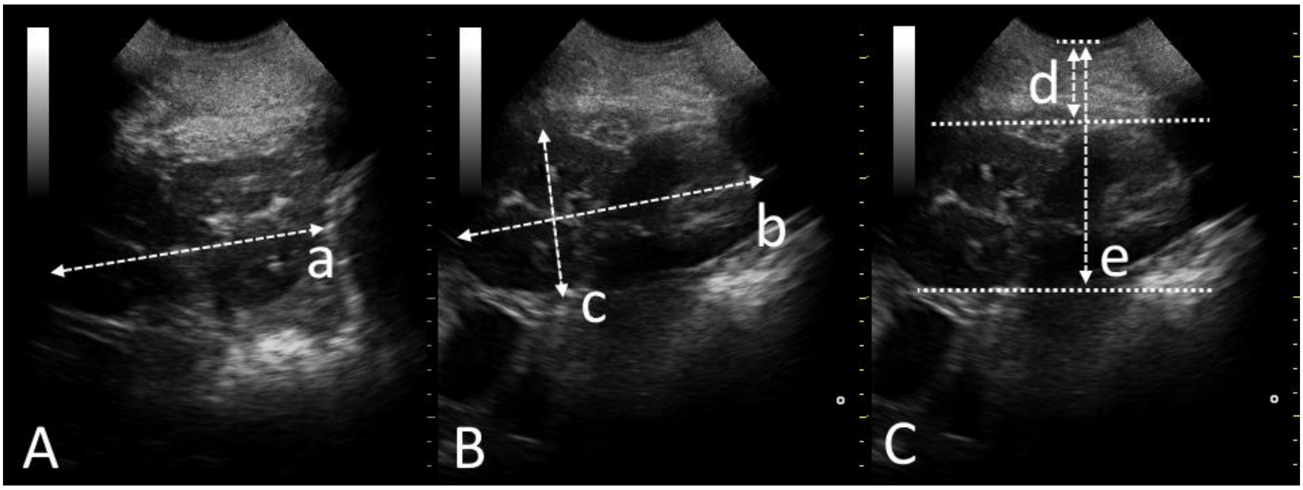

FIGURE 18. Bidimensional images of the kidney in transverse (A) and longitudinal (B, C) scans. Measurement of the renal width (a), length (b), height (c), minimum (d) and maximum depth (e).

ellipsoid structures described in the literature (Matoon & Nyland; 2015).

Using the transverse images with better cortical-medullary definition, the thickness of the cortical and medullar area was measured. Both measurements were performed at the same level, avoiding measurement in the extremities. The minimum and maximum depth of each kidney was also obtained in relation to the skin (Figure 18).

2.3. Statistical analysis

Statistical analyzes were performed using computational program GraphPad Prism v. 7.03 for Windows (GraphPad Software, La Jolla California, USA), considering significant values with only P value smaller than 0.05. All quantitative variables were submitted to normality test and descriptive analysis, obtaining mean, median, standard deviation (SD), standard error (SE), minimum and maximum values. Unpaired T test was used to observe difference between the kidneys (right and left) and between animals (mule and horse). For the allometric analysis it was used the Pearson correlation test between renal volume:Wt, renal height:WHt and renal width:ThC.

3. RESULTS

The right kidney (RK) is located between the level of the 16th intercostal space (ICS) - 3rd lumbar vertebra (LV) in mules and 15th - 3rd LV in horses and the left kidney (LK) is located between 17th ICS - 4th LV in mules and 16th - 4th LV in horses. The DKLC, DKCT, minimum and maximum depth of the kidneys can be found in table 4. Unrelated to the animal it was observed difference of DKLC and DKCT between kidneys, but only of DKLC between the animals. The minimum and maximum depth were different between the RK and LK, but not between mules and horses.

Renal volume, height, length, width and thickness can be found in table 5. In mules it is only observed difference of length between the kidneys (P value < 0.05) and in horses only of width (P value < 0.05). While it was not observed difference of the cortical thickness between mules and horses it was observed with medulla thickness and cortical:medulla ratio (P value < 0.05). It was not observed difference of cortical or medulla thickness between kidneys (P value > 0.05).

It was not observed difference of Wt, WHt and ThC between mules and horses, with the values described in table 6. In the allometric analysis it was only observed correlation of the renal width with Wt and with ThC. In both cases the correlation was negative and of moderate degree (width:Wt, Pearson r: - 0.79; width:ThC, Pearson r: - 0.75), with a regression analysis that indicates in mules a moderate relationship between the variables (width:Wt, R^2: 0.63; width:ThC, Pearson r: 0.56) and allows the creation of the following regression equations: $renal\ width = Wt\ x\ (-0.31) + 229.1$ and $renal\ width = ThC\ x\ (-1.92) + 434.8$.

TABLE 4. Descriptive analysis of renal topography measurements (cm) of 12 mules and 12 horses.

	Mule					
	Right kidney			*Left kidney*		
	Mean	**SD**	**IC 95%**	**Mean**	**SD**	**IC 95%**
DKLC	23	2.8	21 – 24	25	2.8	23 – 27
DKCT	23	2.3	22 - 24	15	1.5	14 - 16
MinD	3.08	0.79	2.74 – 3.41	5.59	1.06	5.14 – 6.04
MaxD	11.41	1.30	10.86 – 11.96	13.94	1.48	13.32 – 14.57
	Horse					
	Right kidney			*Left kidney*		
	Mean	**SD**	**IC 95%**	**Mean**	**SD**	**IC 95%**
DKLC	19	2.5	17 – 21	23	3.2	21 – 25
DKCT	21	2.1	20 - 23	15	1.9	14 - 17
MinD	2.70	0.62	2.42 – 2.97	6.01	1.40	5.44 – 6.57
MaxD	11.84	1.62	11.12 – 12.56	14.50	1.61	13.85 – 1.15

Abbreviations: SD, standard deviation; IC, interval of confidence; DKLC, distance kidney-lumbar column; DKCT, distance kidney-coxal tuberosity; MinD, minimum depth; MaxD, maximum depth.

TABLE 5. Descriptive analysis of renal dimensions of 12 mules and 12 horses.

		Mean	**Median**	**SD**	**Min**	**Max**	**IC 95%**
				Mule			
Length (cm)	*Right*	14.62	14.71	1.08	11.21	16.84	14.22 – 15.00
	Left	15.23	15.08	1.12	13.1	18.78	14.82 – 15.65
Height (cm)		7.20	7.00	0.89	5.74	9.65	6.97 – 7.43
Width (cm)		11.04	11.26	1.67	7.68	14.39	10.61 – 11.46
Volume (ml)		619.13	624.35	155.48	310.50	975.50	574.94 – 663.32
CT (cm)		1.19	1.18	0.16	0.94	1.68	1.15 – 1.24
MT (cm)		1.99	1.93	0.41	1.33	3.00	1.89 – 2.10
CT:MT ratio		1.67	1.60	0.32	1.20	2.60	1.59 – 1.76
				Horse			
Length (cm)		15.14	14.85	1.54	10.72	18.61	14.69 – 15.58
Height (cm)		7.38	7.52	1.09	5.40	9.50	7.07 – 7.70
Width (cm)	*Right*	10.73	10.40	12.22	9.05	13.25	10.17 – 11.29
	Left	11.89	12.21	16.14	9.16	14.89	11.25 – 12.53
Volume (ml)		692.63	657.20	198.09	307.30	1211.50	624.54 – 760.67
CT (cm)		1.11	1.10	0.17	0.75	1.53	1.06 – 1.15
MT (cm)		1.74	1.71	0.35	1.14	2.61	1.66 – 1.83
CT:MT ratio		1.50	1.50	0.20	1.00	2.10	1.51 – 1.63

Abbreviations: SD, standard deviation; IC, interval of confidence; CT, cortical thickness; MT, medulla thickness. Length and height were obtained in longitudinal images and width in transversal scans.

TABLE 6 . Descriptive analysis of weight, wither's height and thoracic circumference of 12 mules and 12 horses.

	Mule			*Horse*		
	Mean	**SD**	**IC 95%**	**Mean**	**SD**	**IC 95%**
Wt (kg)	385	32	365 – 405	413	40	388 – 439
ThC (cm)	170	5.2	167 – 173	173	6.7	169 - 177
WiH (cm)	140	4.1	138-143	138	3	136 - 141

Abbreviations: SD, standard deviation; IC, interval of confidence; Wt, weight; ThC, thoracic circumference; WiH, withers height.

4. DISCUSSION

In mammals the kidneys are located within the retroperitoneal space and are covered by a layer of fat tissue to protect against mechanical trauma (Sertich & Turner, 1998; König *et al*., 2004). In domestic horses they are found immediately ventral to the extensions of the last ribs and the first lumbar vertebras, with a more superficial location of the right (between 14th and 17th ICS) and a more deep of the left due the spleen (between 16th ICS and point of the hip) (Sertich & Turner, 1998; König *et al*., 2004; Schott II & Woodie, 2012). Nowadays there are few studies that provide objective information about the kidneys' location in equines. Some authors use an imaginary line between the coxal tuberosity and the humeral-scapular joint to help locate the kidneys, since they always can be found at or above this level, while others first locate de liver or the spleen to help find the kidneys (Penninck *et al*., 1986; Reff *et al*., 1998; Draper *et al*., 2012).

Some studies have provided information of the renal site according to the kidney's distance to coxal tuberosity or to cutaneous plane (Draper *et al*., 2012; Slovis, 2014). However most if not all studies have been performed in domestic horses and, to the author's knowledge, until the present moment there wasn't information about the location or dimension values of mule's kidneys. In this study it was possible to observe similar topography between mules and horses, with the exception of a more ventral localization. Even though small, the higher DKLV in mules is statistical significant and could be related to the slight inclined pelvis, that mules inherit from donkeys and that differ them from horses (Burnham. 2002; Burden & Thiemann, 2015). Regarding the depth of the mules' kidneys, it was observed similar values to those described by the literature (right: 2 – 6cm; left: 2 – 15cm from skin), indicating an absence of paternal influence (Draper *et al*., 2012; Slovis, 2014).

Due the large size of the equine's kidneys it is considered impossible to accurately measure them using ultrasound, although it is possible to estimate the length, height and width (Penninck *et al*., 1986; Matthews & Toal, 1996; Draper *et al*., 2012; Slovis *et al*., 2014). While it underestimates the renal dimensions, the ultrasonographic measurement is still used due the fact that the difference is considered minimal (Walter *et al*., 1987; Nyland *et al*., 1989; Barr, 1990). No difference of renal height, length or width between mules and horses was observed in this study, all measurements obtained were within the normal range described to horses by the literature, and with the exception of the renal length there weren't significant differences between kidneys in mules (Penninck *et al*., 1986; Matthews & Toal, 1996; Reed *et al*., 2010; Draper *et al*., 2012; Schott II & Woodie, 2012; Slovis *et al*., 2014). This difference can be expected, especially when the left one is the longer due to fact it can have a more ovoid shape than the right one (Penninck *et al*., 1986; Matthews & Toal, 1996; Reed *et al*., 2010; Schott II & Woodie, 2012; Slovis *et al*., 2014).

Estimation of renal volume via ultrasound it's widely performed in humans and small animals and considerate a more accurate indicator of renal mass and function than the obtaining of only one measurement (Nyland *et al*., 1989; Barr, 1990). In this study it was observed a renal volume of 619.13 +/- 155.48ml in mules and 692.63 +/- 198.09ml in horses, both at the inferior limit of the reference range (600 - 1000g) set by the literature for adult horses (Reed *et al*., 2010; Schott II & Woodie, 2012). This fact could be related to the fact that the animals of this study are lighter than the ones used to set this range (400 to 500kg) or could be explained by the fact that the formula used in small animals and humans is designed for elliptic structures, and while equine kidneys can have an ovoid shape, the right kidney frequently has a triangular or heart-like shape (König *et al*., 2004; Reed *et al*., 2010; Schott II & Woodie, 2012). Although there are methods that allow the volume measurement of pyramidal structures (Cavalieri principle), they are not routinely used due the use of several sequential parallel images instead of two used in elliptic structures (Nyland *et al*., 1989; Barr, 1990; Pazvant *et al.*, 2009).

Macroscopically the kidneys are composed of two parenchymal regions: cortex and medulla. The first one is located at the periphery and is composed of Bowman's capsules (glomerulus), convoluted tubules, initial portion of the collecting ducts and corresponding vascularization. The last one is located at the center, between cortex and pelvis, composed of Henle loops, collecting ducts and vessels (Hill *et al*., 2012). In horses the renal cortex is 1 to 2cm thick and the corticomedullary junction is not well defined by the ultrasonographic evaluation, but there isn't information about the medulla width or cortex:medulla ratio (Penninck *et al*., 1986; Reff *et al*., 1998; Sertich & Turner, 1998; Slovis *et al*., 2014). The cortical thickness obtained in this study did not differ between mules or horses and was within this reference range. However it was observed difference of the medulla values, being thicker in mule (1.99 ± 0.32cm) than in horses (1.74 ± 0.35cm). It was also observed a higher cortical:medulla ratio and a more defined corticomedullary junction in mules.

Thicker renal medulla is normally found in desert mammals and its related to longer Henle loops, a characteristic that increase water reabsorption and urine concentration (Hill *et al*., 2012). This particularity can be found in donkeys but not in horses, and due to hybrid vigor mules can inherit this particularity (Maloiy, 1970; Kasirer-Izraeli *et al*, 2001; Burnhan, 2002; Smith, 2009; Burden & Thiemann, 2015).

Due to the being a hybrid, mules present intermediary characteristics of both parents. In this study it was possible to observe that the mule's kidneys are very similar to horses, with a similar topography and dimensions, with the exception of a more ventral location and a thicker medulla. These particularities can be due paternal influence, turning necessary new studies evaluating the renal characteristics between donkeys, horses and mules.

Acknowledgements

This work was supported by the National Council for Scientific and Technological Development (CNPq) Brasília, Brazil [grant number 131555/2016-2].

Conflict of Interest Statement

The authors declare that there are no potential sources of conflict of interest.

References

Barr, F. J. (1990). Evaluation of ultrasound as a method of assessing renal size in the dog. *Journal of Small Animal Surgery*,31, 174-179.

Burden, F., & Thiemann, A. (2015). Donkeys are different. *Journal of Equine Veterinary Science*, 35, 5, 376-382.

Burham, S. L. (2002). Anatomical differences of the donkey and mule. *AAEP Proceedings*, 48, 102-109.

Draper, A. C. E., Bowen, I. M., Hallowell, G. D. (2012). Reference ranges and reliability of transabdominal ultrasonographic renal dimensions in thoroughbred horses. *Veterinary Radiology and Ultrasound*, 5, 3, 336-341.

Kasirer-Izraeli, H., Shkolnik, A., Choshniak, I. (2001). Kidney response to rapid rehydration in the donkey. *Israel Journal of Zoology*, 47, 1, 29-39.

König, H. E., Maierl, J., Liebich, H. G. Chapter 9: urinary system (organa urinaria). In: König, H. E., Liebich, H. G. (2004). Veterinary anatomy of domestic mammals: textbook and color atlas (1st ed.) Germany: SchattauerGmbh, 365-380.

Maloiy, G. M. O. (1970). Water economy of the Somali donkey. *American Journal of Physiology*, 219, 5, 1522-1527.

Matoon, J. S., & Nyland, T. G. (2015) Small animal diagnostic ultrasound (3th ed.) Philadelphia: Elsevier Saunders.

Matthews, N. S., Taylor, T. S., Hartsfield, S. M. (2005). Anesthesia of donkeys and mules. *Equine Veterinary Education*, 15, 102-107.

Matthews, N., & Van Loon, J. P. A. M. (2013). Anesthesia and analgesia of the donkey and the mule. *Equine Veterinary Education*, 25, 1, 47-51.

Nyland, T. G., Kantrowitz, B. M., Fisher, P., Olander, H. J., Hornof, W. J. (1989). Ultrasonic determination of kidney volume in the dog. *Veterinary Radiology and Ultrasound*, 30, 3, 174-180.

Pazvant, G., Sahin, B., Kahvecioglu, K. O., Gunes, H., Gezer Ince, N., Bacinoglu, D. (2009). The volume fraction method for the evaluation of kidney: a stereological study. Ankara Üniv Fak Derg, 56, 233-239.

Penninck, D. G., Eisenberg, H. M., Teuscher, E. E., Vrins, A. (2010). Equine renal ultrasonography: normal and abnormal. *Veterinary Radiology and Ultrasound*, 27, 3, 81-84.

Reed, S. M., Bayly, W. M., Sellon, D. C. (2010). Equine internal medicine (3th ed.). St Louis: Saunders Elsevier.

Reef, V. B. (1998). Equine diagnostic ultrasound (1st ed.). Philadelphia: Saunders.

Schott II, H. C., Woodie, J. B. (2012). Chapter 64: kidneys and ureters. In: Auer, J. A., Stick, J. A. (Ed.). Equine surgery (4th ed.). St Louis: Elsevier Saunders.

Sertich, P. L., Turner, R. M. (1998) Equine diagnostic ultrasound (1st ed). Philadelphia: W.B. Saunders Company.

Slovis, N. (2014). Chapter 23: ultrasonography of the liver, spleen, kidney, bladder, and peritoneal Cavity. In: Kidd, J., Lu, K. G., Frazer, M. L. (Ed.). Atlas of equine ultrasonography. West Sussex: John Wiley & Sons, 409–27.

Smith, D. C. (2009). The book of mules: selecting, breeding and caring for equine hybrids. Conecticut: The Lyons Press.

Toribio, R. E. (2007). Essentials of equine renal and urinary tract physiology. *Veterinary Clinics of North American: Equine Practice*. 23, 3, 533-561.

Walter, P. A.; Feeney, D. A.; Johnston, G. R.; Flecther, T. F. (1987) Feline renal ultrasonography: quantitative analyses of imaged anatomy. *American Journal of Veterinary Research*, 48, 569-599.