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"JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA VETERINÁRIA
CÂMPUS DE ARAÇATUBA

MARCEL GAMBIN MARQUES

Ecocardiografia convencional e *speckle tracking* bidimensional em cães saudáveis anestesiados com sevofluorano e submetidos a infusão contínua de nalbufina

Araçatuba
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Tese apresentada à Faculdade de Medicina Veterinária da Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP, Campus de Araçatuba, como parte das exigências para a obtenção do título de Doutor em Ciência Animal (Fisiopatologia Médica e Cirúrgica de Pequenos Animais).

Orientador: Prof. Dr. Paulo Sergio Patto dos Santos
Coorientador: Prof. Dr. Wagner Luis Ferreira

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ORIENTADOR: PAULO SERGIO PATTO DOS SANTOS
COORIENTADOR: WAGNER LUIS FERREIRA

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Prof. Dr. WAGNER LUIS FERREIRA
Departamento de Clínica, Cirurgia e Reprodução Animal / Faculdade de Medicina Veterinária - Câmpus de Araçatuba/Unesp

Prof. Dr. GUILHERME DE PAULA NOGUEIRA
Departamento de Produção e Saúde Animal / Faculdade de Medicina Veterinária - Câmpus de Araçatuba/UNESP

Prof. Dr. CAIO JOSÉ XAVIER ABIMUSSI
Centro Universitário das Faculdades Integradas de Ourinhos-UNIFIO / Ourinhos-SP

Prof. Dr. FÁBIO NELSON GAVA
Departamento de Clínicas Veterinárias / Universidade Estadual de Londrina/UEL

Profa. Dra. BEATRIZ PEREZ FLORIANO
Centro Universitário das Faculdades Integradas de Ourinhos-UNIFIO / Ourinhos/SP

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RESUMO

A nalbufina é um opioide agonista-antagonista com propriedades analgésicas adequadas e poucos efeitos depressores no sistema respiratório. Sua utilização na medicina veterinária é limitada pois muitos veterinários desconhecem suas vantagens. Além disso, seus efeitos na função cardíaca são pouco estudados. Portanto, com o estudo objetivou-se avaliar os efeitos da infusão contínua de nalbufina na função sistólica e diastólica do ventrículo esquerdo em cães saudáveis anestesiados com sevofluorano. Foram utilizados dezoito cães fêmeas de diversas raças ou sem raça definida, com idade média de 2 ± 1 anos e peso médio de 9,9 ± 3,8 kg. Os cães foram aleatoriamente submetidos a dois grupos denominados: nalbufina (G_N) e controle (G_C), com nove animais para cada grupo. Os animais foram induzidos e mantidos sob anestesia com sevofluorano (2V%). No G_N foi administrado bolus intravenoso de nalbufina (0,3 mg/kg), seguido de infusão continua (0,4 mg/kg/h). O G_C recebeu solução salina (NaCl 0,9%), em volumes idênticos em bolus e infusão ao G_N. As variáveis ecocardiográficas de função sistólica e diastólica e os parâmetros hemodinâmicas foram determinadas no momento basal (antes do bolus) e 20, 40, 60 e 80 minutos após o início da infusão contínua. Não houve diferença entre os grupos para os parâmetros de função sistólica e diastólica ventricular esquerda derivados da ecocardiografia convencional e *speckle tracking* bidimensional. Do mesmo modo, as variáveis hemodinâmicas não apresentaram diferença entre os grupos. A Relação E'/A' apresentou aumento aos 20 minutos de infusão com relação ao momento basal apenas no G_C. Os resultados desse estudo indicam que a infusão contínua de nalbufina não alterou a função sistólica e diastólica ventricular esquerda, em cães saudáveis anestesiados com sevofluorano.

Palavras-chave: Função sistólica. Função diastólica. Opioides. Deformação miocárdica.

MARQUES, M.G**. Conventional echocardiography and two-dimensional speckle tracking in healthy sevoflurane-anesthetized dogs undergoing continuous rate infusion of nalbuphine.** 2020. 61f. Tese (Doutorado) Faculdade de Medicina Veterinária, Universidade Estadual Paulista, Araçatuba, 2020.

ABSTRACT

Nalbuphine is an agonist-antagonist opioid with adequate analgesic properties and few depressant effects on the respiratory system. Its use in veterinary medicine is limited due to the unknown of its effects on cardiac function. The aim of this study was to assess the effects of a continuous rate infusion (CRI) of nalbuphine on left ventricular systolic and diastolic function of healthy sevoflurane-anesthetized dogs. Were used eighteen mixed-breed bitches ageging 2 ± 1 years and weighing 9.9 ± 3.8 kg. Dogs were randomly assigned to one of two groups: nalbuphine (G_N, n=9); and control (G_C, n=9). Anesthesia was induced and maintained with sevoflurane (2V%) followed by an intravenous (IV) bolus of nalbuphine (0.3 mg/kg) or 0.9% NaCl at equal volume, then CRI of nalbuphine (0.4 mg/kg/h) or 0.9% NaCl at equal infusion rate. Echocardiographic and hemodynamic variables were determined at baseline and 20, 40, 60 and 80 minutes following start of CRI. No differences were found between groups for left ventricular systolic and diastolic variables obtained through conventional echocardiography and two-dimensional speckle tracking. Likewise, hemodynamic variables did not differ between groups. The E′/A′ ratio significantly increased at 20 minutes compared to baseline only in G_N. Nalbuphine given at a CRI does not influence left ventricular systolic and diastolic function in healthy sevoflurane-anesthetized dogs.

Keywords: Systolic function. Diastolic function. Opioids. Myocardial deformation.

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1 INTRODUÇÃO GERAL

1.1 Nalbufina

A nalbufina é um opioide lipofílico com propriedades agonistas de receptores kappa (κ) e antagonista de receptores mu (μ). Seus efeitos analgésicos são semelhantes aos da morfina, conferindo potência analgésica satisfatória para o tratamento da dor moderada a grave (BEAVER; FEISE, 1978; KUKANICH; WIESE, 2015). Além disso, sua ação depressora no sistema respiratório mínima, confere a principal vantagem com relação aos outros opioides (ZENG et al., 2015).

A nalbufina foi descoberta na década de 80 e desde então, vem sendo estudada e utilizada na medicina humana para o tratamento da dor leve a moderada (WARD, 1981; MILLER, 1980). Zeng et al. (2015) em seu estudo de meta-análise avaliaram os efeitos analgésicos e a segurança da nalbufina comparada a morfina. Os resultados mostraram que a nalbufina promove analgesia semelhante à da morfina, com até 5 horas de duração, entretanto, os efeitos indesejados como náusea, prurido e retenção urinária foram pouco observados. Portanto, os autores concluíram que nalbufina pode ser considerada como uma opção mais segura, principalmente com relação à morfina.

Por isso, a nalbufina é utilizada como analgésico de escolha em diversas circunstâncias na medicina humana, principalmente para o controle da dor leve a moderada durante e após vários procedimentos cirúrgicos e ambulatoriais. Minai e Khan (2003) mostraram que a nalbufina promove adequada analgesia, mantendo a estabilidade hemodinâmica, conferindo recuperação e alívio da dor mais efetivos que a morfina, em mulheres submetidas à histerectomia abdominal total. Outros autores destacam que a adição da nalbufina junto à morfina foi efetiva no controle da dor pós-operatória, reduzindo significativamente o aparecimento de efeitos indesejados, como o prurido (YEH et al., 2008). Lee, Marvin e Heimbach (1989) reportaram que a nalbufina mostrou a mesma efetividade da morfina no controle da dor após o desbridamento de feridas por queimaduras, todavia, sem causar depressão respiratória. Outro estudo avaliou os efeitos da administração de nalbufina no alívio da dor do infarto agudo do miocárdio, lesões traumáticas e por queimaduras. Durante o atendimento prestado por paramédicos, a nalbufina controlou efetivamente a dor, sem demonstrar efeitos adversos importantes. Portanto, os autores concluem que a

nalbufina pode ser seguramente administrada para promover analgesia satisfatória durante o atendimento pré-hospitalar ou ambulatorial (CHAMBERS; GULY, 1994).

Além disso, a nalbufina pode ser utilizada para reverter os efeitos adversos provocados pelos opioides μ-agonistas, principalmente aqueles relacionados à depressão do sistema respiratório. Um estudo avaliou a eficácia de três doses de nalbufina (0,3 mg/kg, 0,6 mg/kg e 1,0 mg/kg) na reversão dos efeitos cardiopulmonares e sedativos da morfina associada à acepromazina em cães. Todas as doses reduziram significativamente o grau de sedação sem produzir alterações significativas na frequência cardíaca, respiratória e pressão arterial média (GOMES et al., 2019).

Em humanos, os efeitos hemodinâmicos da nalbufina parecem ser irrelevantes do ponto de vista clínico (LAKE et al., 1982). A dose de 10 mg de nalbufina em pacientes com doença coronariana não produziu aumento do débito cardíaco, da pressão arterial pulmonar e da pressão arterial sistêmica, mantendo a estabilidade hemodinâmica (GUSTEIN; AKIL, 2006). Entretanto, na medicina veterinária esse fármaco é pouco utilizado. Há relatos de administração de nalbufina empregada em diferentes espécies, como em cavalos (BRUNSON; MAJORS 1987; KULKARNI et al., 2015), burros (TORAD; HASSAN, 2016), ovelhas (O'HAIR et al., 1988) e cães (FOURNIER et al.,2000; LESTER et al., 2003; FRAZÍLIO et al., 2014). Em mamíferos, o efeito analgésico da nalbufina tem duração de 3 a 6 horas, de modo que sua duração é maior que a do butorfanol (GUZMAN et al., 2011). As doses de 0,25 a 1 mg/kg intramuscular, subcutâneo ou intravenoso são recomendadas para o uso em cães e gatos (KUKANICH; WIESE, 2015).

Um estudo comparou a sedação e os efeitos hemodinâmicos da nalbufina ou do butorfanol associados ou não à acepromazina em cães. Os autores concluíram que a administração intravenosa de nalbufina (0,5 mg/kg) associada ou não à acepromazina produziu mínimas reduções na pressão arterial média, frequência cardíaca e respiratória (GOMES et al., 2018). Entretanto, mediante a literatura consultada, não há estudos avaliando os potenciais efeitos da nalbufina na função sistólica e diastólica do ventrículo esquerdo em cães. Esse tipo de estudo é fundamental na compreensão dos efeitos farmacológicos desse opioide no funcionamento do coração e, portanto, embasarão o uso com segurança na medicina veterinária.

1.2 Ecocardiografia bidimensional *speckle tracking*

As variáveis ecocardiográficas convencionais apresentam baixa sensibilidade para a avaliação detalhada da contração miocárdica. Portanto, muitas vezes, a avaliação da função sistólica torna-se subjetiva e imprecisa. Com o objetivo de melhorar a acurácia da quantificação da função sistólica, novas técnicas ecocardiográficas surgem para avaliar a deformação miocárdica. O *strain* e *strain rate* miocárdico representam a deformação e taxa de deformação, respectivamente, de um dado segmento do músculo cardíaco durante as fases de sístole e diástole (D'HOOGE et al., 2000).

O estudo da deformação leva em consideração que o coração possui uma estrutura anatômica helicoidal, ou seja, o músculo cardíaco é formado por uma banda miocárdica única enrolado em si mesmo e ancorado no anel pulmonar e aórtico. Essa banda única distingue-se em: banda basal, envolvendo a região dos anéis mitral e tricúspide, composta por fibras de direção circular, responsáveis pela fase de contração isovolumétrica na sístole; banda descendente, composta por fibras oblíquas e longitudinais, predominantes na região média e apical, refletindo-se no ápice, responsável pela ejeção ventricular esquerda e redução da câmara durante a sístole; e banda ascendente, responsável pelo aumento de tamanho da câmara no momento de sucção ou enchimento rápido ventricular (KOCICA et al., 2006).

Dentro de um plano ortogonal, sabe-se que a deformação do miocárdio ventricular ocorre em três orientações: longitudinal, radial e circunferencial. A ação em conjunto e simultânea proporciona a máxima eficiência e o esvaziamento adequado da câmara ventricular. O estudo individualizado da deformação em cada orientação, permite a avaliação aprimorada da contratilidade cardíaca e, consequentemente, da função sistólica (D'HOOGE et al., 2000).

De acordo com os métodos de avaliação da deformação, a ressonância nuclear magnética utilizando marcadores ionizados, codificação da deformação ou contraste de fase é considerada o padrão ouro (ROSEN et al., 2004; NEIZEL et al., 2009; DELFINO et al., 2008). Entretanto, o custo elevado, o longo tempo para aquisição das imagens, a baixa acessibilidade e a necessidade de anestesia geral são fatores que limitam seu uso na rotina clínica da medicina veterinária.

Portanto, na década de 90 introduziu-se um novo método para quantificação da deformação miocárdica por meio da utilização do Doppler tecidual (HEIMDAL et al.,

1998). Todavia, essa técnica possuiu algumas limitações importantes, visto que a qualidade de sinal do Doppler é dependente do ângulo de insonação, o qual varia constantemente durante o exame ecocardiográfico devido ao movimento translacional do coração. Além disso, pela mesma razão, as regiões apicais também não podem ser devidamente avaliadas por esse método (STORAA et al., 2003).

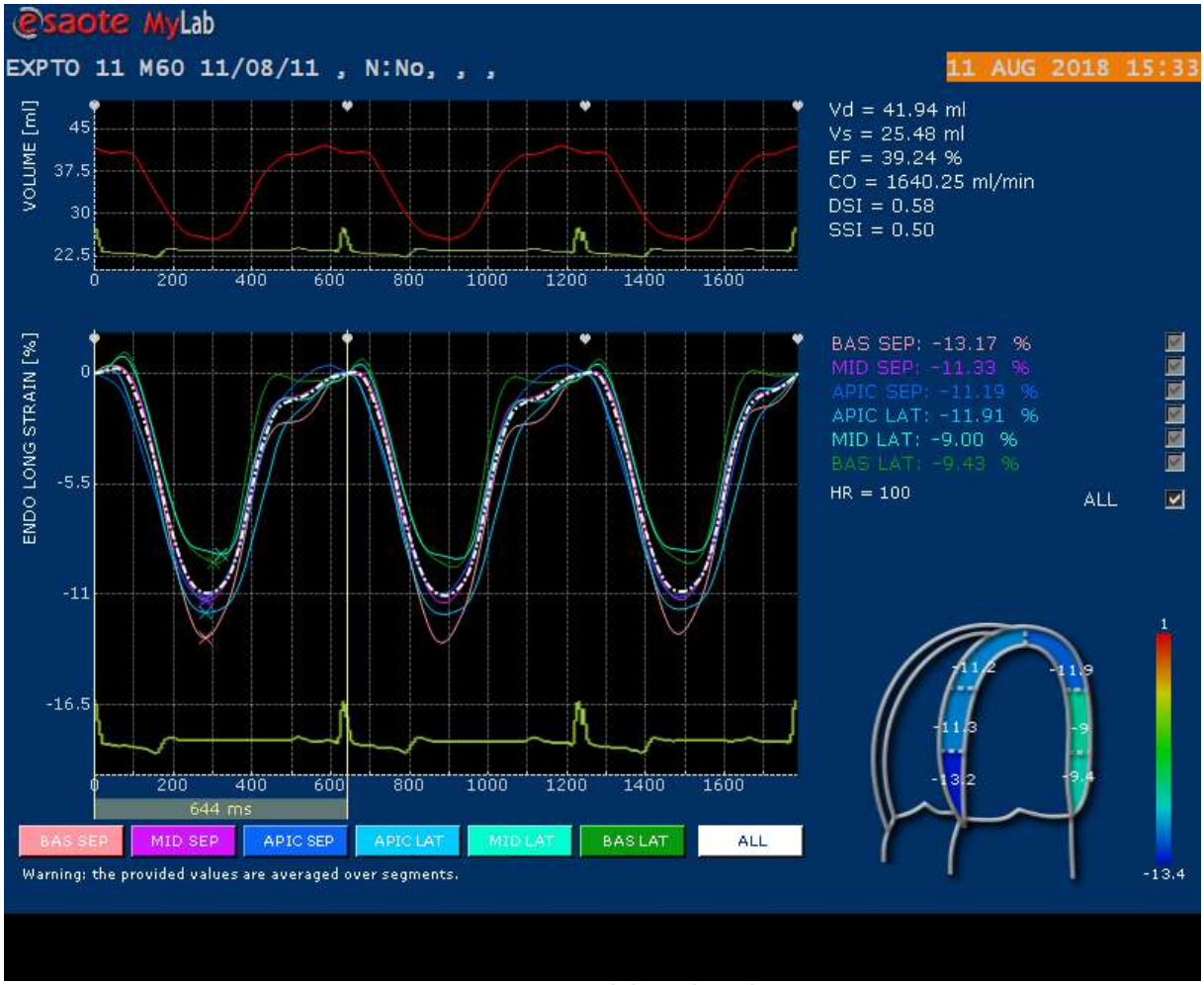
Com a finalidade de superar as limitações do Doppler tecidual, surge a técnica de avaliação da deformação miocárdica pelo método *speckle tracking*. Esse método se fundamenta no mecanismo de rastreamento de pontos que são marcas acústicas digitais originadas pela interação entre o ultrassom e o músculo cardíaco, criando pontos brilhantes (*speckles*) no miocárdio, os quais podem ser monitorados ou seguidos (*tracking*) quadro a quadro, ao longo do ciclo cardíaco. A técnica de *speckle tracking* bidimensional é ângulo-independente e não sofre influência da movimentação translacional do coração, ou seja, os valores obtidos refletem diretamente a deformação miocárdica, permitindo a avaliação inclusive da região apical (PAVLOPOULOS; NIHOYANNOPOULOS, 2008; CHETBOUL, 2010; COLLIER; PHELAN; KLEIN, 2017).

O deslocamento dos *speckles* gera um *loop* com mudanças instantâneas de direção e velocidade, as quais são representadas por vetores que alteram de tamanho e direção, conforme o miocárdio contrai ou relaxa (DEL CASTILLO et al., 2010). Cada deslocamento é representado graficamente por uma curva em função do tempo, denominado de taxa de deformação ou *strain rate*, expresso em s^{-1} ou 1/s. O cálculo da integral da velocidade abaixo da curva deriva a deformação ou *strain*, que mostra o percentual de deformação miocárdica em relação ao seu ponto inicial (DEL CASTILLO et al., 2010).

A deformação e a taxa de deformação podem ser observadas em diferentes planos. Normalmente, são avaliadas nos planos ortogonais: longitudinal, radial e circunferencial, sendo perpendiculares entre si, englobando fibras miocárdicas em suas diferentes orientações. As fibras longitudinais localizam-se na porção endocárdica do ventrículo esquerdo e podem ser estudadas a partir da imagem apical quatro câmaras. Essas fibras sofrem encurtamento durante a sístole, resultando em valores negativos de *strain* e *strain rate* (Figura 1 e 2). As deformações circunferencial e radial são avaliadas a partir da imagem transversal do ventrículo esquerdo. A deformação circunferencial sofre uma redução de seu diâmetro, resultando em valores

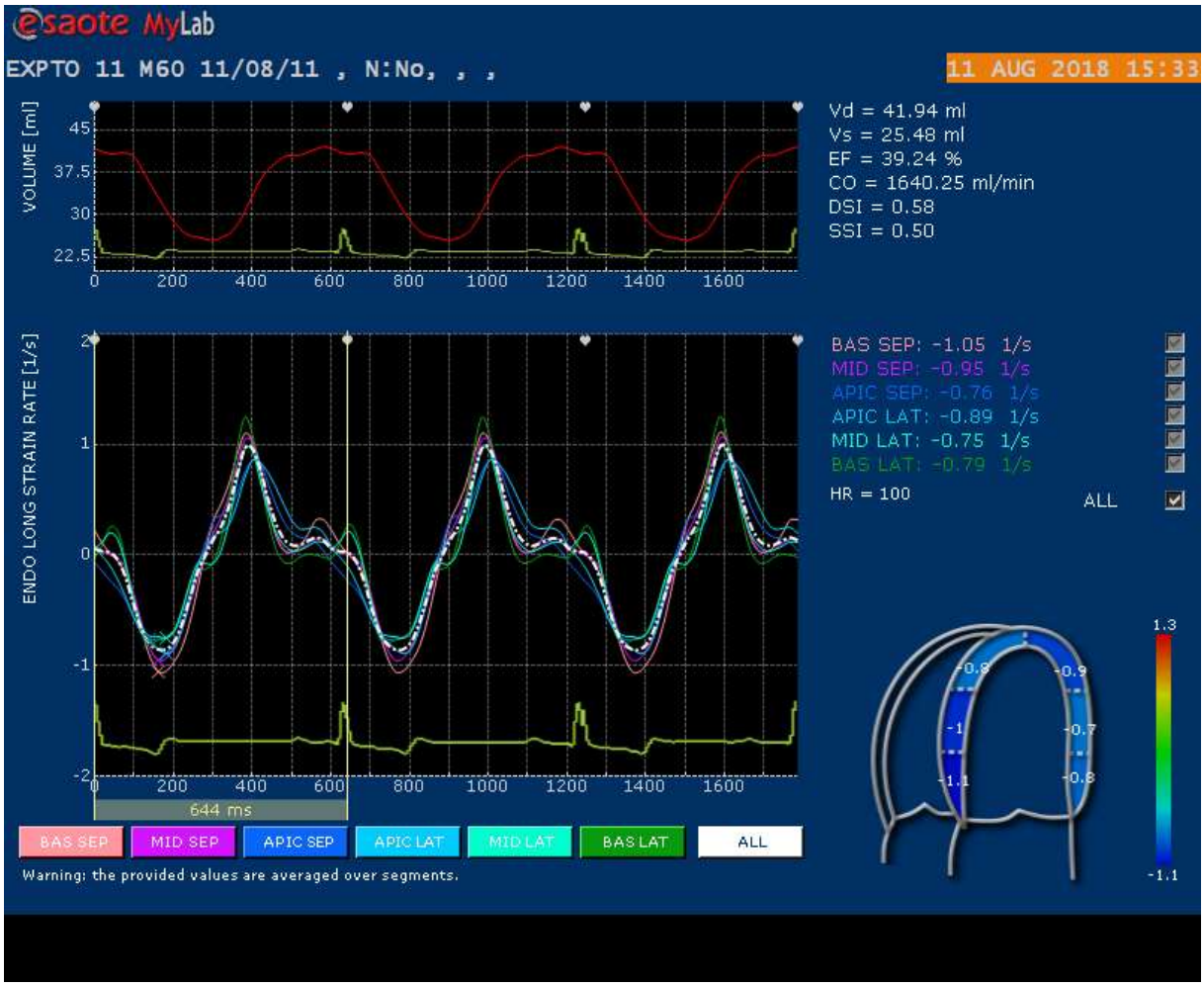
negativos de *strain* e *strain rate* (Figura 3 e 4). Já a radial sofre espessamento durante a sístole originando valores positivos (Figura 5 e 6) (BLESSBERGER; BINDER, 2010; DANDEL et al., 2009).

Figura 1 – *Strain* longitudinal obtido pela técnica de *speckle tracking* bidimensional a partir da janela paraesternal esquerda e imagem apical quatro câmaras, em cão saudável anestesiado com sevofluorano.



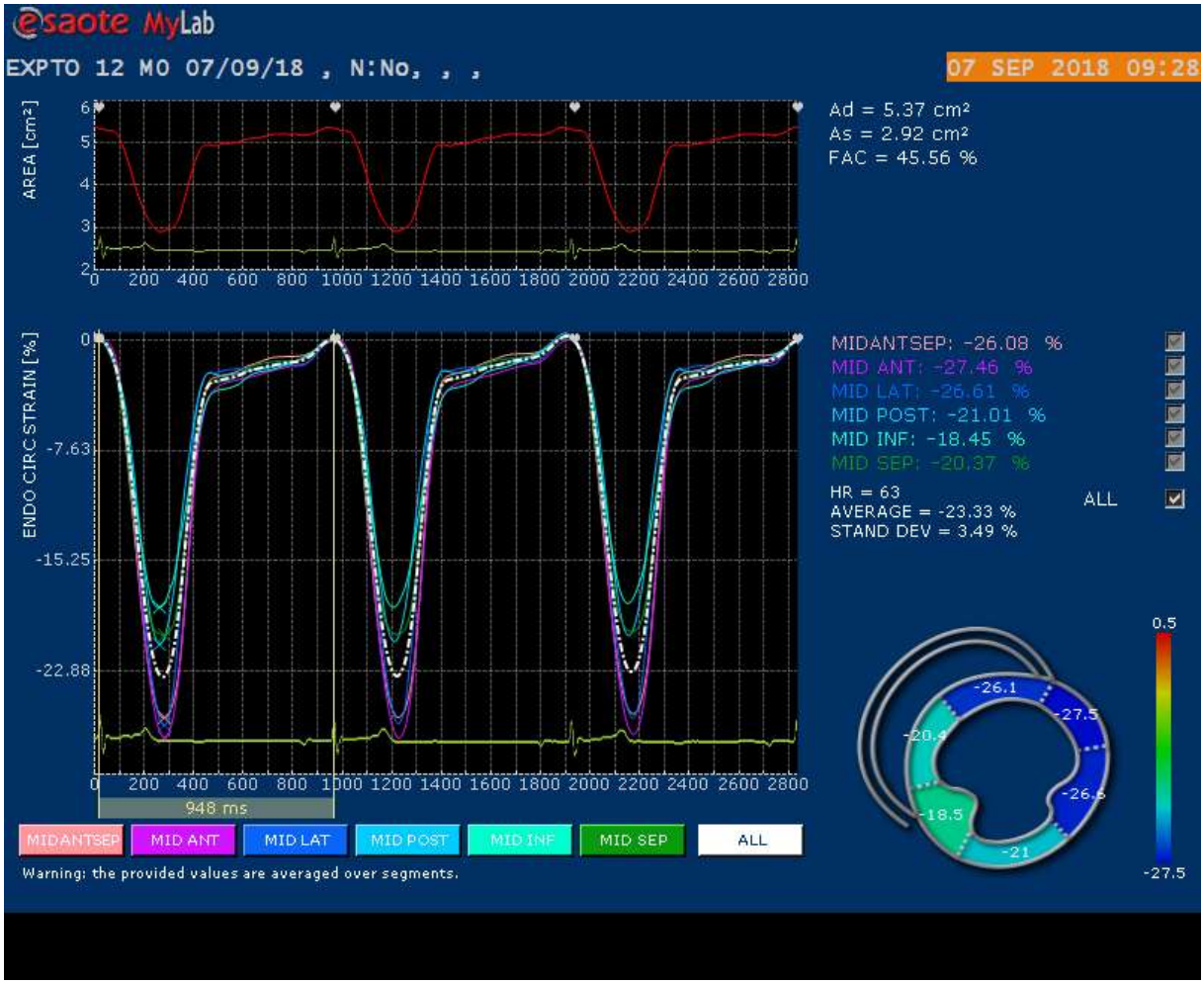
Fonte: Elaborado pelo autor.

Figura 2 – *Strain rate* longitudinal obtido pela técnica de *speckle tracking* bidimensional a partir da janela paraesternal esquerda e imagem apical quatro câmaras, em cão saudável anestesiado com sevofluorano.



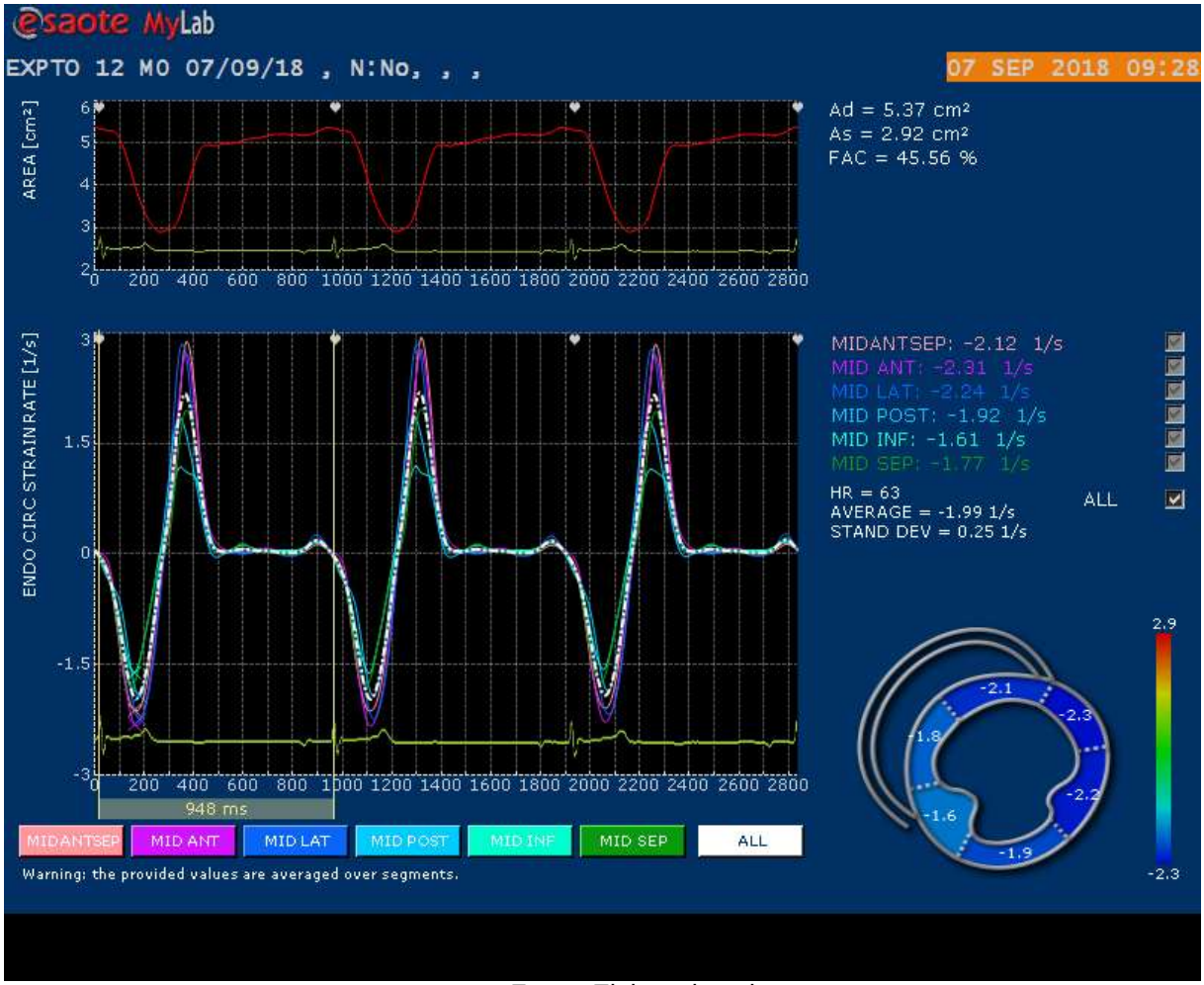
Fonte: Elaborado pelo autor.

Figura 3 – *Strain* circunferencial obtido pela técnica de *speckle tracking* bidimensional a partir da janela paraesternal direita e imagem transversal do ventrículo esquerdo no plano papilar, em cão saudável anestesiado com sevofluorano.



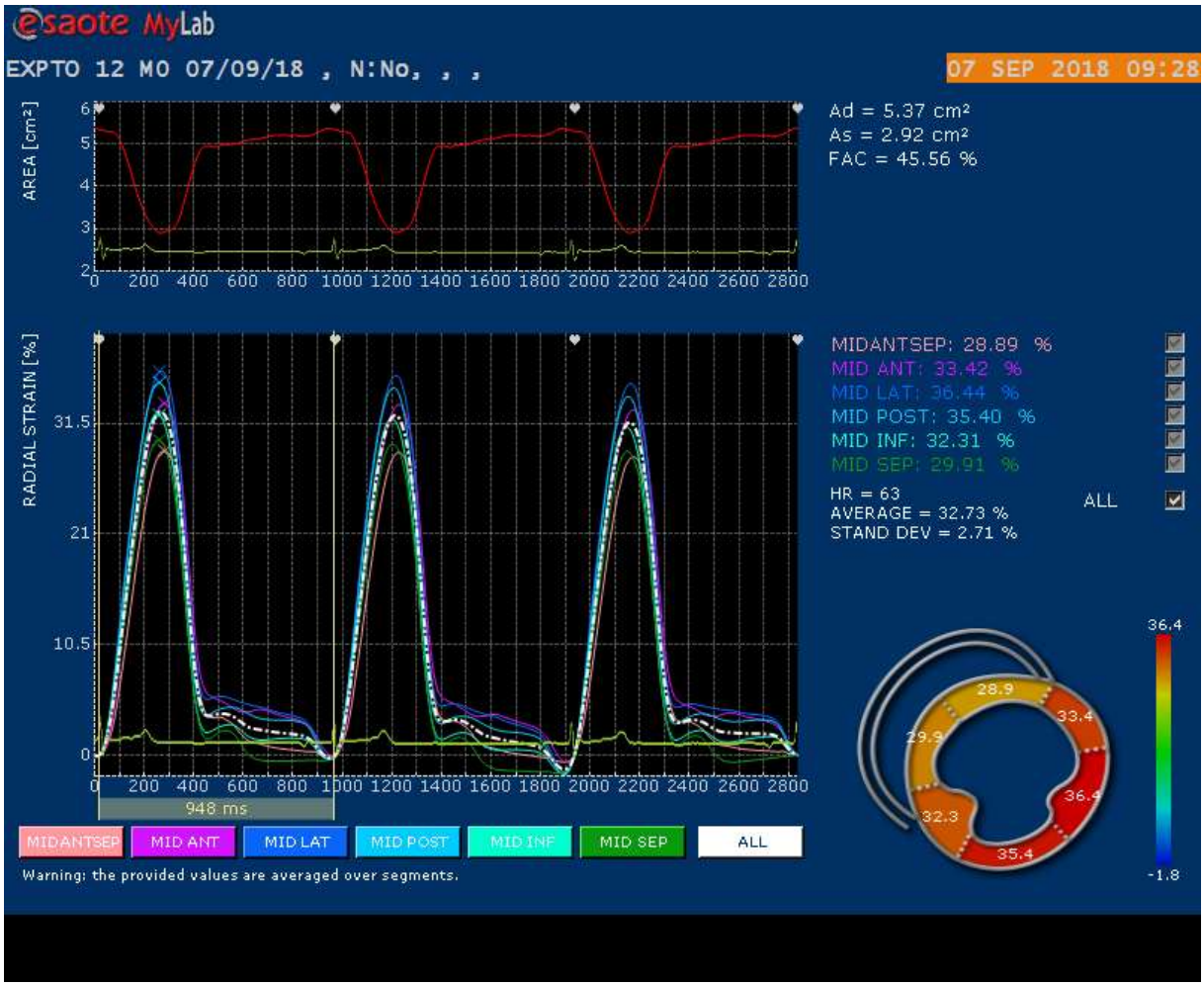
Fonte: Elaborado pelo autor.

Figura 4 – *Strain rate* circunferencial obtido pela técnica de *speckle tracking* bidimensional a partir da janela paraesternal direita e imagem transversal do ventrículo esquerdo no plano papilar, em cão saudável anestesiado com sevofluorano.



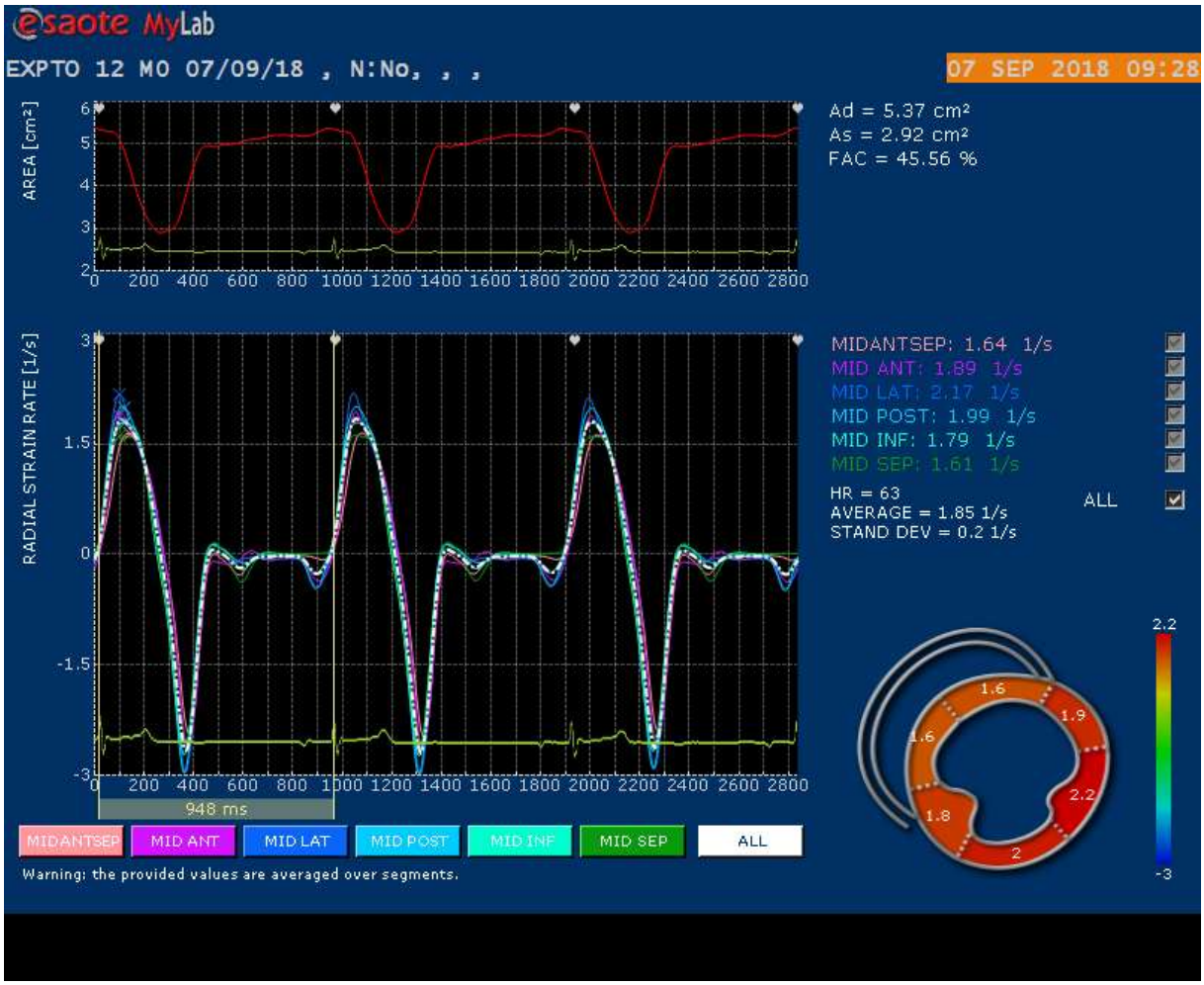
Fonte: Elaborado pelo autor.

Figura 5 – *Strain* radial obtido pela técnica de *speckle tracking* bidimensional a partir da janela paraesternal direita e imagem transversal do ventrículo esquerdo no plano papilar, em cão saudável anestesiado com sevofluorano.



Fonte: Elaborado pelo autor.

Figura 6 – *Strain rate* radial obtido pela técnica de *speckle tracking* bidimensional, a partir da janela paraesternal direita e imagem transversal do ventrículo esquerdo no plano papilar, em cão saudável anestesiado com sevofluorano.



Fonte: Elaborado pelo autor.

Todas as medidas de deformação e taxa de deformação são aferidas de forma segmentar, ou seja, o software divide o miocárdio do ventrículo esquerdo em porções e entrega os valores individuais de deformação. A análise em conjunto, ou seja, a média de todos os valores, permite a avaliação global da deformação e contratilidade cardíaca, originando o *strain* e *strain rate* global, que são os parâmetros mais utilizados no estudo da função sistólica (BLESSBERGER; BINDER, 2010).

A literatura está repleta de exemplos da aplicação prática do *speckle tracking* bidimensional, tanto na medicina humana, quanto na veterinária. Sua utilização entrega a avaliação detalhada da função miocárdica com aplicação em diferentes situações, como: sincronização cardíaca, rejeição aguda de transplantes cardíacos, cardiotoxicidade induzida por quimioterapia, cardiomiopatia hipertrófica e dilatada

entre outros (HERNANDEZ-SUAREZ; CANDALES-LÓPEZ, 2017). Além disso, mais recentemente, tem sido utilizada em outras condições clínicas, como o acompanhamento da função ventricular em pacientes em síndrome da resposta inflamatória sistêmica (CORDA et al., 2019).

A grande vantagem é que a deformação derivada do *speckle tracking*, identifica alterações precoces de disfunção sistólica, antes que haja qualquer mudança das variáveis ecocardiográficas convencionais (DICKSON et al., 2017; EDVARDSEN; HALLE-VALLE; SMISETH, 2006; WANG et al., 2008). Isso é fundamental no âmbito prático, já que a detecção da disfunção sistólica em sua fase inicial gera mudanças de condutas clínicas e de prognóstico, que serão determinantes para as tomadas de decisões. Além disso, o *strain* e *strain rate* são menos influenciados pela pré e pós-carga, conferindo outra vantagem com relação aos parâmetros ecocardiográficos convencionais de função sistólica (MARWICK, 2006).

O *speckle tracking* pode ser utilizado como método de estudo na avaliação dos efeitos de diferentes fármacos na função sistólica do ventrículo esquerdo (WANG et al., 2015; BERLI et al., 2015; SANTARELLI; LÓPEZ; DEL PALACIO, 2017). Alguns autores mostram que essa técnica é capaz de detectar alterações precoces de fármacos na contratilidade cardíaca (MIZUGUCHI et al., 2008). Kang et al. (2013) avaliaram os efeitos cardiotóxicos das antraciclinas no homem por meio da ecocardiografia convencional e *speckle tracking*. Apesar da manutenção dos parâmetros ecocardiográficos convencionais de função sistólica, o *strain* e *strain rate* reduziram significativamente, mostrando a alta sensibilidade do método na detecção de disfunção sistólica inicial.

Na anestesiologia, o *speckle tracking* mostra-se útil na compreensão detalhada dos efeitos de vários fármacos na função ventricular e atrial em diferentes espécies (QUIJANO et al., 2015; MALIK; SUBRAMANIAM; KAPOOR, 2016; BERLI et al., 2015; WANG et al., 2015). Culwell et al. (2011) postularam que a função sistólica ventricular esquerda pode ser avaliada pelo *strain* miocárdico derivado do *speckle tracking* bidimensional, em cães saudáveis anestesiados evidenciando que o *speckle tracking* é uma ferramenta que pode ser aplicada durante a monitoração dos efeitos farmacológicos na anestesia geral. Ainda, alguns estudos mostram que o *speckle tracking* pode ser utilizado na avaliação dos efeitos dos opioides na dinâmica miocárdica. Sabe-se que o miocárdio possui receptores opioides, que podem

influenciar diretamente a função sistólica ventricular. Os receptores κ são os principais reguladores da função miocárdica e, quando estimulados, reduzem as concentrações sarcoplasmáticas de cálcio, podendo resultar em diminuição da contratilidade cardíaca. Um estudo utilizando uma substância opioide com propriedades κ agonista (U-50,488H), mostrou que houve redução significativa da concentração de cálcio sarcoplasmático e consequentemente houve inotropismo negativo (VENTURA et al., 1992). Portanto, a nalbufina por ser tratar de um opioide com propriedades κ agonista, acredita-se que possa haver interações na dinâmica miocárdica.

Até o momento, mesmo com o uso do *speckle tracking*, alguns opioides, como o butorfanol e o sulfentanil mostram-se seguros sob o ponto de vista cardiovascular, já que não alteraram o *strain* e *strain rate* do ventrículo esquerdo (BHAVSAR et al., 2011; SANTARELLI; LÓPEZ; DEL PALACIO, 2017). Entretanto, mediante a literatura consultada, não há estudos avaliando os efeitos da administração de nalbufina na função sistólica e diastólica de cães saudáveis. A utilização dos parâmetros ecocardiográficos oriundos da técnica de *speckle tracking* são de grande valia para a avaliação primorosa (sintonia fina) e detalhada da dinâmica miocárdica.

2 CAPÍTULO 1 – CONVENTIONAL ECHOCARDIOGRAPHY AND TWO-DIMENSIONAL SPECKLE TRACKING IN HEALTHY SEVOFLURANE-ANESTHETIZED DOGS UNDERGOING CONTINUOUS RATE INFUSION OF NALBUPHINE

Marcel G. Marques, DVM, MSc; Carlos Eduardo de Siqueira, DVM; Ana Elisa G.W. Marques, DVM, MSc; Élen AP de Sousa, DVM; Yan S. Ribeiro; Beatriz P. Floriano, DVM, MSc, PhD; Wagner L. Ferreira, DVM, MSc, PhD; Paulo S. P. Santos, DVM, MSc, PhD.

From the Department of Animal Clinic, Surgery and Reproduction, School of Veterinary Medicine of Araçatuba (FVMA) – São Paulo State University (UNESP), 793 Clóvis Pestana st., 16050-680, Araçatuba, São Paulo State, Brazil.

Address correspondence to Mr. Marques (marcelvetcardio@hotmail.com)

2.1 Abstract

Objective: To assess the effects of a continuous rate infusion (CRI) of nalbuphine on left ventricular systolic and diastolic function of healthy sevoflurane-anesthetized dogs.

Animals: Eighteen mixed-breed bitches aged 1-4 years and weighing 9.9 ± 3.8 kg.

Procedures: Dogs were randomly assigned to one of two groups: nalbuphine (G_N, n=9); and control (G_C, n=9). Anesthesia was induced and maintained with sevoflurane (2V%) followed by an intravenous (IV) bolus of nalbuphine (0.3 mg/kg) or 0.9% NaCl at equal volume, then CRI of nalbuphine (0.4 mg/kg/h) or 0.9% NaCl at equal infusion rate. Conventional echocardiography, two-dimensional speckle tracking and hemodynamic variables were determined at baseline and 20, 40, 60 and 80 minutes following start of CRI.

Results: No differences were found between groups for left ventricular systolic and diastolic variables obtained through conventional echocardiography and two-dimensional speckle tracking. Hemodynamic variables did not differ between groups. The E′/A′ ratio significantly increased at 20 minutes compared to baseline only in G_N.

Conclusions and clinical relevance: This is the first study assessing the effects of nalbuphine on conventional and two-dimensional speckle tracking echocardiographic parameters of healthy dogs. According to our results, nalbuphine given at a CRI does not influence left ventricular systolic and diastolic function in healthy sevoflurane-anesthetized dogs.

2.2 Abbreviations

ANOVA – analysis of variance
AVA – aortic valve area
BSA – body surface area
CRI – continuous rate infusion.
DAP – diastolic arterial pressure
DCI – Doppler cardiac index
DEI – Doppler ejection index
EDVI – end-diastolic volume index
EF – ejection fraction
ESVI – end-systolic volume index
GCS – global circumferential strain

GCSSR – global circumferential systolic strain rate

GLS – global longitudinal strain

GLSSR – global longitudinal systolic strain rate

GRS – global radial strain

GRSSR – global radial systolic strain rate

IV – intravenous

IVRT – isovolumic relaxation time

MAP – mean arterial pressure

PVRI – peripheral vascular resistance index

SAP – systolic arterial pressure

SpO_2 – oxyhemoglobin saturation

G_C – control group

G_N – nalbuphine group

VTI – velocity-time integral

2.3 Introduction[1]

Nalbuphine is an opioid drug that acts as an agonist to κ receptors and antagonist to μ receptors, with analgesic potency similar to that of morphine.[1] An important advantage of nalbuphine is its minimal depressant effect on respiratory function. Nalbuphine can also be used to reverse the respiratory depression caused by full μ agonists, while still providing desirable analgesia.[2]

Although nalbuphine has interesting clinical and pharmacological characteristics, its use in veterinary clinical practice is limited by lack of studies regarding its effects on cardiovascular function. A few studies demonstrated a decrease in MAP and HR with the use of nalbuphine in dogs.[2,3] However, no studies were found addressing the effects of a CRI of nalbuphine on left ventricular systolic and diastolic function of healthy dogs.

Among the methods currently used to assess ventricular dynamics, echocardiography stands out for being a noninvasive approach that allows real-time observation of systolic and diastolic function.[4] Also, the possibility of assessing myocardial deformity through two-dimensional speckle tracking has made it possible to further understand myocardial dynamics. This technique provides optimal imaging of myocardial deformation in their variable orientations. The speckles formed during the interaction of the ultrasound with the myocardium are tracked frame by frame, thereby providing the velocity of deformation (strain rate) and the percentage of deformation (strain). This technique is highly accurate for the detection of early changes compared to conventional echocardiographic parameters.[5,6] Other authors also demonstrated the efficacy of conventional echocardiography and two-dimensional speckle tracking when assessing the effects of opioids on human and canine myocardial function.[7,8]

Therefore, the objective of this study was to assess the effects of a CRI of nalbuphine on left ventricular systolic and diastolic function of healthy dogs anesthetized with sevoflurane. The hypothesis was that nalbuphine at 0.4 mg/kg/h, when compared to a CRI of saline,

would not produce clinically relevant changes on echocardiographic parameters reflecting left ventricular systolic and diastolic function of sevoflurane-anesthetized dogs.

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[1] American Journal Veterinary Research (Anexo I).

2.4 Material and Methods

2.4.1 Animals

Eighteen mixed-breed bitches aging 2,0 ± 1,0 years and weighing 9.9 ± 3.8 kg were enrolled in the study. Subjects were classified as ASA I according to the American Society of Anesthesiologists[9] and deemed healthy based on physical examination, cardiovascular assessment (echocardiography and electrocardiography), complete blood count, biochemical analysis and leishmaniasis test. Dogs that participated in the study were scheduled for elective ovariohysterectomy and were included after informed consent by owners. The study was approved by the local Ethics Committee for Animal Usage (protocol No. 00385/2018).

2.4.2 Study Design

Dogs were randomly selected to participate in one of two experimental groups, G_N (n=9) and G_C (n=9). G_N received an IV bolus of nalbuphine (0.3 mg/kg) followed by CRI (0.4 mg/kg/h). CRI of nalbuphine was calculated according to the allometric formula based on the study with rats using 1 mg/kg/h. After the calculations the CRI was determined as 0,4 mg/kg/h. G_C received a bolus and CRI of saline at equal volume and infusion rate of G_N. The order of participation was determined by use of an online random number generator (www.randomization.com).

Prior to each procedure, animals were fasted for 8 hours and water was given ad libitum. Preparation comprised clipping the thoracic area for echocardiographic examination, the metatarsus for invasive blood pressure monitoring and the forearm for intravenous fluid or drug administration. Animals were kept in the exam room at 23°C for 20 minutes prior to the procedures to minimize stress and interferences with cardiac variables.

Anesthesia was then induced via facial mask with sevoflurane[a] at 5V% in 100% oxygen at 5 L/min. Following loss of mandibular tonus and laryngotracheal reflex, subjects were intubated and coupled to the anesthetic circuit to receive sevoflurane at 2V% (0.85 minimum alveolar concentration for sevoflurane in dogs)[10] in 100% oxygen at 50 mL/kg/minute. Sevoflurane concentrations were monitored by a digital gas analyzer[b] (DX-2020; Dixtal Biomédica, AM, Brazil) using the manufacturer's internal calibration and adjusted to maintain anesthesia at stage III, 3rd plane. The dorsal

metatarsal artery and the cephalic vein were catheterized for invasive blood pressure monitoring and fluid administration, respectively. Catheters were coupled to a PRN adaptor and flushed with heparinized saline.

With animals fully instrumentalized, anesthesia was stabilized for 15 minutes prior to data collection and animals were allowed to breathe spontaneously throughout the study. Subsequently, baseline variables were recorded, followed by an intravenous (IV) bolus of nalbuphine[c] (0.3 mg/kg, G_N) or saline at equal volume (G_C) and CRI of nalbuphine (0.4 mg/kg/h) or saline at equal infusion rate. Infusions were administered by an infusion pump[d] in both groups.

Data collection happened at baseline and 20, 40, 60 and 80 minutes following start of CRI. Hemodynamic variables comprised: HR, obtained directly from a multiparameter monitor[b]; and invasive arterial blood pressure (SAP, MAP and DAP), recorded from the same monitor with the transducer zeroed at heart level. The multiparameter monitor was also used to record SpO_2 and a digital thermometer was used to measure rectal temperature.

2.4.3 Conventional Echocardiography

Conventional echocardiography[e] was performed using two multifrequency transducers (1-4 MHz and 7.5-10 MHz) with simultaneous electrocardiographic monitoring. One examiner (MGM) who was unaware of the group performed the recordings according to preestablished standards[4] at the left parasternal window for the four-chamber apical view. End-diastolic and -systolic volumes and EF were obtained by the planimetric Simpson method.[4,11] Volumes were indexed to BSA to obtain the EDVI and ESVI.[12] By placing the Doppler cursor on the extremity of the mitral valve, peak velocities of early left ventricular filling (E wave) and atrial contraction (A wave) were measured and the E:A ratio was calculated. Using a five-chamber apical view with the Doppler cursor on the midpoint between left ventricular outflow and transmitral flow, the IVRT was recorded. Finally, the VTI of the aortic flow was obtained with the cursor placed distally to the aortic valve.

Tissue Doppler assessment was performed on the four-chamber apical view with the sample volume placed on the lateral border of the mitral annulus. Two negative peak velocities were obtained: early left ventricular filling (E′ wave) and atrial

contraction (A′ wave) and the E′:A′ ratio was calculated. The S′ wave was determined as the positive peak during ventricular systole.

At the right parasternal window, the AVA was measured at the two-dimensional view of the left ventricle in its short axis and the transducer inclined to allow visualization of the aortic plane.

Recorded variables were further used to calculate DEI, DCI and PVRI, as follows:

DEI (mL/beat/m²) = (VTI [cm] × AVA [cm²]) / BSA, where BSA = weight (g)$^{0.67}$/1000; DCI (L/minute/m²) = (DEI [mL/m²] × FC [beats/minute]) / 1000; and PVRI (dyne × s/cm^5/m²) = (MAP [mmHg] / DCI [L/minute/m²]) × 80.

2.4.4 Two-Dimensional Speckle Tracking Echocardiography

The same echocardiographer was used to assess two-dimensional speckle tracking with two multifrequency transducers of 1-4 MHz and 7.5-10 MHz and simultaneous electrocardiography recording. Only optimal images obtained between 50 and 110 Hz for at least three consecutive cycles were used. Images were stored digitally for further offline analysis using specific software[f]. One examiner (MGM) was responsible for obtaining and analyzing the images. Global circumferential and radial strain (GCS and GRS, respectively) and global circumferential and radial systolic strain rate (GCSSR and GRSSR, respectively) were obtained from the left ventricular short axis at the level of the papillary muscles. Two points were manually inserted, first on the level of the posteromedial papillary muscle and second at lateral wall. The software then traced the area of interest comprising the endocardial and epicardial margins of the left ventricle. If a given point had been misplaced, the examiner performed manual corrections to ensure all margins of interest were included. The algorithm of the software then automatically segmented the left ventricle and performed myocardial tracing along six segments: anterior, anterolateral, inferolateral, inferior, inferoseptal and anteroseptal. Six strain and systolic strain rate profiles were obtained, which corresponded to the mean values of each myocardial segment, and GLS, GRS, GLSSR and GRSSR were calculated automatically.

Global longitudinal strain (GLS) and global longitudinal systolic strain rate (GLSSR) were obtained from the four-chamber apical view with two points manually placed on the left ventricle, one at each side of the mitral annulus, and the third point on the apical region of the endocardial margin. The software then traced the region of interest and

the examiner adjusted the points as needed. The left ventricle was divided into six segments: anterior, anterolateral, inferolateral, inferior, inferoseptal and anteroseptal. The algorithm of the software traced the left ventricle and provided values for strain and systolic strain rate, allowing calculation of GLS and GLSSR.

Each variable was measured in triplicate from three consecutive images and the mean of each time was used to test repeatability. At the end of data collection, subjects were referred to the surgical team for elective ovariohysterectomy.

2.4.5 Statistical Analysis

Variables were first tested for normal distribution using the Shapiro-Wilk test. Group comparisons were performed using two-way analysis of variance (ANOVA) and time comparisons were performed using ANOVA for repeated measures, followed by Dunnett post-hoc test for multiple comparisons. Differences were considered significant when $p<0.05$. All analyses were performed using a commercial software.[g]

Intraobserver repeatability tests were performed for two-dimensional speckle tracking variables as variation coefficient = (standard deviation/mean) $\times$ 100.

2.5 Results

The mean age of the subjects in G_N was 1.6 ± 0.9 years and in G_C 2.4 ± 1.0 years. Mean weight in G_N and G_C was respectively 10.2 ± 4.1 kg and 9.6 ± 3.6 kg.

Variables reflecting left ventricular volumes and systolic function, such as EDVI, ESVI, EF, DEI, DCI and S′ wave did not differ among time points compared to baseline or between groups (Table 1). Speckle tracking variables reflecting systolic function, such as GCS, GCSSR, GRS, GRSSR, GLS and GLSSR were not different between groups or time points (Table 2). With regard to diastolic function, only the E′:A′ ratio showed a significant increase at 20 minutes compared to baseline in G_N. Nevertheless, there were no differences between groups (Table 3).

Hemodynamic variables HR, MAP, SAP, DAP and PVRI did not differ between groups or time points (Table 4) and SpO_2 remained at 100% throughout the experiment in both groups. Also, rectal temperature remained stable between 36.5 and 38.5°C in both groups.

Intraobserver repeatability testing showed a variation coefficient of 1.58-18.95% for two-dimensional speckle tracking variables.

2.6 Discussion

To the best of our knowledge, this study is the first to assess left ventricular systolic and diastolic function of dogs undergoing a CRI of nalbuphine. The results showed that nalbuphine did not produce clinically relevant changes on echocardiographic parameters of healthy sevoflurane-anesthetized dogs. The combined use of conventional echocardiography and speckle tracking allowed a detailed analysis of the effects of nalbuphine on myocardial dynamics. In addition to the known advantages of nalbuphine compared to other opioid drugs, there seems to be no influence of the drug on left ventricular function of healthy dogs, which renders nalbuphine a safe choice from a cardiovascular standpoint. Our results are important in the practical scenario, since the literature showed that the myocardium has opioid receptors that could influence left ventricular function, thus resulting in negative inotropic effect.[13]

Echocardiography has been used previously in animals and humans to assess the effects of various drugs on cardiac function.[14–17] There is a good correlation between echocardiography and standard methods used for hemodynamic monitoring[18] and its main advantage is the safety of its noninvasive approach. In addition, it allows real-time observation of many parameters of the cardiac cycle, thus improving comprehension of left ventricular systolic and diastolic function.

Myocardial deformation occurs in different orientations. The combined action of longitudinal, radial and circumferential myocardial deformation enables the heart to function properly. Individual assessment of each deformation orientation provides a complete understanding of myocardial dynamics.[19] Therefore, we used two-dimensional speckle tracking to assess deformation (strain) and deformation rate (strain rate) of the three main orientations of myocardial deformations. Furthermore, the technique is highly sensitive in detecting early systolic dysfunction compared to conventional methods used in echocardiography.[6] One study showed that by assessing strain and strain rate of dogs undergoing general anesthesia, left ventricular systolic function can be accurately investigated. Therefore, speckle tracking can replace invasive monitoring methods in dogs.[20] Two-dimensional speckle tracking has also proved useful to assess the effects of opioids on cardiac function in both animals and humans.[7,8]

Variables reflecting left ventricular systolic and diastolic function obtained through conventional echocardiography and speckle tracking did not change during infusion of nalbuphine, compared to G_C. These findings demonstrate clearly that nalbuphine at 0.4 mg/kg/h does not influence systolic function of healthy dogs anesthetized with sevoflurane. Previous studies showed that mammalian myocardium can be directly influenced by opioids, given the presence of opioid receptors μ, σ and κ.[21–23] Kappa receptors seem to be the main regulators of cardiac function. By stimulating κ receptors with an opioid substance (U-50,488H), the sarcoplasmic concentrations of calcium decrease, which impairs cardiac contractility.[13] However, although nalbuphine acts as a κ receptor agonist, the stability found in this study can be ascribed to the infusion rate. Other authors showed that higher infusion rates (8 mg/kg/h) can reduce contractility and influence systolic function directly in dogs.[24] One study demonstrated that an IV bolus of 0.5 mg/kg was capable of producing minimal reductions in a few hemodynamic variables,[3] although systolic function was not assessed through echocardiographic examination. Therefore, it is not possible to assume that the effects of nalbuphine on left ventricular systolic function are dose-dependent.

Both EF and S′ wave were close to the lower acceptable limit for the canine species[25] in G_N and G_C. However, there was no difference between groups. Volatile anesthetic agents are known to inhibit sodium and calcium exchange in myocardial fibers, resulting in a negative inotropic effect.[26] So, this could be an effect of sevoflurane anesthesia, not nalbuphine infusion. Similar findings were reported by other authors, thus demonstrating that volatile anesthesia decreases some of the echocardiographic variables of healthy dogs and should be used with caution in patients with systolic dysfunction.[15,16]

While speckle tracking is a validated technique[27] that has been widely used in dogs,[5] current literature shows a significant discrepancy regarding the values of strain and systolic strain rate obtained using software from different manufacturers.[28] The discrepancy hinders the establishment of reference values for the canine species, thus compromising interpretation of the results obtained in this study. However, since no differences were found between groups, it is possible to infer that nalbuphine at 0.4 mg/kg/h does not influence global dynamics of longitudinal, radial and circumferential deformations during sevoflurane anesthesia. Other studies using the same technique also demonstrated that sufentanil and butophanol do not produce significant changes

on left ventricular function, thereby preserving global indices measured by two-dimensional speckle tracking in humans and dogs.[7,8]

The results of HR and systolic function explain the stability seen on DEI and DCI in both groups. As a consequence, SAP, DAP and MAP remained stable throughout the infusions in G_N and G_C. Therefore, as previously discussed, nalbuphine provides hemodynamic stability when given at a CRI for 80 minutes. These findings corroborate other studies showing that nalbuphine administered at a dosage of 0.5 mg/kg, did not produce important changes on HR and MAP in dogs.[3] In addition, one study in humans demonstrated that patients from the intensive care unit who received nalbuphine showed desirable analgesia with hemodynamic stability compared to those receiving sulfentanil.[29] Other authors, however, demonstrated significant reductions of HR and arterial pressure using CRI of nalbuphine at 1 mg/kg/h during inhalation anesthesia in rats.[30]

Transmitral flow and mitral annular motion were used to assess the effects of nalbuphine on left ventricular diastolic function. The study of diastolic function is crucial, since a dysfunction in ventricular relaxation can influence or precede systolic dysfunction.[31] None of the echocardiographic parameters related to diastolic function differed between groups, demonstrating that nalbuphine does not interfere with left ventricular diastolic function.

Doppler transmitral flow analysis showed slightly lower values of A wave velocity compared to reference limits in both groups along all time points.[32] This result is likely explained by sevoflurane anesthesia, since its negative inotropic effect could have decreased atrial contractility, thereby decreasing the A wave velocity. For that reason, the E:A ratio was high (E:A >2) in practically all measurements in both groups. However, given that atrial contractility itself was not assessed in this study, these assumptions could not be confirmed. Similar findings were reported in other studies in dogs, in which the A wave decreased following sedation with acepromazine and butorphanol.[8] Nevertheless, these changes were not clinically relevant in this study, since the E:A ratio remained within acceptable limits for the species (0.92-2.72).[32] In addition, the use of pulsed tissue Doppler showed that E′ wave velocity remained stable in nalbuphine-treated animals. This demonstrates, as described elsewhere,[32] that the active process of myocardial motion during systole remained stable even

under the effects of nalbuphine and sevoflurane. Other parameters from spectral and tissue Doppler analysis also remained within acceptable ranges for dogs.[32]

A few limitations are worth mentioning in this study. While 18 subjects are statistically sufficient to detect differences among groups and time points, a higher number of subjects could elicit significant changes that could not be evidenced in this study. Furthermore, speckle tracking, although an advantageous technique, is extremely dependent on two-dimensional high-resolution imaging, which was not always possible in the apical portion of the left ventricle, thus hindering the process of tracking some myocardial points. Finally, the use of combined sevoflurane limits assessment of the effects of nalbuphine alone on left ventricular systolic and diastolic function.

In conclusion, nalbuphine given at a continuous rate infusion of 0.4 mg/kg/h did not influence conventional and two-dimensional speckle tracking echocardiographic parameters of left ventricular systolic and diastolic function in healthy sevoflurane-anesthetized dogs along 80 minutes of observation.

2.7 Footnotes

a. Sevocris 1 mL/mL, Cristália – Produtos Químicos Farmacêuticos Ltda, Itapira, São Paulo State, Brazil.

b. Dixtal - mod. DX-2020 - Manaus, Amazonas State, Brazil.

c. Nubain 10 mg/mL, Cristália – Produtos Químicos Farmacêuticos Ltda, Itapira, São Paulo State, Brazil.

d. Syringe pump 680 – Samtronic, São Paulo, São Paulo State, Brazil.

e. MyLab 30 Gold VET, Genoa, Italy.

f. MylabTM Desk algorithm X-strainTM, Genoa, Italy.

g. GraphPad Prism, Inc version 8.2.0 2019 software.

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Table 1 – Mean ± standard deviation of echocardiographic variables reflecting left ventricular volumes and systolic function in 18 bitches anesthetized with sevoflurane and undergoing continuous rate infusion of nalbuphine (G_N) or saline (G_C) over 80 minutes of observation.

Variable	Group	Time (minutes)				
		Baseline	**20**	**40**	**60**	**80**
EDVI (mL/m²)	G_N	34.9 ± 9.6	34.1 ± 10.3	34.7 ± 8.8	33.3 ± 7.6	35.2 ± 9.7
	G_C	32.5 ± 6.0	33.3 ± 4.8	32.7 ± 4.7	32.3 ± 6.6	33.3 ± 5.1
ESVI (mL/m²)	G_N	16.7 ± 5.0	15.7 ± 5.2	15.5 ± 3.5	15.1 ± 3.4	15.8 ± 4.4
	G_C	15.5 ± 3.2	14.3 ± 2.3	14.8 ± 3.5	14.7 ± 3.5	15.6 ± 2.9
EF (%)	G_N	51 ± 9	53 ± 8	55 ± 9	54 ± 8	54 ± 7
	G_C	52 ± 7	57 ± 6	55 ± 9	55 ± 6	53 ± 7
S′ wave (m/s)	G_N	0.07 ± 0.02	0.08 ± 0.02	0.09 ± 0.03	0.09 ± 0.03	0.08 ± 0.03
	G_C	0.08 ± 0.03	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
DEI (mL/beat/m²)	G_N	37.7 ± 8.1	42.9 ± 9	43.1 ± 8.5	44.8 ± 10.1	43.3 ± 9.5
	G_C	32.9 ± 10.0	34.1 ± 11.5	33.7 ± 10.6	32.5 ± 9.2	34.1 ± 11.3
DCI (L/minute/m²)	G_N	4.0 ± 1.3	3.9 ± 1.7	4.1 ± 1.46	4.4 ± 2.1	4.1 ± 1.7
	G_C	3.5 ± 1.2	3.5 ± 1.3	3.5 ± 1.2	3.2 ± 1.0	3.5 ± 1.2

Variables do not differ according to ANOVA ($p>0.05$). EDVI, End-diastolic volume index; ESVI, end-systolic volume index; EF, ejection fraction; S′ wave, peak systolic mitral annular velocity derived from tissue Doppler; DEI, Doppler ejection index; DCI, Doppler cardiac index.

Table 2 – Mean ± standard deviation of echocardiographic variables reflecting left ventricular systolic function obtained through two-dimensional speckle tracking in 18 bitches anesthetized with sevoflurane and undergoing continuous rate infusion of nalbuphine (G_N) or saline (G_C) over 80 minutes of observation.

Variable	**Group**	**Time (minutes)**				
		Baseline	**20**	**40**	**60**	**80**
GCS (%)	G_N	-19.8 ± 6.8	-21.2 ± 3.9	-22.4 ± 5.9	-20.2 ± 5.3	-21.3 ± 4.4
	G_C	-19.5 ± 3.7	-21.1 ± 5.3	-21.4 ± 4.3	-20.7 ± 4.3	-20.8 ± 4.0
GCSSR (s^{-1})	G_N	-1.0 ± 0.7	-1.8 ± 0.5	-1.9 ± 0.7	-1.6 ± 0.6	-1.8 ± 0.6
	G_C	-1.7 ± 0.4	-1.7 ± 0.5	-1.8 ± 0.4	-1.7 ± 0.4	-1.7 ± 0.3
GRS (%)	G_N	23.9 ± 6.3	24.1 ± 6.5	27.8 ± 5.8	24.7 ± 6.8	27.0 ± 7.5
	G_C	22.5 ± 6.6	30.1 ± 6.2	28.7 ± 7.1	30.6 ± 7.0	26.2 ± 6.1
GRSSR (s^{-1})	G_N	1.4 ± 0.4	1.4 ± 0.2	1.8 ± 0.5	1.6 ± 0.4	1.6 ± 0.5
	G_C	1.6 ± 0.3	2.0 ± 0.5	1.8 ± 0.5	1.9 ± 0.4	1.6 ± 0.4
GLS (%)	G_N	-12.2 ± 2.1	-13.5 ± 1.8	-13.7 ± 2.6	-13.3 ± 2.7	-13.4 ± 2.1
	G_C	-14.4 ± 3.2	-14.0 ± 2.3	-15.6 ± 1.8	-15.1 ± 2.0	-14.8 ± 2.2
GLSSR (s^{-1})	G_N	-1.0 ± 0.2	-1.1 ± 0.2	-1.1 ± 0.2	-1.0 ± 0.2	-1.1 ± 0.2
	G_C	-1.3 ± 0.3	-1.1 ± 0.1	-1.3 ± 0.2	-1.1 ± 0.1	-1.2 ± 0.2

Variables do not differ according to ANOVA ($p>0.05$). GCS, Global circumferential strain; GCSSR, global circumferential systolic strain rate; GLS, global longitudinal strain; GLSSR, global longitudinal systolic strain rate; GRS, global radial strain; GRSSR, global radial systolic strain rate.

Table 3 – Mean ± standard deviation of echocardiographic variables reflecting left ventricular diastolic function in 18 bitches anesthetized with sevoflurane and undergoing continuous rate infusion of nalbuphine (G_N) or saline (G_C) over 80 minutes of observation.

Variable	Group	Time (minutes)				
		Baseline	**20**	**40**	**60**	**80**
E wave (m/s)	G_N	0.64 ± 0.20	0.68 ± 0.18	0.71 ± 0.20	0.70 ± 0.19	0.68 ± 0.22
	G_C	0.63 ± 0.09	0.63 ± 0.09	0.63 ± 0.10	0.63 ± 0.11	0.59 ± 0.10
A wave (m/s)	G_N	0.30 ± 0.09	0.27 ± 0.09	0.34 ± 0.11	0.30 ± 0.13	0.33 ± 0.12
	G_C	0.30 ± 0.09	0.27 ± 0.10	0.32 ± 0.08	0.28 ± 0.05	0.33 ± 0.08
E:A ratio	G_N	2.32 ± 1.06	2.75 ± 1.15	2.28 ± 0.96	2.68 ± 1.39	2.30 ± 1.16
	G_C	2.23 ± 0.59	2.59 ± 1.00	2.03 ± 0.42	2.34 ± 0.61	1.87 ± 0.46
IVRT (ms)	G_N	55 ± 9	53 ± 9	60 ± 18	57 ± 13	63 ± 16
	G_C	55 ± 6	57 ± 10	60 ± 9	59 ± 7	63 ± 12
E′ wave (m/s)	G_N	0.11 ± 0.03	0.12 ± 0.02	0.12 ± 0.05	0.12 ± 0.03	0.13 ± 0.04
	G_C	0.12 ± 0.03	0.12 ± 0.03	0.12 ± 0.03	0.11 ± 0.02	0.11 ± 0.03
A′ wave (m/s)	G_N	0.06 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.01
	G_C	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	0.06 ± 0.02
E′:A′ ratio	G_N	1.94 ± 1.00	2.52 ± 1.06*	2.14 ± 0.92	2.23 ± 1.00	2.31 ± 0.98
	G_C	2.17 ± 0.93	2.09 ± 0.85	1.77 ± 0.52	1.94 ± 0.55	1.85 ± 0.51

*Significantly different compared to baseline according to Dunnett test ($p<0.05$). E wave, Peak velocity of early left ventricular filling; A wave, peak velocity of atrial contraction; IVRT, isovolumic relaxation time; E′ wave, peak early left ventricular filling derived from tissue Doppler; A′ wave, peak velocity of atrial contraction derived from tissue Doppler.

Table 4 – Mean ± standard deviation of hemodynamic variables in 18 bitches anesthetized with sevoflurane and undergoing continuous rate infusion of nalbuphine (G_N) or saline (G_C) over 80 minutes of observation.

Variable	Group	Time (minutes)				
		Baseline	**20**	**40**	**60**	**80**
HR (beats/minute)	G_N	103 ± 18	89 ± 20	94 ± 18	95 ± 23	93 ± 21
	G_C	108 ± 17	104 ± 23	103 ± 13	99 ± 13	101 ± 10
SAP (mmHg)	G_N	105 ± 19	99 ± 24	105 ± 20	107 ± 23	105 ± 27
	G_C	93 ± 33	101 ± 16	100 ± 10	106 ± 12	106 ± 18
DAP (mmHg)	G_N	57 ± 11	55 ± 12	57 ± 11	58 ± 12	58 ± 11
	G_C	59 ± 10	57 ± 12	55 ± 6	58 ± 6	61 ± 8
MAP (mmHg)	G_N	71 ± 12	68 ± 15	72 ± 14	72 ± 15	72 ± 13
	G_C	74 ± 11	71 ± 13	70 ± 6	73 ± 7	76 ± 10
PVRI (dyne × s/cm^5/m^2)	G_N	1575 ± 589	1540 ± 496	1525 ± 513	1446 ± 444	1597 ± 605
	G_C	1877 ± 806	1870 ± 938	1832 ± 777	2005 ± 787	2009 ± 865

Variables do not differ according to ANOVA ($p>0.05$). HR, Heart rate; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; PVRI, peripheral vascular resistance index.

Apêndice A – Referências da Introdução Geral

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Anexo I – Normas da revista

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The AVMA has adopted the following policy on editorial independence of the *AJVR*:

> The AVMA recognizes and fully accepts the need for editorial independence of the AVMA journals and grants the editor-in-chief full authority over the editorial content of the journals, including the selection of content for publication and the timing of publication of that content. For these purposes, editorial content is understood to include research articles, other types of scientific reports, opinion articles, news, and advertising. Opinions and statements expressed in the AVMA journals are those of the contributors and do not represent the official policy of the AVMA, unless so stated. AVMA management does not interfere in the evaluation, selection, or editing of individual articles published in the AVMA journals, either directly or by creating an environment that strongly influences decisions of the editor-in-chief.

Funding and support

All funding, other financial support (eg, grant support), and material support (eg, provision of equipment or supplies) received directly or indirectly (via an author's institution) from any third party (eg, any government agency, foundation, or commercial enterprise) in connection with the study or writing of the manuscript must be clearly and completely described in the Acknowledgments section of the manuscript. If no third-party funding or support was received, the following statement or an equivalent should be included: No third-party funding or support was received in connection with this study or the writing or publication of the manuscript.

The authors must also include a relevant statement in the Acknowledgments section if any funding organization or sponsor had any role in the design or conduct of the study; collection, analysis, or interpretation of the data; writing or approval of the manuscript; or decision to

submit the manuscript for publication. Alternatively, the following statement or an equivalent should be included: Funding sources did not have any involvement in the study design, data analysis and interpretation, or writing and publication of the manuscript.

Failure to fully disclose sources of financial and other support may be grounds for rejection or retraction of the manuscript.

Humane animal care and use

To be considered for publication in the *AJVR*, all research studies involving animals must have been performed in compliance with guidelines outlined in the US Animal Welfare Act (jav.ma/AWA), US Public Health Service Policy on the Humane Care and Use of Laboratory Animals (jav.ma/HCULA), National Research Council's Guide for the Care and Use of Laboratory Animals (jav.ma/GCULA), or Guide for the Care and Use of Agricultural Animals in Research and Teaching (jav.ma/GCUAART) or in compliance with equivalent guidelines. If animals were euthanized, the method of euthanasia must be indicated in the manuscript. Methods of euthanasia must comply with AVMA Guidelines for the Euthanasia of Animals (jav.ma/GEA). If a method not recommended by the AVMA Guidelines on Euthanasia was used, a justification for use of this method must be provided.

A manuscript containing information that suggests animals were subjected to adverse, stressful, or harsh conditions or treatments will not be considered for publication unless the authors demonstrate convincingly that the knowledge gained was of sufficient value to justify these conditions or treatments.

Institutional oversight and owner consent

With the exception of reports of retrospective studies based solely on reviews of medical records, manuscripts describing studies that involved the use of animals, including studies that involved the use of privately owned animals (eg, animals owned by clients, staff members, students, or private entities), must include a statement that the study protocol was reviewed and approved by an appropriate oversight entity (eg, an animal care and use committee or institutional review board) or was performed in compliance with institutional or other (eg, governmental or international) guidelines for research on animals.

Manuscripts describing prospective studies that involved privately owned animals must also include a statement indicating that informed owner consent was obtained. Manuscripts describing research involving human subjects, including surveys of human subjects, must include a statement that the research was performed under appropriate institutional review board oversight.

NIH Public Access Policy

The AVMA journals are in compliance with the National Institutes of Health Public Access Policy (jav.ma/NIHPAP) and with the open access policies of other research funders. To assist authors of manuscripts subject to the NIH Public Access Policy (jav.ma/PAPA), the AVMA has arranged to submit articles to PubMed Central on behalf of the authors at the time of publication. Authors should not submit the accepted or any other version of their manuscript to PubMed Central, as this will preclude submission of the published version.

Patient confidentiality and the right to privacy

Authors have an obligation to protect the personal privacy of patients and clients and to maintain the confidentiality of patient-client information. For any manuscript containing patient information (eg, patient descriptions, photographs, or pedigrees) that would allow specific animals or their owners to be identified, the authors must obtain a signed statement of informed consent to publish the information (in print and online) from the owners. Generally, such consent should include an opportunity for the owner to read the manuscript to be submitted for publication. If necessary, nonessential identifying data can be removed, unless clinically or epidemiologically important. However, identifying data may not be altered or falsified. Cropping or altering photographs to remove nonessential identifying information is acceptable, so long as the photographs are not otherwise altered. Patient identifiers may not appear in photographs. Authors must also obtain informed consent to publish from any identifiable person appearing in photographs. Importantly, these guidelines also apply to any materials (eg, text, photographs, or videos) submitted for posting as supplementary materials.

Prior publication

A manuscript is received with the understanding that the information has not been published or submitted for publication in any compiled printed (eg, journals, symposia, proceedings, newsletters, or books) or electronic (eg, preprint servers, conference or university websites, blogs, or social media posts) format in English or any other language and will not be published or submitted for publication elsewhere while the manuscript is under consideration by the *AJVR*.

A manuscript containing previously published information may be rejected on the grounds of prior publication. Publication of abstracts containing 250 words or fewer will not be considered to constitute prior publication, but publication of longer abstracts in any compiled printed or electronic format may be (note that this includes posting of poster presentations to conference or university websites). Authors are encouraged to consult the guidelines for preparation of scientific abstracts (jav.ma/GPSA) when preparing scientific abstracts for publication or presentation at meetings. In general, figures, tables, footnotes, and references should not be included in abstracts.

At the time of manuscript submission, the cor-

responding author must include copies of any abstracts of the manuscript that have been published or submitted for publication or that are expected to be submitted for publication, along with copies of any closely related manuscripts or manuscripts with substantially similar content.

Publication fees and open access

All manuscripts accepted for publication in the *AJVR* are subject to an article publication charge of $1,000.

All articles published in the *AJVR* are posted to the AVMA journals website (jav.ma/AJVR) at no charge to the authors. However, access to the full text of these articles is restricted to subscribers or available on a pay-per-view basis. Authors can elect to have their manuscripts made freely available online for all to read, download, and share. The fee for this open access is an additional $1,000 per manuscript.

Reporting guidelines

To ensure thoroughness of reporting, authors are strongly encouraged to make use of the following guidelines, if applicable, when preparing manuscripts:

- CONSORT (Consolidated Standards of Reporting Trials)—for clinical trials
- REFLECT (Reporting Guidelines for Randomized Controlled Trials for Livestock and Food Safety)—for clinical trials in livestock and food safety
- STARD (Standards for the Reporting of Diagnostic Accuracy Studies)—for diagnostic test evaluation
- STROBE (Strengthening the Reporting of Observational Studies in Epidemiology)—for cross-sectional, case-control, and cohort studies
- PRISMA (Preferred Reporting Items of Systematic Reviews and Meta-analyses)—for systematic reviews and meta-analyses
- ARRIVE (Animal Research: Reporting of In Vivo Experiments)—for all studies involving laboratory animals
- SRQR (Standards for Reporting Qualitative Research)—for all studies involving qualitative research

These guidelines and more are available through the EQUATOR (Enhancing the Quality and Transparency of Health Research) Network (www.equator-network.org).

Scientific misconduct

The *AJVR* strongly supports and upholds the code of conduct espoused by the international Committee on Publication Ethics (COPE) to promote integrity in the conduct and reporting of research. The journal views gravely instances of scientific misconduct, which COPE defines as "the intention to cause others to regard as true that which is not." Such misconduct includes but is not limited to data fabrication or falsification, deceptive image manipulation, and plagiarism. In signing the Copyright Assignment Agreement and Authorship Form, authors attest that their works are original and free of scientific misconduct.

The *AJVR* is ethically obliged to investigate all suspicions or allegations of scientific misconduct, including plagiarism. Therefore, authors are expected to know and understand the definition of plagiarism as well as the consequences. The *AJVR* considers plagiarism to be the intentional or unintentional use of another's ideas or words as one's own, without attribution to the original source. Such use can range from copying of brief passages from previous publications (with or without changing a few words) to copying of entire portions of text, data, or both.

Detection of plagiarism and other instances of scientific misconduct will result in notification of the primary author, the coauthors, and possibly the author's institution, depending on the extent of misconduct and nature of the deception (eg, intentional, reckless, or negligent). Further sanctions for misconduct detected prior to publication will depend on the author's response to the allegations and may range from admonition by the editor to rejection of the manuscript, barring of the author(s) and their institution from future considerations, referral to the author's institution for further disciplinary action, and informing of other editors and the indexing authorities. For misconduct detected after publication, these sanctions can extend to retraction of the report, with full explanation of the reason, and reporting to other authorities.

Special consideration is given to the practice of text recycling, also known as self-plagiarism, which refers to an author's use of his or her own previously published text. Although text recycling may be acceptable in select instances (eg, in the Materials and Methods section of a scientific report), it generally will not be considered acceptable. The *AJVR* supports the text recycling guidelines developed by BioMed Central in collaboration with the COPE (jav.ma/COPE-TR).

Manuscript Categories

Reports of original research, review articles, and letters to the editor will be considered for publication in the *AJVR*; clinical reports that describe features of 1 or more clinical cases will generally not be considered. For reports of original research, preference is accorded to those that provide novel findings that could be expected to have clinical or practical value within the next several years. Review articles should focus on subject areas in which important advances have been made during the past 5 years.

Readers who submit letters to the editor must limit them to 500 words (longer letters will be condensed as needed) and 6 references. Letters must be original and cannot have been published or submitted for publication elsewhere. Not all letters are published; all letters accepted for publication are subject to editing. Those pertaining to anything published in the *AJVR* should be received within 1 month after the date of publication of the material to which they refer. Submission via email (JournalLetters@avma.org) is encouraged; authors should give their full contact information, including address,

daytime telephone number, fax number, and email address. Letters containing defamatory, libelous, or malicious statements will not be published, nor will letters representing attacks on or attempts to demean veterinary societies or their committees or agencies.

Manuscript Preparation

Authors should pay close attention to the following guidelines for manuscript preparation and format. Manuscripts that are not prepared in accordance with these guidelines will be returned to the authors for amendment and resubmission.

Format

Manuscripts (including footnotes, references, figure legends, appendices, and tables) should be prepared with the following attributes:

- 8.5 X 11-inch (or A4) page size
- Double-space typed
- 12-point font
- 1-inch (2.5-cm) margins
- Left justification
- Sequential line numbering

Organization and contents

Manuscripts should be organized as follows:

- Title page
- Structured abstract (when applicable; letters to the editor and review articles do not have a structured abstract)
- Abbreviations list (when applicable)
- Text
- Acknowledgments
- Footnotes
- References
- Figure legends
- Appendices
- Tables

Title page

The title page must include the manuscript title and the first name, middle initial, and last name of each author, along with each author's professional degree and highest earned academic degree (eg, MS or PhD, MPVM). Do not list academic degrees lower than the bachelor's degree (eg, associate degrees), specialty board certifications, fellowship designations, and honorary degrees; a bachelor's degree should be listed only if it is the author's only degree. Professional affiliations (full mailing addresses) of the authors at the time of the study should be indicated. If an author's affiliation has changed since the study was performed, the author's new affiliation must be identified as well. Finally, the name and email address of the corresponding author must also be included on the title page.

Structured abstract

With the exception of review articles, all manuscripts must include a structured abstract of 250 or fewer words, organized under the following headings:

- Objective
- Animals (or Sample)
- Procedures
- Results
- Conclusions and Clinical Relevance

Abbreviations list

All abbreviations except for standard abbreviations (see jav.ma/StdAbbr for full list) and units of measure should be listed in alphabetical order at the beginning of the manuscript text (after the Structured Abstract and before the introductory section), along with their definitions. These abbreviations should then be used without expansion in the text, figures, appendices, and tables, except at the start of a sentence, in which case the expanded term should be used.

Text

The text should begin with an introduction (which does not have a heading) and then be organized under the following headings:

- Materials and Methods
- Results
- Discussion

The introduction should supply sufficient pertinent background information to allow readers to understand why the study was performed. It must include the rationale for the study, a clear statement of the purpose of the study, and the investigators' hypothesis or hypotheses. The introduction is not intended to be a thorough review of the published literature on a subject. Rather, it should be brief (often, 2 or 3 paragraphs will suffice) and should focus on identifying the specific problem the study is meant to address; describing how the study addresses the problem, differs from previous studies, or improves our understanding; and explaining what the present study was meant to do and what hypotheses it was meant to test.

The Materials and Methods section should describe the study design in sufficient detail to allow others to reproduce the study. A subsection detailing statistical methods used to summarize data, evaluate data distributions, and test hypotheses, along with a statement regarding the cutoff for significance used for hypothesis testing, should be provided. Appendices and methods-related figures should be cited parenthetically. Products (including software), equipment, and drugs should be identified in the text by chemical or generic names or descriptions. For all statistical tests, authors are required to indicate whether

applicable test assumptions were met. When citing software products, a footnote should be used to cite the software (eg, PROC GLM, SAS, version 9.2, SAS Institute Inc, Cary, NC) and a reference should be used to cite a user's guide (eg, *SAS/STAT 9.2 user's guide*. Cary, NC: SAS Institute Inc, 2008;page number).

The Results section should provide data that are clearly and simply stated without discussion or conclusions. Tables and figures should be cited parenthetically. Authors should refrain from repeating within the text data that are also presented in tables and figures and are encouraged to report the number of subjects included in any statistical calculations (eg, means, medians, and results of statistical tests), particularly if that number differs among described variables. For each percentage, the numerator and denominator used in the calculation should also be reported. Authors of manuscripts reporting gene sequences should submit those sequences to an appropriate data bank.

The Discussion section should focus on findings in the manuscript and should be brief (generally no more than 2,000 words), containing only discussion that is necessary for the interpretation of findings. The major findings, including whether hypotheses stated in the introduction were supported, should be given in the first paragraph. Strengths and weaknesses of the study should be acknowledged, and the discussion should end with the principal points that readers should take away. The Discussion section should concentrate mainly on what is known in nonhuman animals, with less emphasis on what is known in humans. It should not contain any subheadings.

In general, the main text should be brief and focus on the main issues. Although there are no word limits for reports in the *AJVR*, the main text (ie, all text other than the acknowledgments, footnotes, references, figure legends, appendices, and tables) for most manuscripts should consist of no more than 3,000 to 4,000 words. Manuscripts that are excessively long may be returned for removal of nonessential information.

Acknowledgments

The Acknowledgments section is where information on sources of funding and support and conflicts of interest must be listed, along with any disclaimers, any acknowledgments of individuals who made important contributions to the study but did not meet the criteria for authorship, and any previous presentations of the findings at scientific meetings. In addition, for studies involving multiple institutions, a statement indicating where the work was done may be included, if applicable. For information on listing sources of funding and support and conflicts of interest, see the editorial policies on *Funding and support* and *Conflicts of interest and financial disclosures*.

The Acknowledgments section should be used to identify specific individuals who had an important role in or made important contributions to the study but who do not meet the criteria for authorship. In general, this includes individuals who contributed intellectually to the study or report but whose contributions do not justify authorship, individuals who provided technical assistance (eg, individuals who performed special tests or research), and individuals who assisted with the statistical analyses.

The Acknowledgments section should not be used simply as a method of expressing gratitude to individuals who had a minor role in the study. The acknowledgments should not include individuals whose only contribution to the study or report involved the routine performance of their normal job duties and who did not provide any unusual or extraordinary intellectual contribution or technical expertise to the study. Acknowledgments of nonspecific groups (eg, the intensive care unit technicians) and unidentifiable groups (eg, the anonymous contributors or study participants) are not allowed. Individuals named in the acknowledgments must have given their permission to the authors to be listed, because readers may infer their endorsement of the data and conclusions.

Footnotes

Footnotes are to be used when referencing each of the following types of information:

- Abstracts
- Conference presentations
- Online databases
- Personal communications
- Products, drugs, equipment, and other materials
- Statistical and computer software
- Theses and dissertations
- Other unpublished materials (eg, preliminary reports)

Specific products, equipment, or drugs should be included in the footnotes only if they were essential to the outcome of the report or study. Products, equipment, and drugs that are commonly used materials in veterinary medicine need not be footnoted.

Footnotes should be cited in the text as superscript letters and listed alphabetically after the Acknowledgments section and before the references. If more than 26 footnotes are required, continue the sequence with double letters (eg, aa, bb, and cc). For products and equipment, provide complete information in the footnote, including manufacturer's name and location (ie, city, state, and country [if other than the United States]).

References

Authors bear primary responsibility for accuracy of all references. References must be limited to those that are necessary and must be cited in the text by superscript numbers in order of citation. Journal titles in the Reference section should be abbreviated in accordance with the National Library of Medicine and Index Medicus (jav.ma/NLM-JA). For references with more than 3 authors, only the first 3 authors should be listed, followed by

et al. The following is the style used for common types of references:

- **Article in a journal**

1. Lamont LA, Bulmer BJ, Sisson DD, et al. Doppler echocardiographic effects of medetomidine on dynamic left ventricular outflow tract obstruction in cats. *J Am Vet Med Assoc* 2002;221:1276–1281.

- **Book chapter**

2. Muir P, Johnson KA, Manley PA. Fractures of the pelvis. In: Birchard SJ, Sherding RG, eds. *Saunders manual of small animal practice*. 2nd ed. Philadelphia: WB Saunders Co, 2000;1126–1132.

- **Proceedings**

3. Moore MP, Bagley RS, Harrington ML, et al. Intracranial tumors, in *Proceedings*. 14th Annu Meet Vet Med Forum 1996;331–334.

- **Electronic material**

4. Animal and Plant Health Inspection Service. Bovine spongiform encephalopathy (BSE). Available at: www.aphis.usda.gov/lpa/issues/bse/bse.html. Accessed Feb 18, 2016.

Figures

Figures should be limited to those that reduce or clarify the text. Images of clinically normal animals are not usually required, nor are images of equipment unless the equipment has been set up in a special way and the setup is integral to the study. Text and symbols should be large enough that they will still be legible when the figure is reduced to 1 column in width during publication (in general, this means that all text and symbols must be at least 1.5 mm tall when the figure is reduced to 8 cm in width). For text labels, the first word of each label should start with a capital letter, with any remaining words other than proper nouns in lowercase letters (eg, Cranial vena cava).

To ensure high-quality reproduction, symbols used to represent data in graphs should be limited to white and black circles, triangles, and squares; axes should be labeled in Helvetica or Arial font. Keys to data symbols may be placed in a small box inserted into the unused portion of graphs. Symbols used in figures and tables should be assigned in the following order:

- Asterisk (*)
- Dagger (†)
- Double dagger (‡)
- Section indicator (§)
- Double vertical bar (||)
- Paragraph indicator (¶)
- Pound sign (#)
- Two asterisks (**)
- Two daggers (††)
- Two double daggers (‡‡)

Photomicrographs and electron micrographs must include an internal scale marker. For figures that include multiple panels, each panel should be sequentially labeled with a capital letter in the same corner of each panel. If a figure contains 2 or more rows of panels, the letter labels should be applied sequentially from left to right in the first row, then from left to right in the second row, and so on.

Figure legends must be provided at the end of the manuscript, after the references and before any appendices and tables. Sufficient information should be included to allow the figure to be understood without reference to the text. Abbreviations defined in the abbreviations list at the beginning of the text do not need to be expanded; however, newly introduced abbreviations in figures should be defined in the figure legend, in alphabetical order. When applicable, stains used for microscopic examination of specimens must be indicated in the legend as well as the scale of the marker bar (eg, H&E stain; bar = 100 µm). Figure legends for ECG traces must include the paper speed and scale (eg, Paper speed = 50 mm/s; 1 cm = 1 mV). Authors wishing to use any previously published figures must submit written permission from the copyright holder.

Appendices

Appendices may be provided when information pertaining to the Materials and Methods could be more succinctly and clearly summarized in tabular rather than narrative format. Examples of information that might lend itself to an appendix include scoring and classification rubrics; lists of nucleotide sequences; tabular summaries of complex treatment protocols; and compositions of diets or feedstuffs. Copies of questionnaires and surveys also qualify as appendix materials but should instead be submitted in pdf format for publication as online supplementary material.

Tables

Tables are reserved for reporting of findings and not for describing the materials and methods. Submission of excessive tabular data is discouraged, and tables should be limited to those containing data important to understanding and interpreting results of the study. All tables should be placed at the end of the manuscript, after the figure legends. Authors will be asked to delete tables containing data that could be reported more succinctly in the text. Tables that focus solely on findings in individual animals rather than summary data from groups of animals are to be avoided. Authors wishing to use any previously published tables must submit written permission from the copyright holder.

For the order of symbol use in tables, please refer to the instructions for figures. To indicate significant differences between or among values in a row or column, symbols or superscript lowercase letters assigned in alpha-

betical order (a–z) may be used. If additional differentiation is needed (eg, if differences need to be reported in both rows and columns) and lowercase letters have already been used, superscript uppercase letters in alphabetical order (A–Z) may be used.

Supplementary materials

Additional materials that are not in themselves essential to the understanding of the article but provide an important expansion of the article contents may be submitted for publication as supplementary materials. Examples include extended descriptions of experimental methods or statistical analyses, extended bibliographies, additional supporting data or results (eg, tables and figures), reporting checklists, copies of survey instruments or questionnaires, handouts, forms, and multimedia representations (eg, video clips) of relevant content. All published supplementary materials are subject to copyright.

Supplementary materials must be useful to readers and relevant to the article; redundant and extraneous content will not be accepted. Whether supplementary materials will be accepted for publication is solely at the discretion of the editors. Supplementary materials accepted for publication will not appear in the printed version of the journal but will be posted on the journal's website. Ideally, supplementary materials will be sent with the manuscript to external reviewers for peer review. Whether supplementary materials have or have not undergone peer review will be indicated on the landing page where the supplementary materials are posted.

Supplementary materials should be prepared in compliance with the general guidelines for manuscript style. Although supplementary materials may undergo minor copy editing or formatting, they generally will not undergo the same substantive editing provided for manuscripts. Therefore, the authors are responsible for ensuring clarity and accuracy of the content as well as consistency with the printed version.

MANUSCRIPT STYLE

For questions of style, refer to the latest edition of the American Medical Association Manual of Style (www.amamanualofstyle.com; online access requires a subscription; individual subscriptions are available on a monthly basis if desired). Manuscripts should be written in American English. For spelling of lay terms, refer to the latest American edition of the Merriam-Webster Dictionary. For anatomic terms, use anglicized versions of official terms listed in the latest edition of the Nomina Anatomica Veterinaria. Refer to the latest editions of the American Drug Index and USP Dictionary of US Adopted Names and International Drug Names for proper spelling of chemical and drug names and to the latest edition of Dorland's Illustrated Medical Dictionary for proper spelling and use of medical terms. Refer to Bergey's Manual of Determinative Microbiology for spelling and correct taxonomic classifications of microorganisms. For pharmacologic and pharmacokinetic terms, see the AVMA journals style sheet on the subject (jav.ma/pk-terms).

Authors of manuscripts that are not written in their first language or that required substantial language translation in the writing process are encouraged to seek professional language correction or copyediting services prior to submission. Such services can aid with language, grammar, and style in scientific writing and can help ensure the manuscript content can be understood by editors and potential reviewers.

Abbreviations

Overuse of abbreviations can be confusing and frustrating for readers. In general, use of abbreviations other than the journal's standard abbreviations (see jav.ma/StdAbbr for full list) and units of measure should be kept to a minimum.

In the Structured Abstract, a term should be abbreviated only if it is used at least 3 times in the Structured Abstract. The term must be expanded at first mention, with the abbreviation given in parentheses after the expanded term. Similarly, in the manuscript text, figures, appendices, and tables, a term should be abbreviated only if it is used at least 3 times. Abbreviations used in the Structured Abstract must be defined again in the abbreviations list. All abbreviations should be derived directly from the word or words that make up the expanded term.

Abbreviations that appear only in the figures or tables should be defined in the figure or table legend. Except for the abbreviations ELISA, ACTH, EDTA, DNA, and RNA, abbreviations should not be used in titles.

Products, equipment, drugs, and other materials

All materials used in the study or referred to in the manuscript should generally be identified by chemical or generic names or descriptions. A trade name should be included in a lettered footnote if that specific product, equipment, or drug was essential for the outcome. Trademark and similar proprietary symbols are not needed.

MANUSCRIPT SUBMISSION

Manuscripts must be submitted online at mc.manuscriptcentral.com/avma.

Electronic file specifications

Manuscripts must be submitted in Microsoft Word format (.doc or .docx) or rich text format (.rtf). Tables should be included at the end of the manuscript in the same electronic file; however, if necessary, they can be saved as separate files.

Figures

All figures should be saved as separate electronic files with the name of the figure used as the file name (eg, Figure 1); figures should not be embedded in the manuscript. Gray scale or black and white should be used; color should be used only when important information would

otherwise be lost (eg, when certain tissue-staining patterns are poorly visible in gray scale or when a color-flow Doppler ultrasonogram is provided). For figures that include multiple panels, each panel should be sequentially labeled with a capital letter in the same corner of each panel. If a figure contains 2 or more rows of panels, the letter labels should be applied sequentially from left to right in the first row, then from left to right in the second row, and so on. Simple figures such as line drawings, bar graphs, and line graphs prepared in Excel should be saved and submitted as .TIF files; however, .JPG, .GIF, .EPS, and .BMP files are also acceptable. Figures created with software programs that use proprietary graphic formats (eg, SigmaPlot or Statistix) cannot be used; most such software programs have the capability to save figures in one of the aforementioned formats. Minimum resolution for figures is 300 dots per inch when displayed at the size at which they will be reproduced.

Digital images (eg, photographs, photomicrographs, and radiographs) must be provided at a minimum resolution of 300 dots per inch. Images that are not available in a digital format should be scanned on a flatbed scanner, also at a resolution of at least 300 dots per inch when displayed at the size at which they will be reproduced. Files should be saved as .TIF files; however, .JPG, .GIF, .EPS, and .BMP files are also acceptable. Color figures should be submitted in CMYK, rather than RGB, format to prevent color shift during production.

Additional required materials

At the time of manuscript submission, the corresponding author is responsible for ensuring that each author submits a Copyright Assignment Agreement and Authorship Form (jav.ma/CAA-AF). Manuscripts will NOT be considered for publication until a completed authorship form has been received from each author.

The corresponding author is also responsible for submitting copies of any references listed as in press or submitted, copies of any abstracts containing information from the manuscript that have been published or that have been submitted or are expected to be submitted for publication, copies of any closely related manuscripts or manuscripts with substantially similar content that have been published by the authors or submitted for publication, and copies of signed permission forms from the copyright holders if the manuscript contains any tables, illustrations, or appendices that have been published previously. This material should be submitted electronically (eg, by scanning and uploading with the manuscript or by uploading the electronic file).

Keywords

During manuscript submission, authors will be prompted to supply keywords to describe the topic of the manuscript to facilitate indexing and aid in the selection of reviewers. In choosing their keywords, authors must use the list of manuscript keywords available on the AVMA journals website (jav.ma/keywords) and in Manuscript Central.

Online submission

Once electronic files of the manuscript and all of its parts have been prepared, log on to the AVMA Manuscript Central website at mc.manuscriptcentral.com/avma. If you already have an account with the system, log in with your user ID and password, click on *Author Center*, and select *Click here to submit a new manuscript*. Follow the instructions for submitting your manuscript. After submitting your manuscript, please check that your user information (including mailing address, telephone and fax numbers, and email address) is current. If you do not have an account with the system, click on *Create An Account*. Fill in all fields carefully; all fields in bold are required.

A cover letter is not required; however, authors are given the option of including a cover letter when submitting their manuscripts. The cover letter can be used to explain the importance of the manuscript as well as any points that the editor should consider when reviewing the manuscript. Authors who have discussed their manuscript with an editor prior to submission should indicate this in the cover letter.

During manuscript submission, authors are also given an opportunity to indicate preferred and nonpreferred editors and reviewers for their manuscript. Authors' choices of nonpreferred editors and reviewers are typically honored. Those of preferred editors and reviewers may or may not be honored, depending on circumstances and availability. In research fields in which few experts are available for review or in which experts may not be known by *AJVR* staff, suggestions of preferred reviewers are appreciated.

Peer Review Process

The *AJVR* reserves the right to reject any manuscript. Manuscripts are reviewed initially by an AVMA scientific editor. Because of the large volume of manuscripts submitted to the journal, manuscripts are classified by the editors on the basis of priority for publication, and those not judged to be of sufficient priority are rejected promptly. Manuscripts considered for publication are sent to a minimum of 2 experts for external peer review. Instructions provided to the external reviewers are available on the AVMA website for authors' perusal (jav.ma/PeerRvw). Identity of peer reviewers is kept confidential; identity of authors is not.

Authors are expected to respond to reviewer comments and make appropriate revisions within 14 days (minor revisions) or 21 days (major revisions). Revised manuscripts may be reviewed again by the original peer reviewers or by others when those individuals are unavailable. Manuscripts that pass peer review are accepted for publication provided that authors respond meaningfully to questions and concerns raised by an AVMA scientific editor.

SEQUENCE OF PUBLICATION

Once a manuscript has satisfied all reviewer concerns and passed peer review, a provisional letter of acceptance will be issued. Final acceptance is contingent on the authors responding meaningfully to suggestions and questions raised by the scientific editor at the time of editing. Manuscripts are typically processed for publication in the order that they pass peer review, except for manuscripts dealing with emerging or zoonotic diseases, public health, or biodefense, which are prepared for publication as soon as they pass peer review. Adherence to these author instructions and expedient revision and return of manuscripts will minimize time from submission to publication. The time until a manuscript is edited will vary depending on the number of manuscripts already in line for editing at the time of provisional acceptance.

All manuscripts are subject to editing by a scientific editor prior to publication. Editorial changes are made to maintain consistency, improve clarity, smooth transitions, or make the report more understandable for the journal's general readership. Other changes are made to comply with the journal's editorial style, such as placement of superscript numbers and letters, use of abbreviations, and formatting of footnotes and references. During editing, questions may emerge about the intended meaning of terms or phrases, information that appears to be missing, and other elements that may have been missed during the peer review process.

The edited manuscript will be returned to the corresponding author to ensure that the editorial changes are accurate and acceptable and to address any editor queries. Concerns regarding editorial changes must be addressed at this stage, not at the galley stage. Authors should carefully and thoroughly review the edited manuscript, make any additional changes that are necessary, and upload the final version to the journal's online manuscript-tracking system. The returned manuscript will be reviewed by the editor, who will ensure all queries have been addressed and then issue a letter of final acceptance.

After final acceptance, manuscripts will be sent to the journal's production staff for assignment to an issue, copyediting, and layout. Once a manuscript has been assigned to an issue and copyedited, the corresponding author will be notified via email regarding when the manuscript will be published and when the galley proof will be available for review. The galley proof will be sent via email to the corresponding author (or designee, if alternate arrangements have been made) for approval approximately 4 to 6 weeks after the final acceptance letter is issued. Galley changes must be returned within 48 hours. Changes should be limited to those that affect the accuracy of the information presented and should not include changes in wording that do not affect overall accuracy of the report.

An invoice for the $1,000 article publication charge, due on receipt, will be emailed to the corresponding author approximately 45 days prior to publication.