PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

LETÍCIA MARIA DE LIMA CERAZO

EFICÁCIA CLÍNICA DA PREGABALINA EM CADELAS SUBMETIDAS À MASTECTOMIA UNILATERAL RADICAL EVARIOSALPINGOHISTERECTOMIA

Presidente Prudente - SP
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Dissertação apresentada a Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal – Área de concentração: Fisiopatologia Animal

Orientador:
Profa. Dra. Renata Navarro Cassu

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Presidente Prudente, 24 de Novembro de 2022.

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DEDICATÓRIA

Dedico esse trabalho a minha família, ao meu marido e todos que estiveram ao meu lado durante esse processo.

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“Mas é claro que o sol vai voltar amanhã
Mais uma vez, eu sei
Escuridão já vi pior, de endoidecer gente sã
Espera que o sol já vem

Nunca deixe que lhe digam que não vale a pena
Acreditar no sonho que se tem
Ou que seus planos nunca vão dar certo
Ou que você nunca vai ser alguém
Tem gente que machuca os outros
Tem gente que não sabe amar

Mas eu sei que um dia a gente aprende
Se você quiser alguém em quem confiar
Confie em si mesmo
Quem acredita sempre alcança...”

(Renato Russo)

RESUMO

Eficácia clínica da pregabalina em cadelas submetidas à mastectomia unilateral radical e ovariosalpingohisterectomia

Objetivou-se avaliar a eficácia analgésica da pregabalina como adjuvante no controle da dor pós-operatória em cadelas submetidas à mastectomia. Foram avaliadas 24 cadelas, encaminhadas para mastectomia unilateral radical e ovariosalpingohisterectomia, que foram distribuídas em dois grupos de doze animais cada: GP: administração de pregabalina 4mg/kg por via oral (VO); GC: administração VO de solução placebo. Em todos os animais a medicação pré-anestésica foi realizada com morfina (0,3 mg/kg) por via intramuscular (IM). Quinze minutos após, foi realizada a cateterização da veia cefálica. A indução e manutenção anestésicas foram realizadas com propofol (4 mg/kg, IV) e isofluorano, respectivamente. Meloxicam (0,2 mg/kg, IV), foi administrado cinco minutos antes da incisão cirúrgica. No período pós-operatório o grau de analgesia foi mensurado 0,5, 1, 2, 4, 8, 12 e 24 horas após a extubação traqueal utilizando-se a Escala Analógica Visual Interativa e Dinâmica (EAVID) e a Escala Composta de Glasgow Modificada – Forma Abreviada (CMPS-SF). Nos mesmos momentos, também foi avaliado o grau de sedação através da Escala de Sedação para Cães. A analgesia de resgate foi feita com morfina (0,5 mg/kg IM) em casos do escore de dor igual ou superior a 6 na CMPS-SF. Os dados foram submetidos ao teste de normalidade Shapiro-Wilk para identificar a sua distribuição; os escores obtidos da avaliação do grau de analgesia e de sedação foram avaliados pelo teste Mann-Whitney.Teste de Friedman foi empregado para avaliação ao longo do tratamento para um mesmo grupo. Para comparar o número de cães que receberam analgesia suplementar foi utilizado o teste exato de FisherConclui-se que administração de pregabalina não reduziu o requerimento de resgate analgésico para controle da dor no período pós operatório de cadelas pós mastectomia e ovariosalpingohisterectomia.

Palavras-chave: Analgesia, Dor Aguda, Gabapentinoide, Opioide, Morfina.

ABSTRACT

Clinical effectiveness of pregabalin in dogs submitted to unilateral radical mastectomy and ovarysalpingohysterectomy

The objective was to evaluate the analgesic efficacy of pregabalin as an adjuvant in the control of postoperative pain in bitches submitted to mastectomy. Twenty-four bitches were evaluated, referred for unilateral radical mastectomy and ovariosalpingohysterectomy, which were divided into two groups of twelve animals each: GP: administration of pregabalin 4mg/kg orally (VO); GC: VO administration of placebo solution. In all animals, pre-anesthetic medication was administered with morphine (0.3 mg/kg) intramuscularly (IM). Fifteen minutes later, cephalic vein catheterization was performed. Anesthetic induction and maintenance were performed with propofol (4 mg/kg, IV) and isofluorane, respectively. Meloxicam (0.2 mg/kg, IV) was administered five minutes before the surgical incision. In the postoperative period, the degree of analgesia was measured at 0.5, 1, 2, 4, 8, 12 and 24 hours after tracheal extubation using the Iteractive and Dynamic Visual Analog Scale (DIVAS) and the Modified Glasgow Composite Scale - Form Abbreviated (CMPS-SF). At the same times, the degree of sedation was also evaluated using the Sedation Scale for Dogs. Rescue analgesia was performed with morphine (0.5 mg/kg IM) in cases of pain score equal to or greater than 6 on the CMPS-SF. The data were submitted to the Shapiro-Wilk normality test to identify their distribution; the scores obtained from the evaluation of the degree of analgesia and sedation were evaluated by the Mann-Whitney test. Friedman's test was used to evaluate the treatment during the same group. Fisher's exact test was used to compare the number of dogs that received supplemental analgesia. It was concluded that the administration of pregabalin did not reduce the analgesic rescue requirement for pain control in the postoperative period of dogs after mastectomy and ovariosalpingohysterectomy.

Keywords: Analgesia, Acute Pain, Gabapentinoid, Opioid, Morphine.

LISTA DE SIGLAS

EAVID – Escala Analógica Visual
ECGM – Escala Composta de Glasgow Modificada -forma abreviada
$ETCO_2$ – Concentração Final Expirada de Dióxido de Carbono
ETiso – Concentração Final Expirada de Isofluorano
f – Frequência Respiratória
FC – Frequência Cardíaca
GC – Grupo Controle
GP – Grupo Pregabalina
H – Hora
IM – Intramuscular
IV – Intravenoso
Kg – Kilo
Mg – Miligrama
Ml – Mililitro
OSH – Ovariosalpingohisterectomia
PAS – Pressão Arterial Sistólica
SpO_2 – Saturação de Oxigênio na Hemoglobina
VO – Via Oral

SUMÁRIO

1 ARTIGO CIENTÍFICO

Eficácia clínica da pregabalina em cadelas submetidas à mastectomia unilateral radical e ovário-salpingo-histerectomia

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Objetivo - Avaliar a eficácia analgésica da administração perioperatória da pregabalina como parte de um protocolo multimodal de analgesia para o controle da dor pós-operatória de cadelas encaminhadas para mastectomia radical unilateral e ovário-salpingohisterectomia.

Delineamento - Estudo clínico, prospectivo, encoberto, randomizado e placebo controlado

Animais – Vinte e quatro cadelas submetidas à mastectomia unilateral radical e ovariosalpingohisterectomia.

Procedimento – Os animais foram distruibuídos em dois grupos (n=12 por grupo), sendo tratados por via oral com suspensão de pregalina (4mg/kg,GP) ou solução placebo (0,1 mL/kg; GC), 60 minutos antes da indução anestésica. Os animais foram pré-medicados com morfina (0,3 mg/kg) por via intramuscular (IM). A indução e manutenção anestésicas foram realizadas com propofol e isofluorano, respectivamente. Meloxicam (0,2 mg/kg, IV), foi administrado após a intubação traqueal. O grau de analgesia e de sedação foram mensurados 0,5, 1, 2, 4, 8, 12, 18 e 24 h após a extubação traqueal utilizando-se a Escala Analógica Visual Interativa e Dinâmica (EAVID) e a Escala Composta de Glasgow – Forma Modificada (CFMS-SF) e escala descritiva numérica. Morfina foi administrada como analgesia de resgate (0,5 mg/kg IM). Os dados foram analisados com teste t, teste de Mann-Whitney, teste de Friedman, teste de Fisher ($P < 0,05$)

Resultados – Os escores de dor e de sedação, bem como o requerimento de suplementação analgésica não diferiram entre os grupos ao longo do tempo ($P > 0.05$). Morfina foi administrada em nove animais de cada grupo ($P = 1,00$), totalizando 13 e 14 resgates nos grupos GC e GP ($P = 0,71$), respectivamente.

Conclusão e Relevância Clínica – A inclusão da pregabalina em um protocolo multimodal de analgesia resultou em efeito analgésico semelhante ao tratamento placebo em cadelas submetidas à mastectomia radical unilateral e OSH.

Abreviações

AINE	Anti-inflamatório Não Esteroidal
CMPS - SF	Escala Composta de Glasgow Modificada – Forma Abreviada
EAVID	Escala Analógica Visual Interativa e Dinâmica
OSH	Ovariosalpingohisterectomia

A mastectomia é uma cirurgia de alta incidência na espécie canina, devido à ocorrência de tumores malignos nas glândulas mamárias[1]. O tratamento mais recomendado têm sido a excisão cirúrgica radical unilateral associada à ovário-salpingo-histerectomia em casos de fêmeas não castradas[2], devido à grande influência hormonal no desenvolvimento de recidivas das neoplasias mamárias[3].

A dor pós-mastectomia é considerada moderada a grave, devido ao extenso trauma cirúrgico, que normalmente tem sido tratada pela associação de opioides e anti-inflamatórios não esteroidas (AINES)[4,5,6]. Contudo, esses fármacos estão associados a efeitos adversos, além de muitas vezes serem insuficientes para o efetivo alívio da dor pós-cirúrgica[7]. Ademais, o uso de opioides em pacientes oncológicos tem sido questionado devido ao efeito imunossupressor de alguns desses

agentes, fator que pode aumentar a probabilidade do desenvolvimento de metástases tumorais[8].

Neste contexto, tem sido crescente a investigação de protocolos de analgesia multimodal, que consistem na associação de fármacos com diferentes mecanismos de ação antinociceptiva, visando incrementar o efeito terapêutico, bem como reduzir os efeitos adversos e o requerimento de opioides[9,4,5,6]. Dentre as classes farmacológicas mais empregados na analgesia multimodal perioperatória destacam-se os opioides, os AINES, os anestésicos locais, os anatagonistas NMDA e os alfa$_2$ agonistas adrenérgicos[10,11]. Contudo, estudos recentes têm demonstrado que a inclusão de gabapentinoides, como pregabalina e gabapentina em protocolos de analgesia multimodal para pacientes cirúrgicos também pode contribuir efetivamente para o controle da dor pós-operatória[12,13].

A pregabalina, ácido (S) -3-aminometil-5-metilhexanóico, é um composto neuroativo usado para o tratamento de várias afecções, incluindo distúrbios convulsivos[14,15,16,17], dor neuropática[18,19,20,21,22] e ansiedade[23,24]. Embora seu mecanismo de ação ainda não tenha sido completamente esclarecido, estudos tem demonstrado que a pregabalina está estruturalmente relacionada ao ácido gama aminobutírico (GABA)[25] e acredita-se que a ligação desse medicamento aos canais de cálcio voltagem-dependentes, parece ser um dos fatores mais determinantes para a indução do seu efeito antinociceptivo, inibindo o influxo de cálcio dentro das células, reduzindo à liberação de neurotransmissores excitatórios como a norepinefrina, glutamato e substância P.[26,27].

A pregabalina tem sido descrita por ter um perfil farmacocinético vantajoso em comparação a gabapentina tanto no homem como no cão, pois possui melhor absorção linear independente da dose, bem como uma faixa de dosagem terapêutica estreita. Outra vantagem potencial da pregabalina comparada a gabapentina é a melhora da

eficácia analgésica e com poucos efeitos colaterais.[28,29]. Os efeitos adversos mais comuns da pregabalina relatados na medicina humana são tontura, sonolência, fadiga, edema periférico, boca seca; ganho de peso e alopecia também foram descritos.[30,31,32]. Um estudo realizado sobre a farmacocinética da pregabalina oral em cães demonstrou que não houve efeitos adversos após o uso da medicação[33].

Até a data vigente, há apenas um estudo publicado referente à utilização da pregabalina como parte de um protocolo multimodal de analgesia para o controle da dor cirúrgica em cães[34]. Na medicina, mulheres submetidas à mastectomia, tanto a gabapentina como a pregabalina proporcionaram redução do consumo de opioides e dos escores de dor no período pós-operatório[13]. Dessa forma, o atual estudo justifica-se pela escassez de estudos na temática proposta, bem como pelos resultados promissores previamente relatados em pacientes cirúrgicos[34,13].

Objetivou-se avaliar a eficácia analgésica da administração perioperatória da pregabalina como parte de um protocolo multimodal de analgesia para o controle da dor pós-operatória de cadelas encaminhadas para mastectomia radical unilateral e ovário-salpingo-histerectomia. A hipótese é de que o tratamento com pregabalina possa proporcionar analgesia superior em relação ao tratamento placebo, resultando na redução dos escores de dor, bem como na suplementação analgésica pós-operatória.

Materiais e Métodos

Animais

Após aprovação da Comissão de Uso de Animais em Experimentação (CEUA, protocolo n° 6703/2021) e termo de consentimento livre e esclarecido assinado pelo tutor do animal, foram avaliadas 24 cadelas, sem raça definida, adultas, de idade e peso variável, provenientes da rotina cirúrgica do Hospital Veterinário da Universidade do

Oeste Paulista, encaminhadas para realização de mastectomia unilateral radical e ovariosalpingohisterectomia. Os animais estudados foram selecionados mediante exames físico, laboratoriais (hemograma, dosagem sérica de uréia, creatinina, enzimas alanino aminotransferase, aspartato aminotransferase e fosfatase alcalina), raio X de tórax, eletrocardiograma e citologia aspirativa da massa tumoral. Critérios de exclusão: animais com metástases pulmonares, doença cardiovascular avançada, alterações das funções hepáticas e renais e utilização de anticonvulsivantes.

Delineamento Experimental e Grupos estudados

Em estudo clínico, prospectivo, encoberto, randomizado e placebo controlado, os animais foram distribuídos de forma aleatória usando como ferramenta de auxílio um programa de software online[a] sendo alocados em dois grupos de tratamentos: GP (n=12): tratamento com suspensão oral de pregabalina[b] 0,4% na dose de 4mg/kg por via oral, 60 min antes da cirurgia, seguindo – se a administração da mesma dose a cada 8 h, durante o período de 24 h subsequentes a cirurgia; GC (n=12): administração de suspensão oral de placebo[c] nos mesmos momentos descritos para o GP . A pregabalina e a solução placebo foram adminisradas no volume de 1 mL/kg e ambos foram manipulados na mesma farmácia[d]. As soluções foram preparadas pela farmacêutica do Hospital Veterinário e encaminhadas para o centro cirúrgico para que um pesquisador não envolvido nas avaliações pós-operatórias fizesse a administração nos animais.

Procedimento cirúrgico e anestésico

Todos os procedimentos anestésicos foram realizados pelo mesmo anestesista. Os animais foram internados no hospital veterinário 24 h antes do procedimento cirúrgico para melhor adaptação com o ambiente e familiarização com o avaliador.

Após o período de jejum sólido de 12 h todos os animais foram tratados pela via intramuscular (IM) com morfina [e](0,3 mg/kg). Vinte minutos após, foi realizada a cateterização[f] da veia cefálica, iniciando-se a infusão contínua intravenosa (IV) de morfina (0,1 mg/kg/h), mediante bomba de infusão de seringa [g], diluída em solução de Ringer Lactato. A infusão contínua IV foi iniciada 10 min antes da indução da anestesia, sendo mantida até o término do procedimento cirúrgico. A indução anestésica foi realizada com propofol[h] (dose-efeito IV). Ato contínuo, foi realizada a intubação endotraqueal, mantendo-se a anestesia inalatória com isofluorano[i], em circuito circular valvular semi-fechado do aparelho de anestesia[j], pelo qual foi fornecido oxigênio a 100%, com fluxo de oxigênio de 1L/ min. A concentração do isofluorano foi ajustada com analisador de gases, em monitor multiparamétrico[k], visando à manutenção dos animais em plano anestésico cirúrgico (rotação do globo ocular, relaxamento mandibular e ausência de reflexo palpebral).

O antiinflamatório não esteroidal, meloxicam[l] (0,2 mg/kg, IV) foi administrado após a intubação traqueal.

As cirurgias foram realizadas pelo mesmo cirurgião, que empregou a mesma técnica cirúrgica e o mesmo material de sutura para todos os animais.

Durante todo o procedimento cirúrgico os animais foram monitorados em relação aos parâmetros cardiorrespiratórios, sendo avaliados: frequência cardíaca (FC), ritmo cardíaco, saturação de oxigênio na hemoglobina (SpO_2) e concentração final expirada de dióxido de carbono ($ETCO_2$), concentração final expirada de isofluorano (ETiso) e frequência respiratória (*f*), mediante monitor multiparamétrico[k]; pressão arterial sistólica não invasiva (PAS), por meio de Doppler vascular[m], com adaptação do manguito no membro anterior direito, respeitando-se uma relação de 0,4 entre a largura do manguito e a circunferência do membro. Todos os animais foram anestesiados com o

uso do colchão térmico[n] mantendo a temperatura do paciente durante todo o procedimento cirúrgico.

Foram registrados os tempos de anestesia (tempo decorrido entre a administração do propofol e interrupção do isofluorano), de extubação (tempo decorrido entre a interrupção do isofluorano e a presença do reflexo laringo-traqueal) e de cirurgia (tempo decorrido entre a incisão da linha alba e o último ponto de sutura de pele).

Avaliação do grau de analgesia e de sedação

Avaliação da dor pós-operatória

Todos os animais foram avaliados pelo mesmo observador que desconhecia o tratamento administrado. As avaliações foram feitas utilizando-se a Escala Analógica Visual Interativa e Dinâmica (EAVID) e pela Escala Composta de Glasgow Modificada – Forma Abreviada (CMPS-SF)[35]. As avaliações foram feitas 24 h antes da cirurgia (basal), aos 0,5, 1, 2, 4, 6, 8, 12 e 24 h após a extubação traqueal, período em que foram mantidos sob internação no hospital veterinário.

Para a avaliação mediante EAVID foi utilizada uma linha de 100 mm, onde o extremo esquerdo representa o animal sem sinais de dor e o extremo direito o máximo de dor. Inicialmente, o animal foi observado dentro do canil individual, durante 1 min pelo pesquisador. Posteriormente, o pesquisador abriu a porta do canil, estimulando verbalmente o animal a se locomover, de modo a observar as reações e o comportamento do animal nesse momento. Foi notificada a presença de vocalização, inquietação ou desconforto. Na sequência, o pesquisador fez uma leve pressão digital na ferida cirúrgica, sendo observada a resposta: o animal que não apresentasse objeção à pressão da área afetada, com comportamento calmo e sem movimento durante a manipulação recebia escore 0 (ausência de dor), enquanto que, a ocorrência de

vocalização intensa, com tentativa de morder o pesquisador e esquivar-se no ato da pressão, foi avaliado como escore 100 (dor máxima). Após estes testes, a EAVID foi registrada na planilha do animal.

A CMPS-SF envolve a avaliação de critérios de comportamento associados à dor, sendo atribuídos escores para cada item, dentro de cada variável, de modo que o mínimo escore obtido será 0 (animal sem dor) e o máximo será 24 (máxima dor) (Anexo 1).

Todos os animais, com valor igual ou superior a 6 na CMPS-SF receberam analgesia complementar com morfina na dose de 0,5 mg/kg (IM). Transcorridos 40 min após o primeiro resgate, se a analgesia ainda fosse insuficiente um novo resgate seria realizado com 0,5 mg/ kg de morfina, IM. O número total bem como o intervalo entre as administrações adicionais de morfina foi registrado.

Avaliação do grau de sedação

O grau de sedação (escore de 0-12 pontos) foi avaliado nos mesmos momentos de aferição do grau de analgesia, por meio da Escala de Sedação para Cães (forma abreviada)[36] (Anexo 2).

Análise Estatística

Considerando-se uma falha de resposta ao tratamento de 75% no grupo controle (GC) e 20% no grupo tratamento (GP) e adotando-se como parâmetros adicionais, a proporção entre grupos de 1:1, poder do teste de 80% e nível alfa de 5%, estimou-se que seria necessário no mínimo 12 cadelas em cada grupo. Os cálculos foram realizados com auxílio do software Biostat 5.3.

Os dados foram submetidos ao teste de normalidade Shapiro-Wilk para identificar a sua distribuição. Para comparação dos dados demográficos (peso, idade, tempo de anestesia, tempo cirúrgico e tempo de extubação) foi empregado o teste *t* não pareado. Os escores obtidos da avaliação do grau de analgesia e de sedação foram avaliados pelo teste Mann-Whitney para comparação entre grupos, enquanto que o teste de Friedman foi empregado para avaliação ao longo do tratamento para um mesmo grupo, com contrastes verificados pelo teste de Dunn. Para comparar o número de cães que receberam analgesia suplementar foi utilizado o teste exato de Fisher. O número de doses administradas de morfina no período pós-operatório foi avaliado pelo teste de Mann-Whitney, seguindo-se o pós-teste de Dunn. O nível de significância utilizado em todos os testes foi de 5%. Foi utilizado o programa Instat Graphpad 5,1 para a análise estatística.

Resultados

Foram encaminhadas para o projeto 27 cadelas para a realização de mastectomia e OSH. Dessas, 3 foram excluídas pois não atenderam os fatores de inclusão do estudo (uma possuía comportamento agressivo e as outras duas possuíam cardiopatia).

Não houve diferença estatística entre os grupos em relação ao peso, idade, tempo cirúrgico, tempo de anestesia e tempo de extubação (Tabela 1).

Os escores de dor e de sedação não diferiram entre os grupos ao longo do tempo ($P > 0.05$). Em relação ao momento basal, os escores avaliados pela EAVID foram superiores nas primeiras 4 e 6 h nos grupos GP e GC, respectivamente, enquanto que os escores avaliados pela CMPS-SF foram superiores até 2 h nos dois grupos ($P <$

0,0001) Em relação ao grau de sedação, os escores foram superiores em relação ao basal entre 0,5 e 1 h em ambos os grupos ($P < 0,0001$) (Tabela 2).

Não houve diferença entre os grupos em relação ao número de cadelas que necessitaram de resgate analgésico ($P = 1,00$) e nem em relação ao número de doses de morfina ($P = 1,00$) administradas ao longo das 24 h de avaliação, sendo necessária suplementação analgésica em 9 animais de cada grupo, totalizando 13 e 14 resgates nos grupos GC e GP, respectivamente ($P = 0,71$) (Tabela 3).

Discussão

A hipótese do atual estudo não foi confirmada, sendo demonstrado que a administração oral de pregabalina resultou em efeito similar à de solução placebo, em termos de escores de dor e de suplementação analgésica.

Este é o primeiro estudo que investigou a eficácia analgésica da pregabalina em cadelas submetidas à mastectomia unilateral radical e ovariosalpingohisterectomia. Até a data vigente há apenas um estudo publicado com foco na utilização da pregabalina (4 mg/kg, a cada 8 h) para o controle da dor pós-cirúrgica em cães, no qual foram constatadas redução dos escores de dor e do limiar nociceptivo mecânico, durante cinco dias após a realização de hemilaminectomia[34]. Em contrapartida, no presente estudo, a mesma posologia de pregabalina não conferiu nenhum benefício analgésico em relação ao tratamento controle. A divergência entre os resultados pode estar relacionada ao tipo de cirurgia realizada em cada estudo, onde a hemilaminectomia induz maior compressão de fibras nervosas comparativamente à mastectomia/OSH, determinando maior sensibilização central. Dados da ginecologia humana demonstraram que em procedimentos cirúrgicos que determinam pouca compressão de fibras nervosas, a inclusão da pregabalina no protocolo analgésico não reduziu significativamente a dor

pós-operatória ou o consumo de opioides[37]. Como o principal mecanismo de ação da pregabalina está relacionado à inibição do influxo de cálcio, reduzindo à liberação de neurotransmissores excitatórios, entre os quais glutamato, norepinefrina e substância P, que estão intimamente associados à dor neuropática[26], é possível que a o efeito antinociceptivo desse medicamento não seja tão efetivo para redução da dor nociceptiva incisional e visceral desencadeada pela mastectomia/OSH. Apesar da mastectomia ser um procedimento cirúrgico com extensa lesão tecidual[2], no atual estudo o procedimento foi unilateral, sem a necessidade de remoção de linfonodos axilares, o que provavelmente reduziu a risco de compressão de fibras nervosas. Em um estudo similar placebo-controlado desenvolvido em cadelas submetidas à mastectomia, o tratamento com gabapentina reduziu o consumo de morfina durante as 72 h de avaliação pós-operatória[12]. Contudo, nesse estudo o período de avaliação foi mais longo, possibilitando a avaliação da dor neuropática em resposta ao edema, além do procedimento cirúrgico ter sido realizado de forma bilateral radical em 40% dos animais, o que pode ter favorecido maior injuria das fibras nervosas.

Embora as escalas EAVID e CMPS-SF sejam consideradas ferramentas subjetivas confiáveis para o reconhecimento da dor aguda e já tenham sido extensamente empregadas na espécie canina[38,39,40], esses sistemas de pontuação podem ser afetados por alguns fatores, como por exemplo o efeito sedativo residual do protocolo anestésico utilizado[39]. A sedação pode gerar viés de interpretação em ambas as escalas, reduzindo a resposta do animal frente à palpação da ferida cirúrgica, e consequentemente diminuindo os escores de dor, bem como interferindo na avaliação de mobilidade e comportamento, podendo resultar em elevação dos escores, devido à presença de apatia e letargia, além de resistência ao movimento[36]. No atual estudo, o pico máximo de sedação foi detectado na primeira hora após a extubação, período em

que foram efetuados resgates analgésicos em 7/12 e 8/12 dos animais do GP e GC, respectivamente, dos quais 42,8% (3 de 7) e 25% (2 de 8) apresentavam grau de sedação moderado a profundo. Esses dados sugerem que a sedação possa ter interferido no diagnóstico da dor, sobretudo nos animais tratados com pregabalina, visto que entre 0.5 e 1 hora após a extubação, foi observada sedação em 58,3% (7/12) e 33,3% (4/12) dos animais dos grupos GP e GC, respectivamente. A incidência de sedação observada no GP foi semelhante à relatada por Dewey et al[41], onde 60% dos animais tratados com pregabalina (4 mg/kg, a cada 8 h) apresentaram sedação de graus moderado a profundo.

Os medicamentos empregados no protocolo anestésico com potencial analgésico também podem prejudicar a avaliação da dor pós-operatória. No atual estudo, 55,5% (69,2% no GC e 50% no GP) do total de resgates analgésicos foram administrados na primeira hora após a extubação traqueal, período em que provavelmente a concentração plasmática da morfina começou a declinar. Em cães, a infusão contínua intravenosa de morfina na taxa de 0,17 mg/kg/h durante o período de 4 horas, resultou em meia vida de eliminação de 27±14 min[42]. Além disso, no atual estudo por questões éticas, o meloxicam também foi incluído no protocolo analgésico, o que pode ter dificultado ainda mais a detecção de diferenças significativas entre os grupos, visto que esse anti-inflamatório tem longa duração de ação, cuja meia-vida de eliminação é cerca de 24 h. Ademais, a eficácia clínica desse anti-inflamatório já foi demonstrada em cadelas submetidas à mastectomia e OSH[5,6].

A ausência de diferença estatística entre os grupos também pode estar relacionada ao perfil farmacocinético da pregabalina. Dados provenientes de um estudo desenvolvido em cães demonstraram que a máxima concentração plasmática de pregabalina (4 mg/kg) foi alcançada cerca de 1,5 h após a administração oral, enquanto a meia-vida de eliminação foi aproximadamente 6 h[33]. No atual estudo, o intervalo entre

a administração da pregabalina e as primeiras avaliações da dor foi aproximadamente 4 h. Em vista desses dados, é possível que a concentração plasmática da pregabalina já estivesse declinando na primeira hora de avaliação, justificando a alta prevalência de suplementação analgésica nesse período. Contudo, apesar da repetição das doses da pregabalina, o efeito analgésico não foi incrementado quando comparado ao tratamento controle, provavelmente devido à redução dos escores EAVID e CMPS-SF pela suplementação analgésica efetuada ao longo do tempo, a qual pode ter mascarado as diferenças entre os grupos. O viés gerado pela suplementação analgésica poderia ter sido evitado pela exclusão dos dados obtidos dos animais após a administração do primeiro resgate analgésico. Contudo, no atual estudo não foi possível a exclusão desses dados, devido à alta prevalência de resgate analgésico em ambos os tratamentos, tornando o número de animais insuficiente para uma análise estatística confiável.

Dentre os fatores limitantes do atual estudo, podemos ressaltar o reduzido tamanho amostral. No cálculo do tamanho amostral foi estimada falha de tratamento em 75% no grupo controle (GC) e 20% no grupo tratamento com pregabalina (GP). No entanto, a falha de tratamento foi similar entre os grupos, reduzindo o poder do cálculo do estudo. Além disso, o tratamento estatístico sem a exclusão dos dados após a suplementação analgésica pode ter mascarado as diferenças entre os grupos, devido à redução dos escores de dor após o resgate analgésico. Todavia, esse método de avaliação estatística evita o viés de seleção, além de preservar o tamanho amostral inicial, pois a exclusão dos dados dos animais após a administração de resgate analgésico, implicaria em menor amostra populacional, o que poderia reduzir ainda mais o poder de cálculo desse estudo. Paralelamente, deve-se considerar a possibilidade de falhas na avaliação da dor em animais devido a impossibilidade da expressão verbal, bem como pela variação da resposta comportamental de cada paciente. No atual estudo,

a avaliação foi feita por um único pesquisador, com treinamento prévio em vídeos. Contudo, é possível que a falta de experiência clínica do pesquisador com as escalas de comportamento possa ter comprometido os resultados desse estudo.

Em conclusão, a inclusão da pregabalina em um protocolo multimodal de analgesia resultou em efeito analgésico semelhante ao tratamento placebo em cadelas submetidas à mastectomia radical unilateral e OSH, sem nenhuma evidência de incremento analgésico durante 24 h de avaliação pós-cirúrgica.

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Nota de Rodapé

[a] Research Randomizer, Computer software, http://www.randomizer.org/, Pennsylvania,EUA

[b] Composição: Pregabalina 4mg/ml, xarope de morango 30% e xarope simples)

[c] Composição: Glicerina 5%, Goma Xantana 0,4%, Acessulfame 0,75%, Sacarina 0,1%, Aspartame 0,1%, Aroma Flavorizante de Creme de Morango 0,5%, Solução Conservante 1,6%, Stevia 0,75%, Aroma Morango Alimentício 0,5%, q.s.q. Água destilada)

[d] Farmácia de Manipulação Magistral, Presidente Prudente, Brasil

[e] Dimorf, Cristália, São Paulo, Brasil.

[f] Cateter Intravenoso Nipro 22G Safelet, São Paulo, Brasil

[g] Bomba de Seringa Universal, Vet Syringe Pump, modelo TR400VET, Wellkang Ltda, Irlanda.

[h] Propovan, Cristália, São Paulo, Brasil

[i] Isoforine, Cristalia, São Paulo, Brazil

[j] SAT 500, Takaoka, SãoPaulo-SP.

[k] Monitor Multi-Parâmetro LifeWindow™ LW9xVet, Digicare Animal Health, Flórida, EUA.

[l] Maxicam, Ouro Fino, São Paulo, SP.

[m] Doppler Vascular Portátil dv 610V, MedMega, São Paulo, Brasil

[n]Sistema de Aquecimento de Ar Automático Veterinário, Warm Air WA-7001, Hefei *Longshore* Tech Co., Ltd., Anhui Province, China

372 **Referências**

373 1. Lutful KFM, Alvarez CE, Bird RC. Canine Mammary Carcinomas: A
374 Comparative Analysis of Altered Gene Expression. Vet Sci 2015; 25: 3(1).
375 2. Sleeckx N, De Rooster H, Veldhuis KE, et al. Canine mammary tumours, an
376 overview. Reprod Dom Anim 2011; 46: 1112–1131.
377 3. Sorenmo KU, Shofer FS, Goldschmidt MH. Effect of spaying and timing of
378 spaying on survival of dogs with mammary carcinoma. J Vet Intern Med 2000;
379 14: 266–270.
380 4. Sarrau S, Jourdan J, Dupuis-Soyris, F, et al. Effects of postoperative ketamine
381 infusion on pain control and feeding behaviour in bitches undergoing
382 mastectomy. J. Small Anim. Pract 2007; 48: 670–676.
383 5. Teixeira RC, Monteiro ER, Campagnol D, et al. Effects of tramadol alone, in
384 combination with meloxicam or dipyrone, on postoperative pain and the
385 analgesic requirement in dogs undergoing unilateral mastectomy with or without
386 ovariohysterectomy. Vet Anaesth Analg 2013; 40: 641-649.
387 6. Minto BW, Rodrigues LC, Steagall PV. Assessment of postoperative pain after
388 unilateral mastectomy using two different surgical techniques in dogs. Acta Vet
389 Scand 2013; 55: 60-65.
390 7. Monteiro-Steagall BP, Steagall PVM, Lascelles BDX. Systematic review of
391 nonsteroidal anti-inflammatory drug-induced adverse effects in dogs. J Vet
392 Intern Med 2013; 27(5):1011-1019.
393 8. Gach K, Wyrębska A, Fichna J, et al. The role of morphine in regulation of
394 cancer cell growth. Naunyn Schmiedebergs Arch Pharmacol 2011; 384: 221–
395 230.

9. Gilron I, Orr E, Tu D, et al. A placebo-controlled randomized clinical trial of perioperative administration of gabapentin, rofecoxib and their combination for spontaneous and movement-evoked pain after abdominal hysterectomy. Pain 2005; 113: 191–200.
10. Yeng LT. Pharmacological treatment of neuropathic pain. Drugs Today 2009;45(Suppl C):7-12.
11. Tornero TC, Fernández RLE, Orduña VJ. Multimodal analgesia and regional anaesthesia. Rev Esp Anestesiol Reanim 2017; 64(7):401-405.
12. Crociolli GC, Cassu RN, Barbero RC, et al. Gabapentin as an adjuvant for postoperative pain management in dogs undergoing mastectomy. J Vet Med Sci 2015; 77: 1011-1015.
13. Rai AS , James S K , Jasneet D, et al. Preoperative pregabalin or gabapentin for acute and chronic postoperative pain among patients undergoing breast cancer surgery: A systematic review and meta-analysis of randomized controlled trials. J Plast Reconstr Aesthet Surg 2017; 10: 1317-1328.
14. Arroyo S, Anhut H, Kugler AR, et al. Pregabalin add-on treatment: a randomized, double-blind, placebocontrolled, dose-response study in adults with partial seizures. Epilepsia 2004; 45: 20–27.
15. Beydoun A, Uthman BM, Kugler AR, et al. Safety and efficacy of two pregabalin regimens for add-on treatment of partial epilepsy. Neurology 2005; 4: 475–480.
16. Elger CE, Brodie MJ, Anhut H, et al. Pregabalin addon treatment in patients with partial seizures: a novel evaluation of flexible-dose and fixed-dose treatment in a double-blind, placebo-controlled study. Epilepsia 2005; 46: 1926–1936.

17. Kanner AM, Ashman E, Gloss D, et al. Practice guideline update summary: Efficacy and tolerability of the new antiepileptic drugs I: Treatment of new-onset epilepsy Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology and the American Epilepsy Society. Neurology 2018; 91:74-81.
18. Dworkin RH, Corbin AE, Young JP, et al. Pregabalin for the treatment of postherpetic neuralgia: a randomized, placebo-controlled trial. Neurology 2003; 60:1274–1283.
19. Lesser H, Sharma U, LaMoreaux L, et al. Pregabalin relieves symptoms of painful diabetic neuropathy: a randomized controlled trial. Neurology 2004; 63: 2104–2110.
20. Sabatowski R, Galvez R, Cherry DA, et al. Pregabalin reduces pain and improves sleep and mood disturbances in patients with post-herpetic neuralgia: results of a randomised, placebo-controlled clinical trial. Pain 2004; 109: 26–35.
21. Freynhagen R, Strojek K, Griesing T, et al. Efficacy of pregabalin in neuropathic pain evaluated in a 12-week, randomised, double-blind, multicentre, placebo-controlled trial of flexible- and fixed-dose regimens. Pain 2005; 115: 254–263.
22. Richter RW, Portenoy R, Sharma U, et al. Relief of painful diabetic peripheral neuropathy with pregabalin: a randomized, placebo-controlled trial. J Pain 2005; 6: 253–260.
23. Pande AC, Crockatt JG, Feltner DE, et al. Pregabalin in generalized anxiety disorder: a placebo-controlled trial. Am J Psychiatry 2003; 160: 533–540.
24. Rickels K, Pollack MH, Feltner DE, et al. Pregabalin for treatment of generalized anxiety disorder: a 4-week multicenter, double-blind, placebo-

446 controlled trial of pregabalin and alprazolam. Arch Gen Psychiatry 2005; 62:
447 1022–1030.
448 25. Ben-Menachem E. Pregabalin pharmacology and its relevance to clinical
449 practice. Epilepsia 2004;45(Suppl. 6):13–8.
450 26. Tiippana EM, Hamunen K, Kontinen VK, et al. Do surgical patients benefit
451 from perioperative gabapentin/pregabalin? A systematic review of efficacy and
452 safety. Anesth Analg 2007; 104:1545-1556.
453 27. Calandre EP, Rico-Villademoros F, Slim M. Alpha2delta ligands, gabapentin,
454 pregabalin and mirogabalin: a review of their clinical pharmacology and
455 therapeutic use. Expert Rev Neurother 2016; 16:1263-1277.
456 28. Bockbrader HN, Wesche D, Miller R, et al. A comparison of the
457 pharmacokinetics and pharmacodynamics of pregabalin and gabapentin. Clin
458 Pharmacokinet 2010; 49: 661-669.
459 29. Guay DRP. Pregabalin in neuropathic pain: a more 'pharmaceutically elegant'
460 gabapentin? Am J Geriatr Pharmacother 2005; 3:274-287.
461 30. Freeman R, Durso-DeCruz E, Emir B. Efficacy, safety, and tolerability of
462 pregabalin treatment for painful diabetic peripheral neuropathy: findings from
463 seven randomized, controlled trials across a range of doses. Diabetes Care 2008;
464 31:1448-1454.
465 31. Chen B, Choi H, Hirsch LJ, et al. Cosmetic side effects of antiepileptic drugs in
466 adults with epilepsy. Epilepsy Behav 2015; 42:129-137.
467 32. Hagen EM, Rekand T. Management of neuropathic pain associated with spinal
468 cord injury. Pain Ther 2015; 1:51-65.

469 33. Salazar V, Dewey CW, Schwark W et al. Pharmacokinetics of single-dose oral
470 pregabalin administration in normal dogs. Veterinary Anaesthesia and Analgesia
471 2009; 36: 574–580.
472 34. Schmierer, P. A. et al. Randomized controlled trial of pregabalin for analgesia
473 after surgical treatment of intervertebral disc disease in dogs. Vet. Surg 2020;
474 49: 905–913.
475 35. Murrell JC, Psatha EP, Scott EM, et al. Application of a modified form of the
476 Glasgow pain scale in a veterinary teaching centre in the Netherlands. Vet Rec
477 2008; 162: 403-408.
478 36. Wagner MC, Hecker KG, Pang DSJ. Sedation levels in dogs: a validation study.
479 BMC Vet Res 2017; 13: 1-8.
480 37. Paech MJ, Goy R, Chua ,S et al. A randomized, placebo-controlled trial of
481 preoperative oral pregabalin for postoperative pain relief after minor
482 gynecological surgery.Anesth Analg 2007; 105: 1449-1453.
483 38. Testa B, Reid J, Marian ES, et al. The Short Form of the Glasgow Composite
484 Measure Pain Scale in Post-operative Analgesia Studies in Dogs: A Scoping
485 Review. Front Vet Sci. 2021; 8:751949.
486 39. Reid J, Nolan AM, Hughes JML, et al. Development of the short-form Glasgow
487 Composite Measure Pain Scale (CMPS-SF) and derivation of an analgesic
488 intervention score. Animal Welfare 2007; 16(S): 97-104.
489 40. Comassetto F, Rosa L, Ronchi SJ, et al. Correlation between visual analog scales,
490 Glasgow, Colorado and Melbourne in the evaluation of postoperative pain in dogs
491 undergoing total unilateral mastectomy. Arq. Bras. Med. Vet. Zootec 2017; 69: 355 -
492 363.

41. Dewey, CW, Cerda-Gonzalez, S., Levine, JM, Badgley, BL, Ducoté, JM, Silver, GM, Cooper, JJ, Packer, RA, & Lavely, JA (2009). Pregabalina como adjuvante do fenobarbital, brometo de potássio ou uma combinação de fenobarbital e brometo de potássio para o tratamento de cães com suspeita de epilepsia idiopática, Journal of the American Veterinary Medical Association 2009; *235* (12): 1442-1449.

42. Nakagawa K, Miyagawa Y, Takemura N, et al. Influence of preemptive analgesia with meloxicam before resection of the unilateral mammary gland on postoperative cardiovascular parameters in dogs. J Vet Med Sci 2007; 69: 939 – 944.

Tabela 1. Valores médios e desvio padrão das variáveis demográficas e dos tempos de procedimento registrados em cadelas submetidas à mastectomia unilateral radical com ovário-salpingo-histerectomia tratadas com pregabalina (GP, n =12) e solução placebo (GC, n= 12)

Variáveis	GP	GC	Valor de P
Peso (kg)	12 ± 7	10 ± 6	0,37
Idade (anos)	11 ± 3	10 ± 2	0,06
Tempo Anestésico (minutos)	111 ± 21	102 ± 16	0,41
Tempo Cirúrgico (minutos)	80 ± 15	73 ± 13	0,68
Tempo de Extubação (minutos)	5 ± 4	4 ± 2	0,23

536 **Tabela 2.** Escores de dor e de sedação (medianas, mínima e máxima) mensurados antes da cirurgia (BL), 0,5, 1, 2, 4, 6, 8, 12 e 24 horas após a
537 extubação traqueal em cadelas submetidas à mastectomia unilateral radical com ovário-salpingo-histerectomia tratadas com pregabalina (GP, n =12)
538 e solução placebo (GC, n= 12).
539

		Tempo (h)								
Escala	**Grupo**	BL	0,5	1	2	4	6	8	12	24
EAVID	GP	0 (0-5)	27,5 (0-70)*	20 (0-50)*	25 (0-60)*	12,5 (0-35)*	12,5 (0-40)	10 (0-40)	10 (0-20)	7,5 (0-30)
	GC	0 (0-5)	30 (10-50)*	32,5 (5-50)*	20 (5-50)*	12,5 (0-50)*	12,5 (0-30)*	12,5 (0-35)	20 (0-40)	15 (0-50)
CMPS-SF	GP	0 (0-2)	4 (3-10)*	3,50 (2-7)*	2,50 (0-8)*	2,50 (0-5)	2 (0-8)	2,50 (0-6)	2 (0-4)	1 (0-6)
	GC	1 (0-5)	4 (0-16)*	4,50 (0-13)*	3 (2-8)*	2 (0-13)	2 (0-5)	2 (0-6)	1,50 (0-7)	1,50 (0-8)
Sedação	GP	0 (0-1)	4 (2-8)*	3 (1-7)*	1,50 (0-4)	1,50 (0-4)	0,50 (0-3)	0,50 (0-3)	0,50 (0-2)	0 (0-2)
	GC	0 (0-2)	2,50 (1-8)*	2 (0-8)*	1 (0-6)	0,50 (0-3)	0 (0-3)	0 (0-3)	0 (0-3)	0 (0-2)

540 EAVID = Escala Analógica Visual, (0-100 mm); CMPS-SF = Escala Composta de Glasgow Modificada – Forma Abreviada (0-24 pontos), Sedação = Escala de Sedação
541 para Cães (0-12 pontos).
542 *Diferença significativa em relação aos valores basais (Teste de Friedmann, $P < 0,0001$)

543
544

Tabela 3. Suplementação analgésica administrada no período pós-operatório de cadelas submetidas à mastectomia unilateral radical com ovário-salpingo-histerectomia tratadas com pregabalina (GP, n =12) e solução placebo (GC, n= 12)

	Período pós-operatório (h)									
Grupo	0,5	1	2	4	6	8	12	24	n°doses (morfina)	n°cadelas (total)
GP	4	3	2	0	2	2	0	1	14	9/12
GC	3	5	1	1	0	1	1	2	13	9/12

553 **Anexo 1** - Escala Composta de Dor de Glasgow (forma abreviada)

Categoria	**Variável**	**Escore e descrição**
A1.	Vocalização	0 Cão quieto 1 Cão choramingando 2 Cão gemendo 3 Cão gritando
A2.	Atenção à área da ferida cirúrgica	0 Cão ignora a área da ferida cirúrgica 1 Cão olha para a área da ferida cirúrgica 2 Cão lambe a área da ferida cirúrgica 3 Cão esfrega a área da ferida cirúrgica 4 Cão morde a área da ferida cirúrgica
B.	Mobilidade	0 Cão caminha normalmente 1 Cão claudica 2 Cão com dificuldade para se movimentar (lento) 3 Cão apresenta rigidez do membro afetado 4 Cão recusa se movimentar
C.	Resposta ao toque	0 Cão não responde 1 Cão olha para a ferida cirúrgica 2 Cão recua ao toque 3 Cão rosna 4 Cão tenta mor;der o avaliador 5 Cão chora
D1.	Comportamento	0 Cão feliz 1 Cão apático 2 Cão indiferente ao ambiente 3 Cão nervoso, ansioso ou com medo 4 Cão letárgico, sem respostas aos estímulos
D2.	Postura	0 Cão confortável 1 Cão agitado 2 Cão inquieto 3 Cão curvado ou tenso 4 Cão rígido

Anexo 2. Escala de sedação para cães (forma abreviada)[36]

1. Postura
• em pé = 0 • sonolento, mas em pé = 1 • deitado, mas capaz de se levantar = 2 • deitado, com dificuldade para se levantar = 3 • incapaz de se levantar = 4
2. Posição dos olhos
• central = 0 • rotacionado, sem a terceira pálpebra = 1 • rotacionado e encoberto pela terceira pálpebra = 2
3. Resposta ao ruído (palmas)
• reação normal (gira a cabeça em direção ao ruído) = 0 • reação reduzida (gira pouco a cabeça/ mínimo movimento) = 1 • reação mínima = 2 • ausência de reação = 3
4. Aparência geral
• excitado = 0 • acordado e normal = 1 • tranquilo = 2

ANEXO A– NORMAS DE PUBLICAÇÃO DA JAMA INSTRUCTIONS FOR AUTHORS

JAVMA Instructions for Authors

The *Journal of the American Veterinary Medical Association* is a semimonthly peer-reviewed general veterinary medical journal owned by the American Veterinary Medical Association. The journal publishes manuscripts dealing with any subject germane to the practice of veterinary medicine. Specifically, the mission of the *Journal of the American Veterinary Medical Association* is to promote the science and art of veterinary medicine and to provide a forum for discussion and dissemination of ideas important to the profession.

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Individuals should be listed as authors only if they (1) made a substantial contribution to the conception and design of the study, the acquisition of the data used in the study, or the analysis and interpretation of that data; (2) were involved in drafting or revising the manuscript critically for important intellectual content; and (3) approved the submitted version of the manuscript and will have an opportunity to approve subsequent revisions of the manuscript, including the version to be published. All 3 conditions must be met. Each individual listed as an author must have participated sufficiently to take public responsibility for the work. Acquisition of funding, collection of data, or general supervision of the research team does not, alone, justify authorship. Requests to list a working group or study group in the byline will be handled on a case-by-case basis. All authors must complete and submit the authorship form (https://www.avma.org/News/Journals/Pages/journalscaa-instructions.aspx), confirming that they meet the criteria for authorship.

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, websites, CD-ROMs, DVDs, or blogs) 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 *JAVMA*. Any exceptions must be clearly described at the time of manuscript submission.

A manuscript containing previously published information may be rejected on the grounds of prior publication. Publication of abstracts containing less than 250 words will not be considered to constitute prior publication, but publication of longer abstracts may be. Authors are encouraged to consult the guidelines for preparation of scientific abstracts (https://www.avma.org/News/Journals/Pages/journals-scientific-abstracts. aspx) when preparing scientific abstracts for publication. In general, figures, tables, footnotes, and references should not be included in abstracts.

At the time of manuscript submission, the corresponding 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.

Copyright

The *JAVMA* is covered by copyright. All authors will be required to transfer copyright to the AVMA (https://www.avma.org/News/Journals/Pages/journals-caa- instructions.aspx) prior to publication of any manuscript or letter. Requests to copy, reprint, or use portions of published material (including information in figures and tables) should be addressed to the editor-in-chief. Authors must obtain and submit a statement of permission from the copyright holder (most often, the author or publisher) if they wish to include items such as figures, tables, or appendices that appeared or will have appeared in other published reports prior to publication of the manuscript, regardless of the originating source.

Original artwork (eg, drawings or photographs) that was created specifically for use in the manuscript must be accompanied by a letter explaining the conditions under which the work was created. The letter must be signed by the artist and specify the rights given to the authors for use of the artwork and the rights retained by the artist (if any). If rights are retained by the artist, the letter must include a statement that allows the journal to use the material for publication in print and online.

Commercial availability of products used

A manuscript reporting results of a study that involved evaluation of the efficacy or safety of a pharmaceutical, biologic, or other product or in which such products were relevant to the diagnosis, treatment, or outcome will be considered only if the product is commercially available in the United States and can legally be used in the species of interest. For all studies, but particularly for studies involving food animals, any extralabel drug use must comply with the provisions of the Animal Medicinal Drug Use Clarification Act (https://www.avma.org/KB/Resources/Reference/Pages/ AMDUCA.aspx).

Editorial independence

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

> 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 editorin-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 may 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 may 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.

NIH Public Access Policy

The AVMA journals are in compliance with the National Institutes of Health Public Access Policy (https:// publicaccess.nih.gov/) and with the open access policies of other research funders. To assist authors of manuscripts subject to the NIH Public Access Policy (https:// publicaccess.nih.gov/determine-applicability.htm), 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.

Conflicts of interest and financial disclosures

A conflict of interest exists whenever an individual has financial interests or personal relationships that might consciously or unconsciously influence his or her decisions. Conflicts of interest are ubiquitous and cannot be completely eliminated; they do not, by themselves, indicate improper behavior, wrongdoing, or scientific misconduct. Financial relationships are the most easily identifiable conflicts of interest and include, among other things, ownership, employment, consultancies, honoraria, paid expert testimony, grants, patents, stock ownership or options, and service as an officer or board member. Other types of conflicts of interest include personal relationships, academic competition, and intellectual beliefs.

All authors must disclose in the Acknowledgments section of the manuscript any financial or personal relationships that could be perceived to influence or could give the appearance of influencing information in the submitted manuscript. This includes detailed information about all relevant financial interests, activities, relationships, and affiliations (other than affiliations listed on the title page of the manuscript) occurring at the present time or within the 3 years prior to manuscript submission. In this context, *relevant financial interests, activities, relationships, and affiliations* should be interpreted broadly. For example, authors should disclose relationships they have not only with companies that manufacture products that are the subject of research described in the manuscript but also with companies that manufacture competing products. If no such conflicts of interest existed, the following statement or an equivalent may be included: The authors declare that there were no conflicts of interest. The editors reserve the right to reject any manuscript because of conflicts of interest. Failure to fully disclose conflicts of interest may be grounds for rejection or retraction of the manuscript.

Humane animal care and use

To be considered for publication in the *JAVMA*, all research studies involving animals must have been performed in compliance with guidelines outlined in the Animal Welfare Act (http://awic.nal.usda.gov/government- and-professional-resources/federal-laws/animalwelfare-act), US Public Health Service Policy on the Humane Care and Use of Laboratory Animals (http://grants.nih.gov/grants/olaw/references/phspol. htm), National Research Council's Guide for the Care and Use of Laboratory Animals (http://www.nap.edu/ read/5140/chapter/1), or Guide for the Care and Use of Agricultural Animals in Research and Teaching (http://adsa.org/Publications/FASS2010AgGuide.aspx) 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 the AVMA Guidelines for the Euthanasia of Animals (https://www.avma.org/KB/Policies/Document

s/ euthanasia.pdf). 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 must include a statement that the research was performed under appropriate institutional review board oversight.

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 supplemental materials.

Publication fees

Authors are not charged a fee for publication of manuscripts in the *JAVMA*.

Reporting guidelines

To ensure thoroughness of reporting, authors of Scientific Reports 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

Dual-use research of concern

Openness is recognized as a priority when making decisions regarding scientific publishing. Advances in molecular and cellular biology, genetics, microbiology, and other life sciences have made it increasingly possible to manipulate aspects of biological systems to better understand healthy states and mechanisms of disease. However, these advances have also increased the potential that information, products, or technologies resulting from life sciences research may be misused for harmful purposes. The US National Science Advisory Board for Biosecurity (http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/nsabb) has proposed the following definition for dual-use research:

> Dual-use research of concern is research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health, safety, agricultural crops and other plants, animals, the environment, or material.

Accordingly, the *JAVMA* has adopted the following policy regarding assessment of submitted manuscripts with potential dual-use content:

- Any manuscript submitted for publication that raises concerns regarding dual-use potential will be subject to editorial review to determine the risks and benefits to the scientific community and to the public at large that may result from publication. The AVMA scientific editors maintain a strong commitment against withholding scientific or other information unless there are compelling reasons to do so.
- The scientific editors reserve the right to seek special external review of these manuscripts from individuals with technical and biosecurity expertise to assist their decision.
- Authors and reviewers are expected to alert the AVMA scientific editors when submitting or reviewing manuscripts with dual-use potential.
- The final decision for publication as well as the means of communicating manuscripts with dual-use potential will be made by the editor-in-chief. An accompanying editorial may be published.

ManuscriPt catEgoriEs

Authors may submit manuscripts for publication in the *Views*, *Veterinary Medicine Today*, and *Scientific Reports* sections of the journal.

Views

The *Views* section is a forum for exchange of ideas and includes: • **Letters to the Editor** **•Commentaries**

•Viewpoint Articles

Letters to the editor—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 *JAVMA* 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.

Commentaries—Commentaries represent opinionbased articles that relate to any aspect of the veterinary medical profession. Opinions expressed should be focused and clearly presented. The text should generally be less than 1,500 words. References should be

limited, and tables and figures should generally not be included. These manuscripts are typically not sent for external peer review.

Viewpoint articles—Viewpoint articles describe an important issue in clinical medicine, public health, or biomedical research, generally espousing or promoting a particular viewpoint. They should be scholarly, thorough, and well-referenced. Maximum length is typically 5,000 words. Figures and tables may be included as necessary. Viewpoint articles are typically sent for external peer review, with reviewers specifically asked to comment on the overall importance of the topic to the veterinary profession, whether statements of fact in the manuscript are adequately referenced, whether any pertinent references have been omitted, and whether any aspects of the issue have been overemphasized or underemphasized. Authors are allowed to express opinions in Viewpoint articles, and manuscripts do not necessarily have to be balanced or dispassionate. However, authors should clearly indicate when they are stating an opinion versus established fact and should provide a cogent, logical defense of their viewpoint.

Veterinary Medicine Today

The *Veterinary Medicine Today* section promotes continuing education through didactic exercises, case discussions, and updates on clinical topics. Not every feature is published in every issue. Authors who wish to contribute a manuscript to the following features should consult the instructions for those features:

- Anesthesia Case of the Month

  (https://www.avma.org/News/Journals/Pages/ javma-ifa-anesthesia.aspx)
- Animal Behavior Case of the Month

  (https://www.avma.org/News/Journals/Pages/ javma-ifa-behavior.aspx)
- Diagnostic Imaging in Veterinary Dental Practice

  (https://www.avma.org/News/Journals/Pages/ javma-ifa-diagnostic-imaging.aspx)
- ECG of the Month

  (https://www.avma.org/News/Journals/Pages/ javma-ifa-ecg.aspx)
- Pathology in Practice

  (https://www.avma.org/News/Journals/Pages/ javma-ifa-pathology.aspx)
- Theriogenology Question of the Month

  (https://www.avma.org/News/Journals/Pages/ javma-ifa-theriogenology.aspx)
- Timely Topics in Nutrition

  (https://www.avma.org/News/Journals/Pages/ javma-ifa-nutrition.aspx)
- What Is Your Diagnosis?

  (https://www.avma.org/News/Journals/Pages/ javma-ifa-diagnosis.aspx)
- What Is Your Neurologic Diagnosis?

  (https://www.avma.org/News/Journals/Pages/ javma-ifa-neurologic.aspx)

Authors who wish to contribute a manuscript to another feature in this section (eg, Food Animal Economics or Exploring the Bond) should refer to recent issues of the *JAVMA* that contain that feature for general format.

Scientific Reports

The *Scientific Reports* section contains reports of important original research and critical reviews and includes: • **Original Studies** •**Clinical Reports**

•Review Articles

Manuscripts based on original research that involved animals with a naturally developing or experimentally induced disease or condition will be considered for publication as **Original Studies**. This includes manuscripts based on evaluations of case records accumulated during a specific period (ie, case series). Manuscripts that describe features of 1 or more clinical

cases will be considered for publication as **Clinical Reports**. **Review Articles** are concise, critical reviews concerning subject areas in which important advances have been made during the past 5 years and contain information that has, or will have, clinical applications. For scientific manuscripts, preference is accorded to those that have immediate clinical or practical value. Note that reports of prospective or retrospective case series must include a meaningful statement of purpose, clinically relevant data, and clinically useful conclusions or interpretations derived directly from evaluation of the cases described. Except for rare conditions, case series reports should contain information on at least 10 animals.

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, and tables) should be prepared with the following attributes:

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

Authors should avoid using software programs that automatically create endnotes, footnotes, and references in the submitted version of their manuscript, because the embedded formatting used by these programs may not be read by the publication software.

Organization and contents

Manuscripts should be organized as follows:

- Title page
- Structured abstract (when applicable; letters to the editor, commentaries, feature submissions, and review articles do not have a structured abstract)
- Text
- Acknowledgments
- Footnotes
- References
- Figures • 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). Academic degrees lower than the bachelor's degree (eg, associate degrees), specialty board certifications, fellowship designations, and honorary degrees should not be listed; a bachelor's degree should be listed only if it is the author's only degree. Professional affiliations 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 should 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 submitted for consideration as a Scientific Report must include a structured abstract of 250 or fewer words.
For an Original Study, the structured abstract must include the following headings:

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

For a Clinical Report, the structured abstract must include the following headings:

- Case Description
- Clinical Findings

- Treatment and Outcome
- Clinical Relevance

Text

The text for an Original Study begins with an introductory section (which does not have a heading) and then is organized under the following headings:

- Materials and Methods
- Results
- Discussion

The introductory section should supply sufficient pertinent background information to allow readers to understand and interpret results. It must include the rationale for the study, a clear statement of the purpose of the study, and the investigators' hypothesis.

The Materials and Methods section should describe the experimental 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 level of significance used for hypothesis testing, should be provided. Products (including software), equipment, and drugs should be identified in the text by chemical or generic names or descriptions. A trade name may be included in a lettered footnote if that specific product, equipment, or drug was essential for the outcome. 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/STAT9.2 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. 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, containing only discussion that is necessary for interpretation of findings. The Discussion 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.

Formatting of the titles for case series reports differs slightly from that for titles of other original studies. Specifically, the title must include the number of cases and the interval during which cases were treated. The general format is as follows: Behavioral modification for treatment of separation anxiety in dogs: 223 cases (2005–2010). In addition, the Materials and Methods section should typically begin with the subheadings *Case selection criteria* and *Medical records review*.

The text for a Clinical Report should begin with the signalment (eg, age, sex, body weight, and breed) of the animal or animals, followed by a chronological description of pertinent aspects of the diagnostic examination, treatment, and outcome, and should end with a brief discussion. When more than 1 animal is involved, a representative of the group can be described in detail, with important differences among animals addressed separately. For reports in which there are 3 or fewer animals, pertinent abnormal findings should be summarized in the text. For 4 or more animals, a single table that provides a summary of pertinent abnormal findings may be accommodated, provided that such findings are not repeated in the text.

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 provided assistance with the statistical analyses.

The acknowledgments 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 offer any unusual or extraordinary intellectual contribution or technical expertise. 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

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 (http://www.ncbi.nlm.nih.gov/nlmcatalog/journals). 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, 2003.

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). Text labels should start with a capital letter (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.

For preparation of digital versions of figures, please see the section on preparation of electronic files for manuscript submission.

Figure legends must be provided at the end of the manuscript, after the references and before any 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 histologic sections 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 mV = 10 mm). Authors wishing to use any previously published figures must submit written permission from the copyright holder.

Tables

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 order of symbol use in tables, please refer to the instructions for preparation of figures. To indicate significant differences between or

among values in a row or column, symbols or superscript lowercase letters assigned in alphabetical 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.

Supplemental 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 supplemental materials. Examples include extended descriptions of experimental methods, 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.

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

Supplemental materials should be prepared in compliance with the general guidelines for manuscript style. Although supplemental 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.

Manuscript Style

For questions of style, refer to the latest edition of the American Medical Association Manual of Style (http://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 Nomina Anatomica Veterinaria. Refer to the latest editions of the American Drug Index and USP Dictionary of USAN 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.

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

In general, use of abbreviations other than standard abbreviations and units of measures 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, and tables, a term should be abbreviated only if it is used at least 3 times. All abbreviations except for standard abbreviations and units of measure should be listed in alphabetical order at the beginning of the manuscript text (after the structured abstract and before the introduction), along with their definitions. These abbreviations should then be used without

expansion in the text, except when used to start a sentence.
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

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 may 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.

Metric conversions

Body weights and temperatures must be reported in metric units, with traditional US (lb, ºF) units reported afterward in parentheses. Doses and dosages must be given in metric units (eg, mg/kg), followed by standard units (eg, mg/lb). All dosages must include route of administration and interval (eg, 10 mg/kg [4.5 mg/ lb], IV, q 12 h).

ManuscriPt subMission

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

Electronic file specifications

Manuscripts must be 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

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