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

GISMELLI CRISTIANE ANGELUCI

COMPARAÇÃO ENTRE OS TONÔMETROS PORTÁTEIS TONOVET, TONOVET PLUS, TONO-PEN AVIA VET E KOWA HA-2 NA MENSURAÇÃO DA PRESSÃO INTRAOCULAR EM CAVALOS

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

Orientadora:
Prof.ª Dra. Silvia Maria Caldeira Franco Andrade

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Presidente Prudente, 07 de outubro de 2021.

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Este trabalho é dedicado à minha Família, base de tudo: Claide e Adalgiza *in memoriam*, Claitton, Marisa, Mariana e, em especial ao Professor Doutor Cleber, incentivador desse projeto.

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“Quem eu sou,
você só vai perceber
quando olhar nos meus olhos,
ou melhor,
além deles”.
(Clarice Lispector)

RESUMO

Comparação entre os tonômetros portáteis Tonovet, Tonovet Plus, Tono-Pen Avia Vet e Kowa HA-2 na mensuração da pressão intraocular em cavalos

O objetivo do presente estudo foi comparar e avaliar a acurácia dos tonômetros portáteis com diferentes metodologias, Tonovet, Tonovet Plus, Tono-Pen Avia Vet e Kowa HA-2 na mensuração da pressão intraocular (PIO) em cavalos. A mensuração da PIO tem importância para o diagnóstico de oftalmopatias que podem levar à cegueira irreversível do animal, como aquelas que podem levar ao aumento da PIO, como o glaucoma ou à sua diminuição, como as uveítes. O diagnóstico é realizado principalmente com o uso de tonômetros que aferem a PIO, sendo a tonometria de aplanação e rebote as mais utilizadas atualmente na Medicina Veterinária representados pelos tonômetros portáteis Tono-Pen Avia Vet (aplanação), Tonovet (rebote), mais recentemente o Tonovet Plus (rebote) e Kowa Ha-2 (metodologia de Goldmann). Foram avaliados 12 olhos de 6 cavalos sedados, para o estudo *in* vivo comparando a PIO real (manometria) com a tonometria, e 60 olhos sadios de 30 cavalos hígidos não sedados, em um estudo a campo com a PIO mensurada com diferentes tonômetros. No estudo *in vivo* os valores da PIO em mmHg mensurados na manometria ocular foram: 24,9±4,0 (20,0-30,0) e na tonometria: Tonovet 25,7±5,8 (19,5-33,0), Tonovet Plus 24,8±7,1 (13,2-33,2), Tono-Pen Avia Vet 19,2±4,7 (13,1-26,5), Kowa HA-2 24,1±1,2 (22,8-25,8); no estudo a campo os valores da PIO foram: Tonovet 30,7±5,6 (21,7-38,0), Tonovet Plus 29,6±6,7 (16,2-38,6), Tono-Pen Avia Vet 27,3±5,8 (14,6-37,1), Kowa HA-2 23,4±2,2 (20,2-28,7). Foi verificada uma forte correlação entre os valores da PIO com a manometria em todos os tonômetros. Valores mais altos da PIO foram aferidos com o Tonovet Plus e Tonovet e valores mais baixos com o Tono-Pen Avia Vet e o Kowa HA-2. Todos os tonômetros utilizados tiveram precisão na mensuração da PIO dos cavalos, especificamente o tonômetro mais recente, o Tonovet Plus, que mostrou uma excelente correlação com a manometria.

Palavras-chave: tonometria, manometria ocular, tonometria de rebote, tonometria de aplanação, Tonovet Plus, cavalo.

ABSTRACT

Comparison between Tonovet, Tonovet Plus, Tono-Pen Avia Vet and Kowa HA-2 portable tonometers in the measurement of intraocular pressure in horses

The aim of the present study was to compare and evaluate portable tonometers accuracy with different methodologies, Tonovet, Tonovet Plus, Tono-Pen Avia Vet and Kowa HA-2 in measuring IOP in horses. Measurement of intraocular pressure (IOP) is important for ophthalmopathies diagnosis that can lead to irreversible animal blindness, such as those lead to IOP increase, in glaucoma or its decrease, in uveitis. Diagnosis is performed mainly with tonometers that measure the IOP, applanation and rebound tonometry are the most used nowadays in Veterinary Medicine, represented by the portable tonometers Tono-Pen Avia Vet (applanation), Tonovet (rebound), more recently Tonovet Plus (rebound) and Kowa Ha-2 (Goldmann methodology). Twelve eyes from 6 sedated horses were used for in vivo study comparing actual IOP (manometry) with tonometry, and 60 healthy eyes from 30 healthy unsedated horses in a field study with IOP measured with different tonometers. In vivo study, the IOP values in mmHg measured in ocular manometry were: 24.9±4.0 (20.0-30.0) and in tonometry: Tonovet 25.7±5.8 (19.5-33 .0), Tonovet Plus 24.8±7.1 (13.2-33.2), Tono-Pen Avia Vet 19.2±4.7 (13.1-26.5), Kowa HA-2 24 0.1±1.2 (22.8-25.8); in field study the IOP values were: Tonovet 30.7±5.6 (21.7-38.0), Tonovet Plus 29.6±6.7 (16.2-38.6), Tono-Pen Avia Vet 27.3±5.8 (14.6-37.1), Kowa HA-2 23.4±2.2 (20.2-28.7). There was a strong correlation between IOP values and manometry in all tonometers. Higher IOP values were measured with Tonovet Plus and Tonovet and lower values with Tono-Pen Avia Vet and Kowa HA-2. All tonometers used were accurate in measuring the IOP of horses, specifically the most recent tonometer, the Tonovet Plus, which showed an excellent correlation with manometry.

Keywords: tonometry, ocular manometry, rebound tonometry, applanation tonometry, Tonovet Plus, horse.

SUMÁRIO

1 ARTIGO

Comparação entre os tonômetros portáteis Tonovet, Tonovet Plus, Tono-Pen Avia Vet e Kowa HA-2 na mensuração da pressão intraocular em cavalos

Gismelli C. Angeluci, Cláudia L. Ricci, João Victor G. C. Passareli, Giovana J. G. Estanho, Adriana S. Oliveira, Stephany G. A. Santos, Rogério Giuffrida, Mirian Rodrigues, Silvia F. Andrade

Resumo

Finalidade: A mensuração da pressão intraocular (PIO) é importante para o diagnóstico de oftalmopatias que causam aumento ou diminuição da PIO e podem ocasionar cegueira em cavalos. A mensuração da PIO na Medicina Veterinária é realizada com o uso de tonômetros portáteis, principalmente o Tonovet e Tonovet Plus (rebote), Tono-Pen Avia Vet (aplanação) e Kowa HA-2 (aplanação pela metodologia de Goldmann).

Objetivos: Comparar e avaliar a acurácia dos tonômetros portáteis com diferentes metodologias, Tonovet, Tonovet Plus, Tono-Pen Avia Vet e Kowa HA-2, na mensuração da PIO em cavalos.

Delineamento do estudo: Experimento randomizado.

Métodos: A PIO foi aferida em 72 olhos de 36 cavalos. Foi realizado um estudo *in vivo* em cavalos sedados comparando os valores reais de PIO pela manometria versus os valores medidos com a tonometria, e um estudo a campo com cavalos sem sedação, hígidos com olhos sadios, utilizando a tonometria com os diferentes tonômetros.

Resultados: No estudo *in vivo* os valores da PIO em mmHg mensurados na manometria ocular foram: 24,9±4,0 (20,0-30,0) e na tonometria: Tonovet 25,7±5,8 (19,5-33,0), Tonovet Plus 24,8±7,1 (13,2-33,2), Tono Pen Avia Vet 19,2±4,7 (13,1-26,5), Kowa Ha-2 24,1±1,2 (22,8-25,8); no estudo a campo os valores da PIO foram: Tonovet 30,7±5,6 (21,7-38,0), Tonovet Plus 29,6±6,7 (16,2-38,6), TonoPen Avia Vet 27,3±5,8 (14,6-37,1), Kowa HA 23,4±2,2 (20,2-28,7).

Principais limitações: Número reduzido de animais utilizados no grupo *in vivo*.

Conclusões: Foi verificada uma forte correlação entre os valores da PIO com a manometria em todos os tonômetros. Valores mais altos da PIO foram aferidos com o Tonovet Plus e Tonovet e valores mais baixos com o Tono-Pen Avia Vet e o Kowa HA-2. Todos os tonômetros utilizados tiveram precisão na mensuração da PIO dos cavalos, especificamente o tonômetro mais recente, o Tonovet Plus, que mostrou uma forte correlação com a manometria.

Palavras-chave: Tonometria, manometria ocular, tonometria de rebote, tonometria de aplanação, Tonovet Plus, cavalo.

2 INTRODUÇÃO

A mensuração da pressão intraocular (PIO) é necessária no exame clínico oftálmico em cavalos, pois a determinação do aumento (glaucoma) ou diminuição (uveíte) é um fator de importante para o diagnóstico e tratamento de doenças oculares que podem levar à cegueira irreversível nessa espécie,[1-3] sendo o glaucoma de menor incidência com relatos inferiores a 1% em cavalos,[3] e as uveítes especialmente a uveíte recorrente equina, mais comumente diagnosticada e causadora importante de cegueira nessa espécie animal.[4]

A tonometria afere a PIO por meio de tonômetros, podendo ser de contato ou não, fixos ou portáteis. Na Medicina Veterinária são usados os tonômetros portáteis de contato com métodos de aplanação ou rebote.[5,6] O método de aplanação se baseia no princípio de que a força requerida para aplanar determinada área de uma esfera é igual à pressão do interior dessa esfera.[1] São descritos trabalhos em bovinos e em equinos.[7,8] Estudos comparativos entre oTono-Pen XL e Perkins em bovinos e equinos mostraram que não houve diferença significativa entre os valores médios obtidos entre os tonômetros.[9] O método de rebote é baseado no princípio da medição/recuperação, em que uma sonda leve e magnetizada é usada para fazer contato momentâneo com a córnea.[10-12] É bem tolerado, causa mínimo estresse e desconforto, considerado um método rápido e eficaz.[13] Estudos avaliaram a PIO de equinos durante competições de corrida e resistência,[14] a PIO de burros miniaturas[15] e de alpacas[16], demonstrando valores da PIO com o tonômetro de rebote estatisticamente maior do que o tonômetro de aplanação.

A manometria ocular ou direta, é a mais precisa, considerada método de ouro, e afere a PIO real por meio de procedimento invasivo, sendo de uso

experimental no estudo de glaucoma, de drogas que interferem na PIO, na validação e estudo da acurácia de tonômetros.[17-19,7] Foi utilizada para o estudo dos tonômetros Mackay-Marg em cavalos e Tono-Pen em cães e cavalos[20] e Perkins em equinos e bovinos.[8] Outros fatores podem afetar a mensuração da PIO em equinos, como a posição da cabeça, uso de bloqueio auriculopalpebral, sedativos, horário do dia e tipo de tonômetro utilizado.[21-24,9]

O objetivo do estudo foi comparar e avaliar a acurácia dos tonômetros portáteis Tonovet, Tonovet Plus, Tono-Pen Avia Vet e Kowa HA-2 na mensuração da PIO em cavalos. É o primeiro estudo que compara todas as metodologias de mensuração da PIO (rebote, aplanação e aplanação pela metodologia de Goldmann) nessa espécie animal.

3 MATERIAIS E MÉTODOS

No estudo randomizado foram utilizados 36 cavalos (72 olhos), 13 machos e 23 fêmeas, com idade de 30 dias a 20 anos e peso de 60 a 500 kg, sendo 20 cavalos (55,5%) da raça Quarto de Milha e 16 cavalos (44,5%) mestiços entre outras raças, divididos em 2 grupos: estudo *in vivo* (12 olhos sadios de 6 cavalos hígidos e sedados) da rotina de atendimento no Hospital Veterinário Universitário da UNOESTE, comparando a manometria versus a tonometria com os diferentes tonômetros e, estudo a campo (60 olhos sadios de 30 cavalos hígidos e não sedados) da rotina de atendimento no Hospital Veterinário da UNOESTE e de atendimento externo em um Haras da região de Presidente Prudente, SP.

Os cavalos estavam clinicamente saudáveis e foram submetidos inicialmente ao exame oftálmico com a inspeção externa e interna dos olhos utilizando-se lâmpada de fenda (Kowa SL-15, Japão), oftalmoscopia direta (Oftalmoscópio Pocket Jr, USA), teste pupilar fotomotor com lanterna puntiforme, Teste Lacrimal de Schirmer (Ophthalmos, SP, Brasil) e Teste de Fluoresceína (Fluoresceína Strips, Ophthalmos, SP). Somente cavalos com parâmetros oftálmicos normais foram utilizados no estudo. Os proprietários dos cavalos foram orientados e assinaram Termo de Consentimento Livre e Esclarecido para a participação no estudo.

Para a aferição da PIO os cavalos foram contidos em tronco, com uma barra na frente e um portão sólido atrás. Os animais permaneceram em postura normal, com a cabeça posicionada acima da cernelha, ou seja, posicionada acima do nível do

coração, uma vez que a PIO é significantemente afetada quando está abaixo desse nível (Figura 1).[22] Animais que demonstraram resistência para manipulação dos olhos com a necessidade de utilização de cachimbo para contenção, foram utilizados no estudo *in vivo*, no qual os cavalos foram sedados para o experimento, pois a utilização de cachimbo para contenção de cavalos aumenta os valores da PIO de forma significativa.[25]

As mensurações da PIO com os tonômetros foram realizadas pelo mesmo examinador: GCA para Tonovet, Tonovet Plus e TonoPen Avia Vet e SFA para o Kowa HA-2, com a finalidade de diminuir a variação de inter-observador, vez que um estudo analisando a variação entre usuários e intra-usuários de dois tonômetros em cavalos sedados e não sedados, foi observado que para o tonômetro de rebote a sedação não afetou a variação inter-usuário ou intra-usuário, mas para o tonômetro de aplanação a variação inter-usuário foi menor enquanto os cavalos estavam sedados.[26] As leituras da PIO foram feitas no intervalo entre 8 e 16 horas para cada grupo para diminuir a influência da variação diurna.[23] A utilização dos tonômetros tiveram como base as instruções dos fabricantes de cada aparelho.

Para comparar e avaliar a acurácia dos valores da PIO mensurada pelos tonômetros, foi realizado um estudo *in vivo* em cavalos sedados e aferida a PIO real por meio da manometria versus a mensuração da PIO obtida com os tonômetros Tonovet, Tonovet Plus, Tono-Pen Avia Vet e Kowa HA-2. Os cavalos foram sedados com cloridrato de medetomidina (Dormiun V, Agener, São Paulo, Brasil) na dose de 20 µg/kg (0,2 mL do produto/100 kg pv) IV, e realizado bloqueio bilateral do nervo auriculopalpebral com 5mL de lidocaína a 2% sem vasoconstritor (Hipolabor, Belo Horizonte, Brasil). As pálpebras foram afastadas com blefarostato e três leituras foram realizadas da PIO com os tonômetros Tonovet, Tonovet Plus, Tono-Pen Avia Vet e Kowa-HA2 e a média calculada. As leituras dos tonômetros (Figura 2) foram realizadas seguindo uma ordem de uso: 1º Tonovet, 2º Tonovet Plus, 3º Tono-Pen Avia Vet, a instilação prévia de 2 gotas de colírio Anestésico® (cloridrato de tetracaína a 1% + cloridrato de fenilefrina a 0,1%) e 4º Kowa HA-2 sendo necessário previamente instilar 1 gota de colírio fluoresceína a 1% para a formação dos semicírculos de fluoresceína. Estudos relataram que o intervalo de uso entre os tonômetros pode ser de 1 minuto.[15] No presente estudo foi dado 1 minuto de intervalo entre o uso de cada tonômetro.

Após as leituras com os tonômetros, a câmara anterior de cada um dos olhos foi canulada (Figura 3) com um escalpe 23G a 2 mm posterior ao limbo lateral na posição de 10h em olho direito (OD) e 2h em olho esquerdo (OE). A agulha foi conectada a um tubo de polietileno e este conectado a uma torneira de três vias fazendo a ligação através de outro tubo de polietileno a um manômetro aneroide. Após a aferição da PIO a agulha foi imediatamente retirada da câmara anterior e em seguida feita a instilação de uma gotícula de cola de cianoacrilato com auxílio de uma agulha 25x7 no local da punção na córnea com o fim de selar a perfuração e evitar extravasamento do humor aquoso. Após o procedimento os cavalos foram tratados com a instilação de 1 gota de colírio antibiótico a base de tobramicina e de colírio anti-inflamatório a base de diclofenaco 3x/dia durante 1 semana e avaliada, por meio de exame oftálmico diário, a recuperação da lesão corneana induzida.

Na avaliação da acurácia dos tonômetros, foi realizado um estudo a campo em olhos sadios de equinos hígidos, não sedados, provenientes da rotina de atendimento oftálmico do setor de Clínica Médica de Grandes Animais do Hospital Veterinário da UNOESTE e de atendimento externo oftálmico de equinos em haras da região de Presidente Prudente, SP. Os animais foram contidos em tronco, realizado o bloqueio bilateral do nervo auriculopalpebral com 5mL de lidocaína 2% sem vasoconstritor. Na sequência foram realizadas as leituras da PIO com intervalo de 2 minutos entre cada aferição, com os tonômetros Tonovet, Tonovet Plus, feita a instilação de 2 gotas de colírio Anestésico® e aferida a PIO com o Tono-Pen Avia Vet, e feita a instilação de 1 gota de colírio de fluoresceína a 1% para realização da leitura da PIO com tonômetro Kowa HA-2. Todos os tonômetros foram calibrados conforme as instruções do fabricante. Para evitar a transmissão de doenças oculares infecciosas de um animal para outro, após o uso dos tonômetros Tonovet, Tonovet Plus, Tono-Pen Avia Vet foram trocadas as probes e o dispositivo de latex descartável,[5] retirado o prisma do Kowa HA-2® e lavado em solução fisiológica a 0,9% e imerso por 5 minutos em solução de peróxido de hidrogênio a 3%, lavado novamente na solução fisiológica e secado com uma gaze estéril.[27] Após a realização das aferições da PIO, nenhuma alteração ocular foi relatada nos cavalos, e nenhum dos animais necessitou de tratamento oftálmico.

3.1 Análise Estatística

O pressuposto de normalidade dos dados foi validado com o teste de Shapiro-Wilk. No estudo *in vivo* de cavalos sedados foi realizado a análise gráfica de concordância de Bland-Altman para comparar os dois métodos quantitativos de medição da PIO (manometria versus tonometria), uma série de acordos foi definido como viés médio de ± 2 desvios-padrão. No estudo a campo de cavalos não sedados foi realizada a análise de correlação de Pearson entre as pressões intraoculares avaliadas por diferentes tonômetros para verificar se há diferenças entre os olhos (direito e esquerdo) e idade. No mesmo grupo, as diferenças entre as PIOs de machos e fêmeas foram avaliadas pelo teste *t* não pareado. A comparação entre os tonômetros, dentro de cada grupo, foram comparados estatisticamente pela análise de variância (ANOVA) em uma via com contrastes pelo método de Tukey. Todas as análises foram conduzidas no Programa R, adotando-se o nível de significância de 5% ($p<0,05$) (R DEVELOPMENT CORE TEAM, 2020).

4 RESULTADOS

No estudo *in vivo* em cavalos sedados, os valores da PIO em mmHg mensurados na manometria ocular direta foram: 24,9±4,0 (20,0-30,0) e, com os tonômetros foram: Tonovet 25,7±5,8 (19,5-33,0), Tonovet Plus 24,8±7,1 (13,2-33,2), Tono-Pen Avia Vet 19,2±4,7 (13,1-26,5), Kowa HA-2 24,1±1,2 (22,8-25,8). No estudo a campo em cavalos não sedados, os valores da PIO em mmHg mensurados com os tonômetros foram: Tonovet 30,7±5,6 (21,7-38,0), Tonovet Plus 29,6±6,7 (16,2-38,6), Tono-Pen Avia Vet 27,3±5,8 (14,6-37,1) e Kowa HA-2 23,4±2,2 (20,2-28,7).

A avaliação entre a manometria e a tonometria foi dada pela análise gráfica de Bland-Altman, em que a dispersão de pontos dos cálculos das médias e diferenças indicaram que as mensurações dos tonômetros permaneceram dentro dos limites superiores e inferiores, demonstrando melhor concordância com a média (Figura 4).

Nota-se que, no estudo *in vivo*, em cavalos sedados, não houve diferença estatística ($p>0,05$) entre a manometria e os tonômetros Tonovet, Tonovet Plus e Kowa HA-2, e os valores foram significativamente menores com o Tono-Pen Avia Vet demonstrando diferença estatística significativa em relação aos outros tonômetros. Os valores de PIO mais próximos da manometria foram os aferidos com o Tonovet Plus, Tonovet e Kowa (Figura 5).

No estudo a campo de cavalos sem sedação, não houve diferença estatística significativa entre os valores aferidos da PIO entre o Tonovet e Tonovet Plus, e houve diferença estatística (*p<0,05*) entre o Tono-Pen Avia Vet e Kowa HA-2, especificamente este último, com todos os tonômetros (Figura 6).

Na análise de correlação de Pearson referente às leituras da PIO pelos tonômetros em cada olho (esquerdo e direito) (Tabela 1) dos animais não sedados do estudo a campo, observou-se diferença estatística significativa (*p<0,05*) e, em relação a idade dos animais não sedados do estudo a campo (Tabela 2) não foram observadas diferenças estatísticas significativas (*p>0,05*). No mesmo grupo, as diferenças entre as PIOs entre sexo (macho ou fêmea) (Tabela 3) também não foram observadas diferenças estatísticas significativas (*p>0,05*) avaliadas pelo teste *t* não pareado.

5 DISCUSSÃO

Este estudo é o primeiro realizado com os principais tonômetros utilizados na Medicina Veterinária para mensurar a pressão intraocular (PIO) com diferentes metodologias (rebote, aplanação e aplanação pela metodologia de Goldmann) em cavalos e a realizar a mensuração da PIO real pela manometria *in vivo*. Em estudo recente em cães[28] onde foi avaliado o uso dos mesmos tonômetros aqui estudados, foi relatado que os tonômetros Tonovet e Tonovet Plus possuem mais vantagens para a utilização clínica diária, pois não há necessidade de anestesia tópica para aferição da PIO, possuem facilidade de manuseio pelo manipulador, mas todos os tonômetros apresentaram boa acurácia para a mensuração da pressão intraocular, o que foi consistente com nosso estudo em cavalos, vez que todos os tonômetros (Tonovet, Tonovet Plus, TonoPen Avia Vet e Kowa HA-2) apresentaram boa acuraria e, o Tonovet e o Tonovet Plus especificamente, apresentaram facilidade de uso ao manipulador sem a necessidade de utilização de anestesia tópica, não causando desconforto aos animais.

Os valores apurados da PIO com os tonômetros no estudo *in vivo* em cavalos sedados que mais se aproximaram da pressão intraocular real mensurada pela manometria no estudo *in vivo*, em ordem decrescente foram, o Tonovet, o Tonovet Plus, e o Kowa HA-2. O Tonovet apresentou valor um pouco maior que a PIO real e o Tono-Pen Avia Vet apresentou valor menor que a PIO real mensurada pela

manometria, entretanto os valores não demonstraram diferenças estatísticas significativas.

No estudo a campo em cavalos sem sedação, em que foram mensuradas as pressões intraoculares pelos tonômetros, o Tonovet e o Tonovet Plus apresentaram valores de PIO maiores em relação aos valores mensurados pelo Tono-Pen Avia Vet e o Kowa HA-2, sendo que estes dois últimos apresentaram diferenças significativas em relação aos tonômetros Tonovet e Tonovet Plus.

Os valores da PIO mensuradas pelo Tonovet e Tonovet Plus foram em média 5 mmHg maiores do que os valores da PIO mensuradas pelos outros tonômetros e, em relação especificamente ao Tonovet Plus, 0,8 mmHg maior demonstrando diferença significativa mínima. Consistente com esses resultados, testes clínicos realizados em cavalos,[5] o Tonovet demonstrou valores da PIO maiores do que o Tono-Pen Avia Vet.

Em estudo para determinar intervalos de referência para a PIO em olhos de burros miniatura clinicamente normais,[15] a PIO média ± desvio padrão foi de 25,75 ± 5,70 mmHg mensurada pelo Tonovet, o que também foi consistente com nosso estudo *in vivo* ao comparar os valores da PIO real pela manometria com a PIO mensurada pela tonometria, em que o Tonovet teve média ± desvio padrão de 25,7 ± 5,8 mmHg e, ainda demonstrou valores maiores do que os outros tonômetros.

Mais recentemente, comparando o Tonovet Plus com o Tonovet em cavalos saudáveis,[29] foram obtidos valores médios ± desvio padrão com o Tonovet Plus 0,6 mmHg maiores do que com o Tonovet, com diferença estatística insignificante entre os dispositivos, sendo condizente com o presente estudo em que se demonstrou uma diferença estatisticamente insignificante entre os valores da PIO mensurados pelo Tonovet e Tonovet Plus.

Na análise de resultados das leituras em separado do olho direito e esquerdo,[16,25] não foi observada diferença estatística significativa, diferentemente do que ocorreu nesse estudo em que foram observadas diferenças significativas para as mensurações em olho esquerdo e direito entre todos os tonômetros no estudo à campo em animais sem sedação. Quanto à idade dos animais, não foram observadas diferenças significativas no estudo à campo em animais sem sedação, corroborando com o estudo de Mustikka (2020).[29]

Em outro estudo, analisando a idade e sexo pelos tonômetros de rebote e aplanação,[15] foi relatado que não ocorreram diferenças estatisticamente

significativas, o que foi consistente com o presente estudo, que demonstrou não haver diferenças significativas nas mensurações pelos tonômetros em referência a idade e ao sexo dos animais.

Em conclusão, as mensurações da pressão intraocular em cavalos clinicamente normais entre os tonômetros Tonovet, Tonovet Plus, Tono-Pen Avia Vet e Kowa HA-2, demonstraram boa acurácia e concordância com os valores da PIO real mensurada pela manometria. O Tonovet Plus, seguido do Tonovet e Kowa-HA-2 foram os tonômetros que mais se aproximaram dos valores da PIO real. O Kowa HA-2 foi o tonômetro que apresentou menor variabilidade nos valores aferidos da PIO. Entre as metodologias, os tonômetros de rebote apresentaram valores maiores de PIO em relação aos tonômetros de aplanação. Em razão da ocorrência de diferenças quanto aos valores mínimos e máximos entre os tonômetros, a elaboração de uma tabela diferenciada de valores normais para cada tonômetro em cavalos, se justifica.

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CONFLITOS DE INTERESSE

Os autores declaram não haver conflitos de interesse.

CONTRIBUIÇÃO DOS AUTORES

Todos os autores listados acima atendem aos critérios de autoria neste manuscrito, conforme descrito nas diretrizes da EVJ. Todos os autores fizeram contribuições substanciais para a concepção e desenho, aquisição, análise e interpretação dos dados. Todos os autores estiveram envolvidos na sua redação e revisão crítica de conteúdo intelectual e aprovaram a versão final a ser publicada. Os autores assumem a responsabilidade por partes apropriadas do conteúdo e por

todos os aspectos do trabalho garantindo a precisão ou integridade de qualquer parte do trabalho.

ÉTICA EM PESQUISA ANIMAL

Todos os procedimentos foram realizados de acordo com aprovação do Comitê de Ética em Uso de Animais (CEUA) da UNOESTE sob protocolo nº 5895 e conduzidos conforme as Diretrizes da Association for Research in Vision and Ophthalmology (ARVO) para o uso de animais em pesquisas oftálmicas e visuais.

CONSENTIMENTO INFORMADO DO POSSUIDOR

Os dados que apoiam as descobertas deste estudo estão disponíveis com o autor correspondente mediante solicitação razoável.

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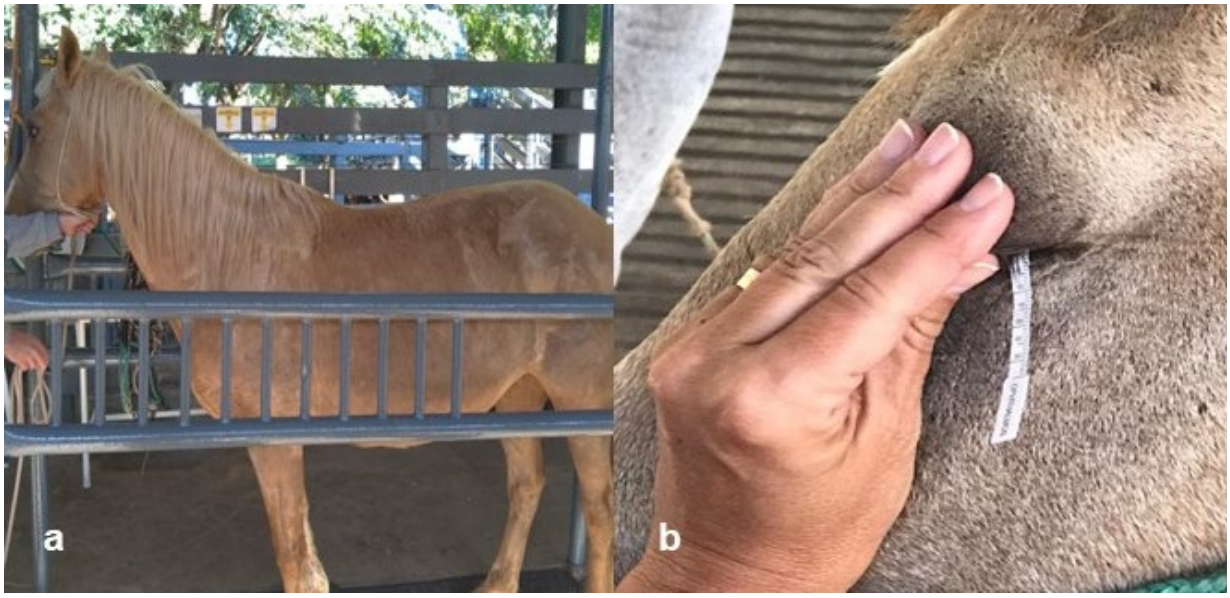

FIGURA 1 (a) Contenção do animal em tronco com posição correta da cabeça para realização da manometria e tonometria. (b) Realização do teste lacrimal de Schirmer (TLS).

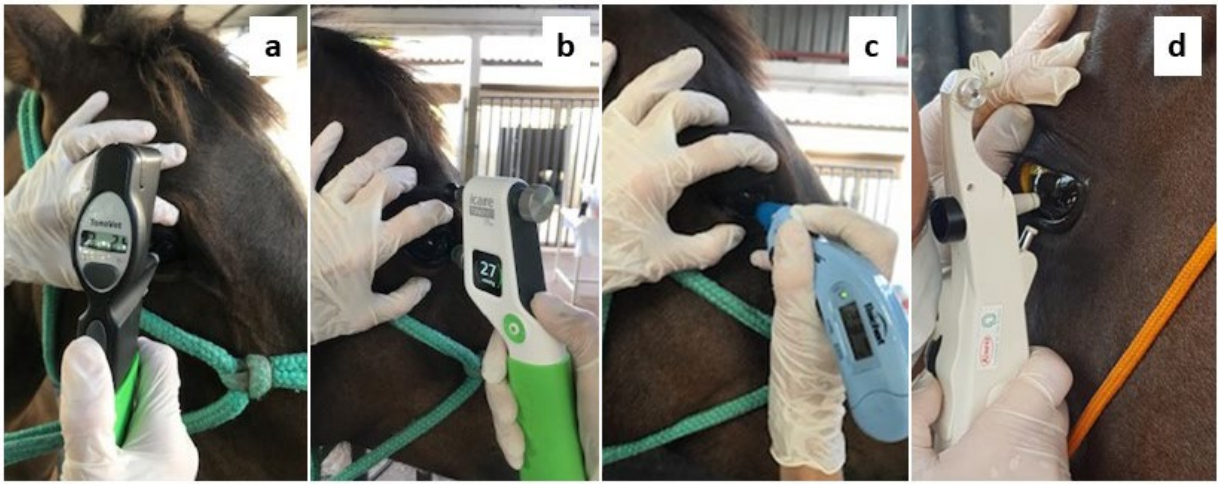

FIGURA 2 Realização das mensurações da PIO do grupo a campo em cavalos não sedados com os tonômetros: (a) Tonovet, (b) Tonovet Plus, (c) Tono-Pen Avia Vet e (d) Kowa HA-2.

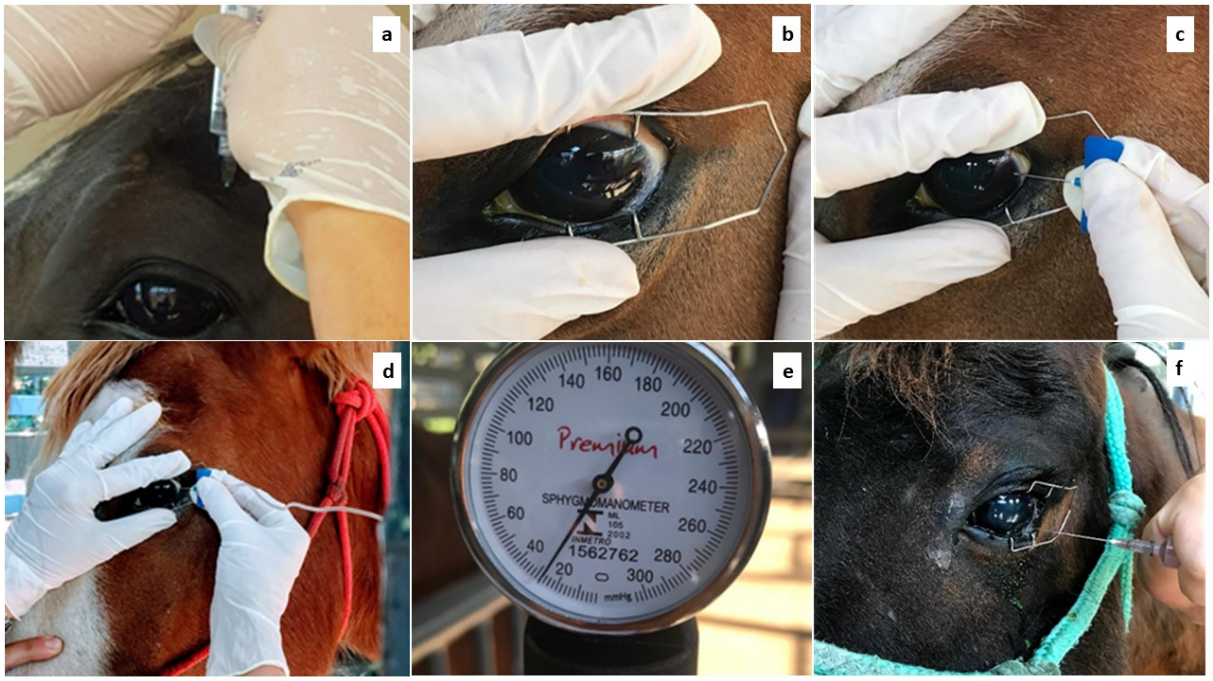

FIGURA 3 Realização da manometria (mmHg) do grupo *in vivo* de cavalos sedados (n = 12 olhos) (a) Bloqueio auriculopalpebral, (b) Posicionamento do blefarostato, (c) Posicionamento para canulação da câmara anterior com escalpe 23G a 2 mm posterior ao limbo lateral na posição de 2h em olho esquedo, (d) Visão da câmara canulada e de uma parte do Sistema para aferição da PIO, (e) Aferição da PIO pelo manômetro aneróide, (f) Lavagem do tampão de cianoacrilato com solução fisiológica a 0,9% após a retirada do escalpe.

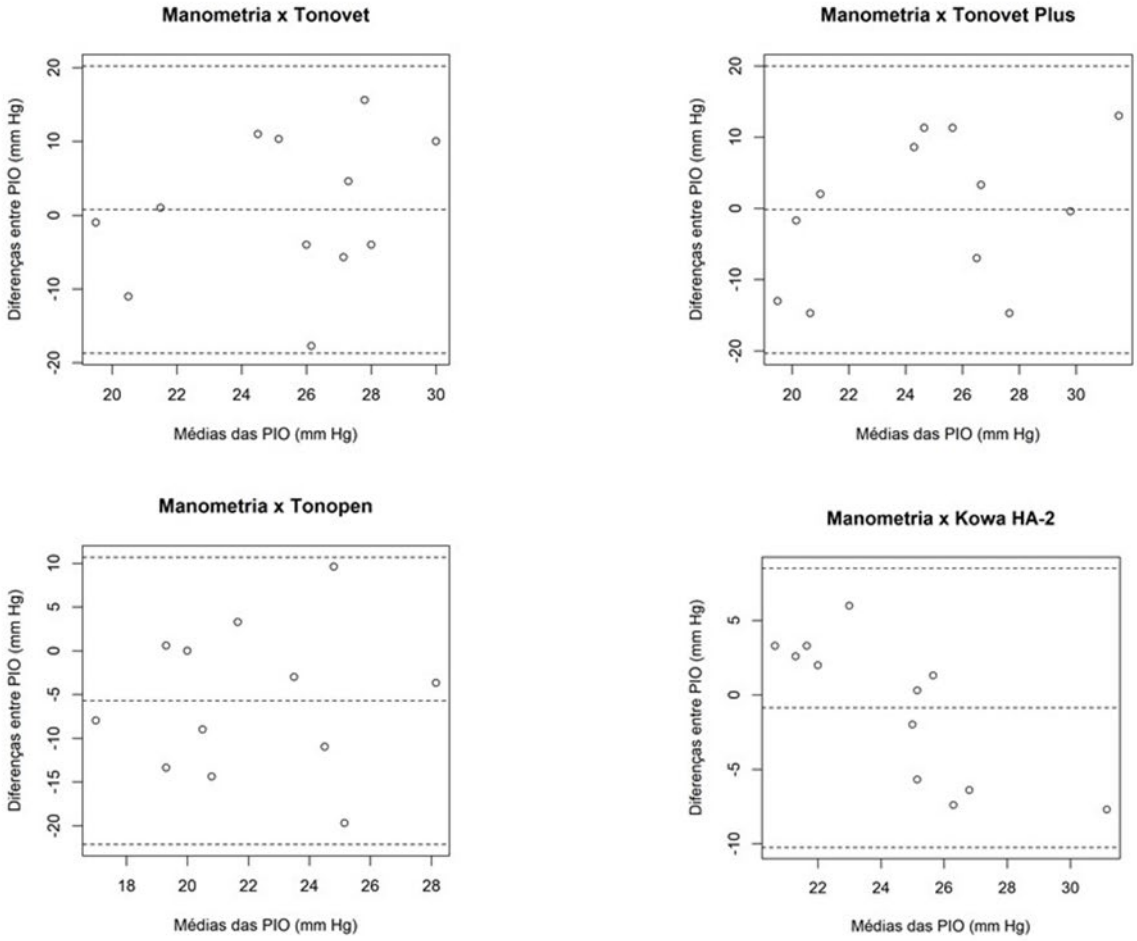

FIGURA 4 Gráfico de Bland-Altman das médias e diferenças entre as leituras da PIO em mmHg obtidas entre a manometria e a tonometria com diferentes tonômetros (Tonovet, Tonovet Plus, Tono-Pen Avia Vet e Kowa HA-2) do grupo *in vivo* em cavalos sedados (n = 12 olhos).

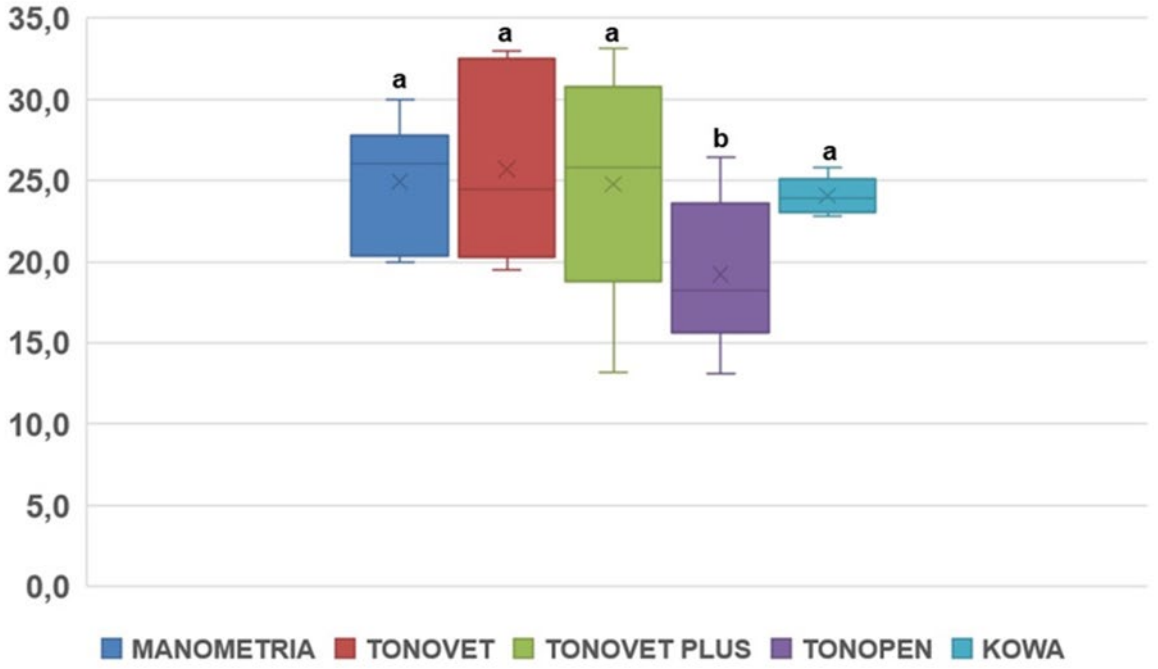

FIGURA 5 Boxplot dos valores das leituras da PIO (mmHg) da manometria versus tonometria com os tonômetros Tonovet, Tonovet Plus, Tono-Pen Avia Vet e Kowa HA-2 do estudo in vivo em equinos sedados.

*letras diferentes indicam diferenças significativas pelo teste de Tukey ($p<0,05$).

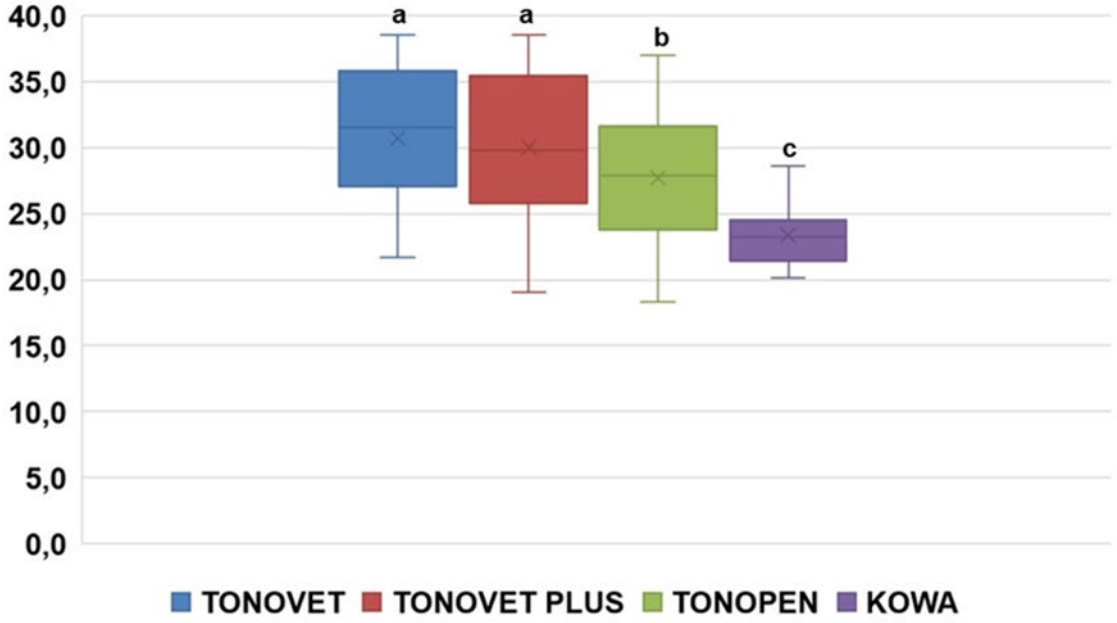

FIGURA 6 Boxplot dos valores das leituras da PIO (mmHg) dos tonômetros Tonovet, Tonovet Plus, Tono-Pen Avia Vet e Kowa HA-2 do estudo a campo em equinos não sedados.

*letras diferentes indicam diferenças significativas pelo teste de Tukey ($p<0,05$).

TABELA 1 Resultados da análise de correlação de Pearson entre PIOs mensuradas para os olhos esquerdo e direito, segundo os diferentes tonômetros no estudo a campo em animais sem sedação.

Tonômetro	r	IC95%	P
TONOVET	0,64	0,36 a 0,81	< 0,001*
TONOVET PLUS	0,69	0,43 a 0,84	< 0,001*
TONOPEN	0,40	0,05 a 0,67	0,026*
KOWA	0,91	0,83 a 0,96	< 0,001*

r = coeficiente de correlação de Pearson; IC95% = estimativa de *r* por intervalo de confiança a 95%; p = significância estatística para hipótese de que p difere de zero; * p<0,05

TABELA 2 Resultados da análise de correlação de Pearson entre PIOs e idade, segundo os diferentes tonômetros no estudo a campo em animais sem sedação.

Tonômetro	r	IC95%	p
TONOVET	0,33	-0,03 a 0,62	0,072
TONOVET PLUS	0,01	-0,35 a 0,37	0,962
TONOPEN	-0,06	-0,41 a 0,31	0,763
KOWA	0,28	-0,09 a 0,58	0,129

r = coeficiente de correlação de Pearson; IC95% = estimativa de r por intervalo de confiança a 95%; p = significância estatística para hipótese de que p difere de zero;

TABELA 3 Médias e desvio-padrões das PIOs em relação ao sexo segundo os diferentes tonômetros no estudo a campo em animais sem sedação.

Tonômetro	Machos	Fêmeas	Geral	p
TONOVET	29,8 ± 5,4	30,0 ± 5,5	29,96 ± 5,37	0,891
TONOVET PLUS	30,0 ± 5,6	28,6 ± 6,9	29,14 ± 6,36	0,545
TONOPEN	25,8 ± 4,9	26,3 ± 5,4	25,97 ± 5,11	0,792
KOWA	24,4 ± 2,6	23,2 ± 3,7	23,69 ± 3,35	0,288

p = significância estatística no teste de ANOVA para comparação entre os tonômetros

ANEXO – NORMAS DE PUBLICAÇÃO DO PERÍODICO EQUINE VETERINARY JOURNAL

Equine Veterinary Journal (EVJ) publishes evidence to improve clinical practice or expand scientific knowledge underpinning equine veterinary medicine. In our bi-monthly issues, *EVJ* publishes original and high quality peer reviewed articles from all over the world.

Author Guidelines

1. SUBMISSION

Authors submitting an article do so with the understanding that the work and its essential substance have not been published in full before and is not being considered for publication elsewhere. If abstracts have been published, in print or online, full articles will be considered only if the published abstract was less than 1000 words and did not contain figures or tables. A copy of the abstract should be uploaded to ScholarOne at the time of submission as a supporting file (see **Section 4.9.i**). *EVJ* accepts material that has been presented orally at conferences that include web-based distribution of video or audio recording of presentations. Manuscripts are screened with software designed to detect plagiarism, including duplication of passages from the authors' previous papers. Reference to previous work within the methods section is generally more appropriate.

Once the submission materials have been prepared in accordance with the Author Guidelines, manuscripts should be submitted online at https://mc.manuscriptcentral.com/evj

The submission system will prompt authors to use an ORCID iD (a unique author identifier) to help distinguish their work from that of other researchers. **Click here** to find out more.

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For help with submissions, please contact: **jane@evj.co.uk**

2. AIMS AND SCOPE

Equine Veterinary Journal (EVJ) publishes and promotes high quality peer-reviewed research that improves equine clinical practice and informs veterinary science. Clinical relevance is the most important criteria against which articles are assessed. This unrivalled international scientific journal is published 6 times per year with over 125 subscription articles per year and a variable number of additional Open Access articles. Contributions are received from sources worldwide.

EVJ expects to publish work of high scientific rigour. To support authors we regularly include editorial material which aims to define our scope and help authors and readers identify standards expected in commonly used study areas. Recent examples include:

How to publish diagnostic imaging studies: common mistakes and recommendations
Designing and performing pharmacokinetic studies for systemically administered drugs in horses
Clinical validation of diagnostic tests
Baysesian versus Frequentist approaches
Bias

Early career researchers are encouraged to make use of our resources on publishing clinical research and writing for veterinary medicine available via our website and can access help with manuscript preparation. Also, EVJ has collected together a number of editorial and tutorial articles on research methods here.

Manuscripts first undergo an Internal Editorial Assessment to ensure that the manuscript content is of interest to our readership, of high scientific quality and the manuscript format conforms to our

guidelines described below. If deemed appropriate, manuscripts are sent for peer review and our review process is double-masked (i.e. the reviewers are not identified to the authors and vice versa, see **Marr, C.M. (2011) Masking the peer review process: better or worse? Equine vet J, 43, 249**). Before being accepted for publication, at least two experts are consulted and many manuscripts also undergo assessment by an individual with expertise in study design and data analysis. Our median time for completing the review process for original submission is around 5 weeks. Accepted manuscripts are pre-published within 10 days of acceptance and immediately submitted for indexing.

3. MANUSCRIPT CATEGORIES AND REQUIREMENTS

EVJ publishes peer-reviewed research articles and reviews on all aspects of equine veterinary science. Categories include General Articles (encompassing both experimental and clinical research and includes systematic reviews and metanalyses), Technical Notes, Review Articles and Correspondence. We welcome submissions from researchers and clinicians from within institutes, hospitals or practices. Reports of novel treatments or diagnostic techniques are unlikely to be considered unless a sufficiently large number of cases have been studied to have reasonable insight in effectiveness and possible harms. Diseases, particularly infectious disease, which have not been reported previously are of interest; however, descriptions of individual cases or small numbers of equids with rare disorders that have been well characterised in other species are unlikely to be of interest, except where the report also illustrates a novel approach to clinical investigation or indicates that the condition is often misdiagnosed, likely to increase in prevalence (e.g. heritable mutation), or shows that novel or conventional treatments or investigations can cause severe harm. Authors should note that fewer than 10% of small case reports that are submitted are accepted.

Authors are encouraged to consult Wiley's Free Online Guide: Writing for Publication in Veterinary Medicine.

3.1 General Articles

General Articles should describe experimental or clinical studies, including systematic reviews and metanalyses. To minimise publication bias, *EVJ* encourages authors to publish negative results, providing the study had adequate power to detect differences and study design is robust.

EVJ readers value conciseness. Our target for General Articles is around 4000-4500 words including figure legends, table legends and references with up to 3 tables and 6 figures, although we are prepared to consider longer articles reporting complex studies. Supplementary items are not included in this word count. Figures must be selected carefully and each must enhance the article. Our reviewers and editors will assess the value of each and where individual figures are not considered essential they may be deleted or moved to online only. Figures should have no more than 6 sub-panels. Our typesetters may choose to set out larger and composite figures across 2 columns but the author must consider the size of the resultant print images particularly where diagnostic images are included in sub-panels. Images will be scaled to fit the page layout by the typesetters. Details of specific formats and requirements are given in **Section 4.4.vi**.

A summary, aiming for around 300 words in total, should be provided with the following headings

- **Background**: The background behind the decision to choose this subject to study.
- **Objectives**: The statement that is being tested, and is testable by the methods (below); or the original aims and study deliverables.
- **Study design:** Concise statement of the study design[§]
- **Methods**: Brief description of materials and methods, including number of subjects, and methods of testing hypotheses.
- **Results**: Brief highlights of the results obtained.
- **Main limitations**: Concise statement of limitations and sources of bias. This will often include comments on the study population, its size and the study generalisability.
- **Conclusions**: Conclusions drawn from results.

§ For concise definitions of clinical study designs see https://en.wikipedia.org/wiki/Clinical_study_design

EVJ's layout includes numbering for each section, i.e. 1. Introduction, 2 Materials and methods etc. which may be further divided into subsections e.g. 2.1. Animals, 2.2. Examination protocol, etc. The introduction should be limited to around 400 words and should be succinct (approximately two paragraphs), conveying why the subject is important and briefly describing what information is known. It is not necessary to provide references for widely accepted clinical practices or knowledge. Authors should bear in mind that most *EVJ* readers have a comprehensive knowledge of equine disease and it is not necessary to include background statements of a basic nature. A comprehensive review of the literature is unnecessary but do state clearly the rationale for your study along with your hypothesis or research question and specific objectives. Summarise how your approach will help fill the gaps in information previously stated. Do not summarise the study findings.

The remainder of the manuscript should be presented in the following sections; Materials and Methods, Results, and Discussion, with subheadings, including data analysis, as appropriate. Additional tables, figures, video material or text describing further details of methods or results can be submitted as Supplementary Items (**see Section 4.5**). Where questionnaires or other similar instruments have been used to collect data a copy of these must be included for online publication, translated into English and with identifying features removed if appropriate. All quantitative results should be analysed by appropriate statistical methods.

Articles must describe discrete studies, using references where appropriate to make methods sections concise. Pairs or larger sets of articles that must be reviewed, and subsequently read, together are not accepted. If appropriate, authors can upload in press articles with identifying details removed, for reviewers' use.

Additionally, although no substitute for obtaining advice on study design and data analysis at the time of planning their work, prior to submission authors must address the points in the **EVJ Manuscript Checklist**. Where any form of data analysis is included authors are required to use the relevant section of our Submission Checklist when assessing their manuscripts prior to submission and a copy of the completed checklist must be uploaded with their submission.

3.1.1 Clinical Audit

Clinical audit is a quality improvement process that seeks to improve patient care and outcomes through systematic review of care against explicit criteria and the implementation of change (see Burgess R. New Principles of Best Practice in Clinical Audit 2. Oxford Radcliffe Publishing 2011). The steps in a clinical audit cycle involve (i) identification of a problem, (ii) definition of a standard against which to judge performance, (iii) collection of data, analysis of these data against the pre-determined standard, (iv) development and implementation of recommendations based on the outcome of this analysis and finally (v) re-audit. To evaluate the impact of these recommendations *EVJ* encourages submission of clinical audit addressing themes around improving outcomes, structures and processes, generating benchmarks and showing clinically important correlations.

Clinical Audits should conform in style and format to General Articles and are most likely to be successful in *EVJ*'s peer-review process when the audit cycle has been completed. However, we will consider clinical audits which seek to generate benchmarks or uncover important data where the dataset is large and highly likely to be transferrable to other clinical centres. Usually, benchmark-generating audit should involve multiple centres to improve generalisability and applicability of the results.

Authors considering submitting Clinical Audit to Equine Veterinary Journal should read our Clinical Audit Guidelines.

3.2. Registered Reports

Registered Reports are designed to eliminate publication bias and incentivise best scientific practice. Registered Reports are a form of empirical article in which the methods and proposed analyses are pre-registered and reviewed prior to research being conducted. This format is designed to minimise bias, while also allowing complete flexibility to conduct exploratory (unregistered) analyses and report serendipitous findings. The cornerstone of the Registered

Reports format is that authors submit as a Stage 1 manuscript an introduction, complete and transparent methods and the results of any pilot experiments (where applicable) that motivate the research proposal, written in the future tense. These proposals will include a description of the key research question and background literature, hypotheses, experimental design and procedures, analysis pipeline, a statistical power analysis and full description of planned comparisons. Submissions will be reviewed by *EVJ* editors, statistical editors and/or external peer reviewers.

Following Stage 1 peer review, manuscripts will be rejected outright (**see the most common reasons why Stage 1 manuscripts are rejected**), offered the opportunity to revise, or be accepted. Proposals that meet the evaluation criteria listed above will be issued an *in-principle acceptance* (IPA), indicating that the article will be published pending completion of the approved experiments and analytic procedures, passing of all pre-specified quality checks, and a sensible interpretation of the findings, discussion of limitations and biases and conclusions that are supported by the data, the manuscript will be published regardless of the results.

Stage 1 protocols will be published following IPA. Stage 1 articles will be referenced into the final Stage 2 manuscript as a footnote, indicating the manuscript is a registered report and will include a link to the paper. In addition, the authors will be required to cite and appropriately reference the Stage 1 report in the final manuscript.

Following data collection, authors prepare and resubmit a Stage 2 manuscript that includes the introduction and methods from the original submission plus their obtained results and discussion. The manuscript will undergo full review; referees will consider whether the data test the authors' proposed hypotheses by satisfying the approved outcome-neutral conditions, will ensure authors adhered precisely to the registered experimental procedures, and will review any unregistered post hoc analyses added by the authors to confirm they are justified, methodologically sound and informative. At this stage, authors must also share their data (see also Wiley's **Data Sharing and Citation Policy**) and analysis scripts on a public and freely accessible archive such as Figshare or Dryad. Data deposited into a repository must be cited.

Additional information can be found by clicking the link to the **Open Science Framework** from the Center for Open Science.

3.3 Technical Notes

A Technical Note is a short article giving a brief description of a specific development, technique or procedure, or it may describe validation studies or a modification of an existing technique, procedure or device applicable to equine veterinary medicine (in a clinical or research setting). The aim of a Technical Note should be to describe, evaluate and assess accuracy of the technique, procedure or device.

The technique, procedure or device described should have practical value and should contribute to clinical diagnosis or management, or be applicable in research. It could also present a software tool or an experimental or computational method. The article must describe a demonstrable advance on what is currently available. The main criteria for publication will be the novelty of concepts involved, the validity of the technique and its potential for clinical or research applications. The method needs to have been well tested in terms of accuracy, repeatability and safety and authors are encouraged to highlight key challenges in development of novel methods and how they addressed these. The Technical Note can demonstrate use of the technique, procedure or device in a research or clinical setting, but Authors are cautioned that a Technical Note is not generally an appropriate format to describe a hypothesis-driven study or study seeking to answer a specific clinical question where a General Article format is usually more appropriate.

Technical Notes should be around 2500 words in length and should contain no more than 2 figures and 2 tables, with a short structured summary in our usual format (**see Section 3.1**).

3.4 Review Articles

Review Articles should normally address important topics relevant to equine veterinary medicine and synthesise results in areas in which there have been large numbers of recent publications. Review Articles are encouraged, however a preliminary discussion with the **Editor** regarding

subject and length of the article is advisable before submission. Please include a list of the main points that the review will cover and a list of approximately 20 key references that you plan to cite. The word count for review articles should be around 10,000 with no more than 6 figures and 2 tables. Note: articles describing systematic reviews and meta-analyses (i.e. secondary research synthesis) are categorised as general articles. A preliminary discussion with the Editor is not required for secondary research synthesis.

3.5 Correspondence

We encourage readers to write letters to the editor for publication. These should usually address one main theme. Appraisals of published work must be measured in tone and ideally supported with specific evidence of weaknesses in the study design, methods, analysis of data and/or conclusions drawn from the results. Correspondence on general issues important to equine veterinary medicine is also welcomed. Correspondence is peer reviewed by at least two individuals, normally *EVJ* editors and/or members of our Editorial Consultant Board, and where correspondence relates to a specific *EVJ* article the authors of that article will automatically be given the opportunity to write a response.

3.6 Editorial

EVJ's editorial material is all commissioned and typically encompasses opinions on issues of current importance in equine veterinary sciences, pieces expanding on studies published within EVJ and editorials which summarise recently published clinical research ('Clinical Insights'). We also include reports of conferences and workshops and short overviews of work that has recently been published in basic science journals that may be of interest to EVJ readers. Editorials in these last two categories are usually described as 'Science-in-brief'. Conference organisers and scientists wishing to draw readers' attention to relevant basic science studies and those who wish to write a Clinical Insight summary are encouraged to contact the Editor to discuss their suggestion [**editor@evj.co.uk**]. Editorial material is not peer-reviewed but is assessed by one or more of the *EVJ* editors prior to acceptance.

4. PREPARING THE SUBMISSION

4.1 Cover Letters

Submissions should include a covering letter stating that the article is original, has not been submitted or published elsewhere, and has the approval of all authors.

4.2 Parts of the Manuscript

The manuscript should be submitted in separate files: title page; main text file including tables; figures; supplementary items; supporting information.

4.3 Title Page

The title page should contain:

i. A short informative and interesting title. Use 8–12 words and encapsulate the content of the article. Give the answer not the question. Put major keywords in first 65 characters and avoid "filler" words like "effects of", "comparison of", "retrospective case series of", "Ten cases of". The title should not contain abbreviations (see Wiley's best practice SEO tips);
ii. The full names of the authors;
iii. The authors' institutional affiliations where the work was conducted;
iv. Declarations
    a. Authorship (see Section 5.8)
    b. Source of Funding (see Section 5.7)
    c. Competing Interests (see Section 5.6)
    d. Ethical Animal Research (see Section 5.4) i.e. Details of ethical review framework

e. Informed consent (see Section 5.4.ii)
f. Acknowledgements (see Section 5.9)
g. Data accessibility statement, including a link to the repository used (see Section 5.2)

The current address of any author, if different from where the work was carried out, should also be supplied.

4.4 Main Text File

As articles are double-masked peer reviewed, the main text file should not include any information that might identify the authors.

The main text file should be presented in the following order:

1. Main text;
2. Manufacturers' addresses;
3. Tables (if relevant, each table complete with title and footnotes);
4. List of Figure legends (if relevant);
5. List of legends for Supplementary items;
6. References
    a. Remove reference manager field codes or convert to plain text before uploading your manuscript (available in the reference manager's tools section) however you may find it helpful to retain an identical copy of the manuscript before removing the field codes in case you need to go back to this to make revisions

Figures and supplementary items should be supplied as separate files. One file per item.

4.4.i Summary and Abstract

A structured summary is required for General Articles and Technical Notes (**see Section 3.1**), an abstract is required for Review Articles. Editorial and Correspondence do not require summaries or abstracts. For details on manuscript types that require abstracts and/or keywords, as well as how to prepare them, please refer to the '**Manuscript Categories and Requirements**' section. See **Section 4.11** for tips on search engine optimisation.

4.4.ii Keywords

Keywords are required for General Articles, Technical Notes and Review Articles. Authors can provide up to six keywords for publication immediately below the title which may help readers find the article in various search engines. These should be different from those used in the title and reflect the manuscript content and should usually include the word 'horse' or similar but not 'Equine' or 'Veterinary' as these terms will be included by most Search Engines as being within the journal's title. Keywords for publication should be listed on the main document. Note during the submission process, you will also be required to choose separate ScholarOne keywords which facilitate the review process – see **Section 4.9.iii**.

4.4.iii References

It is the responsibility of the authors to ensure that all reference details are accurate. References are indicated throughout the text as superscript numbers placed after full stops or commas. The final list of references must correspond with the order in which they appear in the main body of the text.

The format in the reference list is as follows: author(s) name(s) and initials, full title of article, journal title as abbreviated in the World List of Scientific Periodicals, year of publication, volume and page numbers: e.g. Foster BW, Codd J, Smith R Effect of stress on ulcers in foals. Equine Vet J. 1992;35(1):43-52.

The editor and publisher recommend that citation of online published articles and other material should be done via a DOI (digital object identifier), which all reputable online published material

should have - see **www.doi.org/** for more information. If an author cites work that does not have a DOI they run the risk of the cited material not being traceable.

See the **Wiley AMA Manual of Style** for further reference examples and general manuscript style information.

4.4.iv Tables

Tables should summarise data to complement, not duplicate, information contained in the text. Table legends should contain sufficient information to describe the content to a reader who has not read the main text. Tables should be supplied as editable files, not pasted as images. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text. All abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD, 95%CI or IQR should be identified in the headings. Large tables, particularly those listing characteristics of individual cases included in the study, should be submitted as Supplementary Items.

4.4.v Figures and Figure Legends

Figures should be used to clarify methods and study design and to highlight important results. Figure legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement.

4.4.vi Figure Format

Authors are encouraged to send the highest-quality figures possible. **Click here** for figure requirements. We prefer to use colour in graphs and charts rather than shades of grey, and to use pastel rather than primary colours. Avoid colour-fill in diagram and chart backgrounds. There is no fee for figures printed in colour.

4.5 Additional Files

Supporting Information (files for online publication only)

Material that is not essential to the article but provides greater depth and background such as data sets or additional figures, tables or video files can be published online only as supplementary items. For information on data sharing see **Section 5.2**. Supplementary items will not be published in the print edition of the journal but will be viewable via the online edition.

There is no specific limit to the number of supplementary items that can be included but each item will be evaluated by reviewers and editors to ensure that it enhances the article.

Supplementary items are subject to the peer review process and to ensure that the peer review process is masked, if inclusion of identifying information is unavoidable, it may be necessary for authors to upload two copies of some items, one of which is masked for peer review. Each item must be referred to in the main body of the text of the article where appropriate. See **Section 4.9** for details of how to upload these files.

Items submitted for online publication only do not undergo copy editing or typesetting, rather they appear largely as the authors have submitted them with minor corrections being made by the Editors only. Therefore, supplementary items are not sent back to authors at the proof correction stage.

Supplementary items must be prepared as follows:

1. Each individual item (i.e. separate file, table etc) must be uploaded as a separate file under the file designation ‘Files for online publication only’. A supplementary item may also be composed of a brief description of additional methods and results and can include some discussion or references, for example when describing validation of a method used in the study.
2. Articles with associated supplementary items which are not contained within separate files (one per item) will not be sent for peer-review.

3. Number each item in the order that it appears in the text. Supplementary items should be labelled Data S1, Figure S1 or Table S1 as appropriate.
4. Typically, after acceptance supplementary items will be branded by *EVJ* and converted into PDF but PDFs are not acceptable throughout the peer review process.
5. Text: Word (.docx); choose A4 (not US letter), double spaced, top and bottom margins 3.17 cm; left and right margins 2.54 cm, continuous line numbers, page numbers, font – Arial size 10.
6. Figures: GIF, TIFF, EPS, PNG, JPEG, BMP, labelling Arial font, with size selected as appropriate for figure.
7. Tables in Word (.docx) A4, Page set up can be as portrait or landscape as appropriate; top and bottom margins 3.17 cm; left and right margins 2.54 cm, continuous line numbers, Font – Arial, size as appropriate
8. Tables in Excel (.xlsx); use Excel rather than Word for large data tables. Font – Arial, size as appropriate. Data presented in Excel will not usually be converted to PDF as it may be of more use to other researchers in its original format
9. Movies: MPEG, AVI or Quicktime movie format. Any labelling should be in Arial font, with size selected as appropriate. A generous total allowance is available for supplementary video but authors are encouraged to upload several smaller videos rather than use large ones which some readers may have difficulty downloading. The optimal size of each individual supplementary item is around 10Mb.
10. Within the main text (before the reference section), provide a list headed 'List of legends for Supplementary items' with numbers and legends for all Supplementary Items (see Section 4.4).

Note: if data, scripts, or other artefacts used to generate the analyses presented in the article are available via a publicly available data repository, authors should include a reference to the location of the material within their article. **See section 5.2**.

Click here for Wiley's FAQs on supporting information.

4.6 General Style Points

The following points provide general advice on formatting and style. See the **Wiley AMA Manual of Style** for more detailed manuscript style information.

4.6.i Language

Our language of publication is English. The journal uses British spelling as defined in the *Oxford English Dictionary*; we recommend that you choose "English (United Kingdom)" in the spelling preference of your word processing software.

Manuscripts in which the quality of English is insufficient will be rejected without peer review. Authors for whom English is a second language should consider having their manuscript professionally edited before submission to make sure the English is of high quality. A list of independent suppliers of editing services can be found at **http://authorservices.wiley.com/bauthor/english_language.asp.** All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

EVJ provides Chinese and other foreign language translations of selected article summaries online within the Supporting Information section. Authors are welcome to include foreign language translations of their summary for online only publication. Such items should be entitled "Supplementary Item: Summary in French" etc.

4.6.ii Abbreviations, Symbols and Nomenclature

Abbreviations: In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Initially, use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation only. Where possible, only abbreviations that are in common use within the relevant field or discipline should be used. Authors must avoid inventing their own abbreviations for anatomical terms and techniques. Where they are

unavoidable, abbreviations must be explained in both the summary and main text. Spelling should conform to the Oxford English Dictionary, medical terminology to Dorlands Medical Dictionary and units, symbols and abbreviations should conform to the International System of Units defined by Baron, D.N. and McKenzie Clarke, H (Eds) (2008) 'Units, Symbols, and Abbreviations: A Guide for Medical and Scientific Editors and Authors, 6th edn.' Royal Society of Medicine Press, London. Authors should adhere to *EVJ*'s preferred abbreviations for radiography, and preferred terminology and abbreviations for equine upper airway disorders and for equine echocardiography.

Units of measurement: Measurements should be given in SI or SI-derived units. Visit the Bureau International des Poids et Mesures (BIPM) website at **https://www.bipm.org/en/about-us/** for more information about SI units. Doses and measurements should be given in metric (SI) units with /kg bwt added where appropriate. Numbers under 10 are spelt out, except for: measurements with a unit (8 mmol/L); age (6 weeks old), or lists with other numbers (11 dogs, 9 cats, 4 gerbils).

4.6.iii Drug Names and Manufacturers' Details

The generic name of drugs, equipment or other materials should be given in the text, with the product and company name in brackets. Recommended International Non-Proprietary Names (rINNs) **http://www.mhra.gov.uk/Howweregulate/Medicines/Namingofmedicines/ChangestomedicinesnamesBANstorINNs/index.htm** should be used. Brand or trade names should not be used in the title and must be in brackets throughout the text.

4.6.iv Genetic Nomenclature

Sequence variants should be described in the text and tables using both DNA and protein designations whenever appropriate. Sequence variant nomenclature must follow the current HGVS guidelines; see **varnomen.hgvs.org**, where examples of acceptable nomenclature are provided.

4.7 Manuscript File Format

Manuscripts should be submitted as Word documents in double spacing on A4 page size, the pages should be numbered and there should be line numbers continuously throughout the document. Division of the article should be indicated clearly by major headings, subheadings and sub-subheadings. Manuscript text must be uploaded as Word (.docx).

The text file must contain the manuscript including summary, text, tables, list of legends for figures, list of legends for supplementary items, references, but *no* embedded figures. A word count for the entire text including figure, table and supplementary item legends and references (but not including the contents of supplementary items) should be provided. Figures and supplementary items should be provided in separate files.

4.8 Authors' Identity and Declarations

Authors' names, institutes, affiliations and declarations should not be included within the manuscript in order to facilitate double-masked review. To ensure the integrity of this process, authors must create a separate Word document and upload this separately as the 'Title page' document. This document should also contain the author declarations (see **Section 4.3**). Also list here any other details included in the text which might allow the reviewer to identify the authors or host institute, for example, reference to 'in press' publications. In their place, within the main manuscript, state 'masked for review' and highlight these words. All such details must be listed in the 'Title Page' document, with reference to the appropriate line number. With all revisions, authors must re-insert such details into a clean, unmasked version of their manuscript that should be uploaded in addition to the masked version with revisions marked. Copies of 'in press' publications, with relevant identifying details removed, should be uploaded as 'Supporting information' for reviewers' use. It may also be necessary to remove identifying features from supplementary items, such as institute logos on questionnaires.

4.9 ScholarOne Manuscripts

EVJ uses ScholarOne Manuscripts for online manuscript submission and peer review found at **http://mc.manuscriptcentral.com/evj**. Full instructions and support are available on the site and a user ID and password can be obtained on the first visit. If you require assistance then click the **Get Help Now** link that appears at the top right of every ScholarOne Manuscripts page. *EVJ* requires the submitting author (only) to provide an **ORCID iD** when submitting their manuscript.

4.9.i Submission Process

To allow double-masked review, please upload your manuscript in the following manner:

1. Title page (including authors' names and affiliations, corresponding author's email address, declarations, keywords for publication, word count and any details 'masked for review') should be uploaded under the file designation 'Title Page'. Documents uploaded as 'Title Page' will not be viewable in the HTML and PDF format you are asked to review at the end of the submission process.
2. Your manuscript, without a title page, under the file designation 'Main Document'. Please ensure there is no identifying information in this document.
3. Clean non-masked document.
4. Figure files under the file designation 'Figure'.
5. Supplementary items under the file designation 'Files for online publication only'. One file per item.
6. Supporting information (e.g. copies of previously published abstracts or 'in press' publications, with identifying information removed for use by editors and reviewers) under the file designation 'Supporting Information'.

4.9.ii Suggesting a Reviewer

In order to facilitate the review process, *EVJ* welcomes suggestions from authors for appropriate peer reviewers (with e-mail addresses) but these will be followed at the editors' discretion.

EVJ will not normally use a reviewer named as 'non-preferred'.

4.9.iii ScholarOne Keywords

During the submission process, you will be required to select keywords describing the discipline(s) and body system/disease(s) that most closely relate to the content of your article from prescribed lists. These are used by the Editorial Office to match your article with appropriate reviewers. Note: these words can also be used as the 'Keywords for Publication', but this is not obligatory – see **Section 4.4.ii**.

4.9.iv Suspension of Submission Mid-way in the Submission Process

You may suspend a submission at any phase before clicking the 'Submit' button and save it to submit later. The manuscript can then be located under 'Unsubmitted Manuscripts' and you can click on 'Continue Submission' to continue your submission when you choose to.

4.9.v Confirmation of Submission

After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation e-mail after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct, please contact your IT department. The error may be caused by spam filtering software on your e-mail server. Also, the e-mails should be received if the IT department adds our e-mail server (uranus.scholarone.com) to their whitelist.

4.9.vi Manuscript Status

You can access ScholarOne at any time to check your 'Author Centre' for the status of your manuscript. The Journal will inform you by e-mail once a decision has been made.

4.9.vii Submission of Revised Manuscripts

Revised manuscripts must be uploaded within 12 weeks of authors being notified that they are invited to submit a revision. Locate your manuscript under 'Revised Manuscripts in Draft' and click on 'Continue Submission' to submit your revised manuscript. If, however, it is not possible to resubmit within this time frame, please contact the Editorial Office (jane@evj.co.uk). It is essential that you provide a point-by-point response to each of the reviewers' comments.

All articles must be uploaded with:

(i) A main document containing a version which does not have a title page and is masked and in which all changes are highlighted using 'track changes' mode. This version will be sent out to reviewers.

(ii) A document containing a version which includes the title page and in which masking details have been re-inserted and changes are not marked. This should be uploaded with the file designation 'Clean Non-Masked Copy'. If your manuscript is accepted, this version will be uploaded to our Accepted Articles section.

4.10 Pre-submission Checklist

All submissions must be accompanied by a completed copy of **EVJ Manuscript Checklist** which is available to download here.

4.11 Wiley Author Resources

Manuscript Preparation Tips: Wiley has a range of resources for authors preparing manuscripts for submission available **here.** Many students and researchers looking for information online will use search engines. By optimising your article for search engines, you will increase the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in another work. In particular, authors may benefit from referring to Wiley's best practice tips on **Writing for Search Engine Optimization**.

Editing, Translation, and Formatting Support: **Wiley Editing Services** can greatly improve the chances of a manuscript being accepted. Offering expert help in English language editing, translation, manuscript formatting, and figure preparation, Wiley Editing Services ensures that the manuscript is ready for submission.

5. EDITORIAL AND ETHICAL POLICIES

5.1 Editorial Review and Acceptance

The acceptance criteria for all articles are the quality and originality of the research and its significance to journal readership. Except where otherwise stated, manuscripts are double-masked peer reviewed. Articles will only be sent to review if the Editors determine that the article meets the appropriate quality, relevance and ethical requirements.

Wiley's policy on confidentiality of the review process is **available here**.

5.2 Data Storage and Documentation

EVJ expects that data supporting the results in the paper will be archived in an appropriate public repository. Whenever possible the scripts and other artefacts used to generate the analyses presented in the paper should also be publicly archived. Exceptions may be granted at the discretion of the editor for sensitive information such as human subject data or the location of endangered species. Authors are expected to provide a data accessibility statement, including a link to the repository they have used, to accompany their paper.

For information and advice on data sharing see **https://authorservices.wiley.com/author-resources/Journal-Authors/open-access/data-sharing-citation/index.html**

5.2.i Sequence Data

Nucleotide sequence data should be submitted in electronic form to any of the three major collaborative databases: DDBJ, EMBL, or GenBank. It is only necessary to submit to one database as data are exchanged between DDBJ, EMBL, and GenBank on a daily basis. The suggested wording for referring to accession-number information is: 'These sequence data have

been submitted to the DDBJ/EMBL/GenBank databases under accession number U12345'. Addresses are as follows:

- DNA Data Bank of Japan (DDBJ) ddbj.nig.ac.jp
- EMBL Nucleotide Archive: ac.uk/ena
- GenBank ncbi.nlm.nih.gov/genbank

RNA sequence data including small RNA sequencing, microarray and single cell sequencing should be submitted to one of the major repositories for gene expression data:

- GEO https://www.ncbi.nlm.nih.gov/geo/
- Array-express https://www.ebi.ac.uk/arrayexpress/
- ENCODE https://www.encodeproject.org/

Mass spectrometry proteomics data should be deposited to the PRIDE Archive (**http://www.ebi.ac.uk/pride/archive/**) via the PRIDE partner repository with the data set identifier PXDxxxx and 10.6019/PXDxxxx.

Example repositories are:

- Protein Information Resource (PIR): georgetown.edu
- SWISS-PROT: ch/sprot/sprot-top
- PRIDE: https://www.ebi.ac.uk/pride/archive/

Metabolomics data should be submitted following the guidelines. Example repositories are:

- MetaboLights https://www.ebi.ac.uk/metabolights/
- Metabolomics Workbench https://www.metabolomicsworkbench.org/data/index.php

5.3 Research Reporting Guidelines

Accurate and complete reporting enables readers to fully appraise research, replicate it, and use it. Authors are encouraged to adhere to the following research reporting standards.

- CONSORT
- SPIRIT
- PRISMA
- PRISMA-P
- STROBE
- CARE
- COREQ
- STARD and TRIPOD
- CHEERS
- the EQUATOR Network
- Future of Research Communications and e-Scholarship (FORCE11)
- ARRIVE guidelines
- National Research Council's Institute for Laboratory Animal Research guidelines:
- The Gold Standard Publication Checklist from Hooijmans and colleagues
- Minimum Information Guidelines from Diverse Bioscience Communities (MIBBI) website
- Biosharing website
- REFLECT statement

5.4 Ethical Use of Data from Animals in Research

EVJ regularly publishes editorial pieces outlining our position on the use of animals in research and authors are encouraged to ensure that they are familiar with these. More details of *EVJ*'s

current policies regarding the use of animals in research can be found at **http://onlinelibrary.wiley.com/doi/10.1111/evj.12390/full**.

EVJ will reject manuscripts if the editors are not satisfied with the standards of ethical use of animals in research. Editors reserve the right to reject articles if there is doubt as to whether appropriate procedures have been used, and also to insist that information is provided in the text as to the measures taken to protect the welfare of subjects and the outcome of procedures undertaken in respect to any pain or suffering caused. We prioritise owner informed consent regardless of whether it is a national or institutional requirement or not.

All research involving either experimental or clinical research on animals described in manuscripts submitted to *EVJ* must follow international, national, and/or institutional guidelines for humane animal treatment and comply with relevant legislation in the country in which the study was conducted.

Authors are encouraged to adhere to animal research reporting standards, for example the **ARRIVE reporting guidelines** for reporting study design and statistical analysis; experimental procedures; experimental animals and housing and husbandry. Authors should also state whether experiments were performed in accordance with relevant institutional and national guidelines for the care and use of laboratory animals:

- US authors should cite compliance with the US National Research Council's Guide for the Care and Use of Laboratory Animals, the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals, and Guide for the Care and Use of Laboratory Animals.
- UK authors should conform to UK legislation under the Animals (Scientific Procedures) Act 1986 Amendment Regulations (SI 2012/3039).
- European authors outside the UK should conform to Directive 2010/63/EU.

5.4.i Research Ethics Framework

This normally involves review and oversight of the use and care of experimental animals by an institutional ethics committee. In some countries, review and oversight by an institutional, professional or hospital ethics committee is also mandatory for clinical research. Where such a requirement is not in place, *EVJ* strongly encourages researchers to seek informal appraisal of their clinical study protocols by experienced colleagues prior to undertaking any research on client-owned animals as we believe that this will improve the quality of the research, increase the likelihood that the planned outcomes will be achieved and help in the identification and management of adverse effects for the animals involved. The *EVJ* editors may approach institutional ethical committees where we seek further information on local policies, procedures and regulations. Irrespective of whether the study has ethical committee approval or not, authors are expected to satisfy the editors that they have conducted their research in an ethical manner.

5.4.ii Informed Consent

Manuscripts describing research using client-owned animals will be considered for publication only if the work:

- Involves informed client consent for inclusion in the study for all prospective research and informed client consent may be required for some retrospective studies but is not usually expected for retrospective review of medical records.
- *EVJ* accepts that informed client consent may be given by the horses' caretakers providing these individuals have the owners' authority to act as agents for the owner in relation to veterinary matters.
- Client confidentiality must be maintained and authors should select photographs and video material with care to ensure that humans cannot be identified specifically.
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The *EVJ* Editors may require authors to provide copies of signed owner informed consent forms.

Where data has been collected from human subjects, for example in studies involving riders as well as their horses, and data collected by competition of questionnaires and surveys, *EVJ* expects that authors provide a declaration that these subjects have consented to participate in the research.

5.4.iii Materials Derived from Veterinary or Post-mortem Examinations

Research using tissues, fluids or cells that are collected during clinical procedures and are not specific to a patient group (i.e. any other horse could have been substituted) may be used for research provided that the owner has been made aware that these tissues may be retained for research purposes as part of the general consent for the clinical procedure and has been given the opportunity to opt out of research in general.

Where archived material from specific groups of patients (whether defined by age, breed, sex, disease status or any other specific feature) is being studied, owner informed consent is required. Normally, this requirement will be waived for research on samples archived as a result of clinical activities prior to 1 January 2017. For samples collected after that date, authors may be asked to provide evidence that owner informed consent has been obtained. The *EVJ* editors also recognise that some studies include samples from unusual individuals that have been stored for many years and are prepared to discuss exemptions to the requirements for owner informed consent where historical samples are used. Authors should write to the editor setting out their specific concerns.

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Research using tissues, fluids or cells that are derived from commercial abattoirs do not require any form of consent from the previous owner but, if the specific abattoir is identified, the abattoir owner must give permission for publication of the abattoir identity.

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5.4.vi Data in the Public Domain and Research on Animals Derived from Sporting Authorities

Manuscripts describing research including data on animals or humans that are derived from public archives, such as racing result websites, do not require owner informed consent or the consent of Sporting Authorities.

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When the research involves the analysis of genetic material from horses which has been obtained via Sporting Authorities' mandatory or regulatory activities, *EVJ* advises researchers that they should obtain owner informed consent for genetic analysis, except where the researchers can provide explicit evidence that the Sporting Authority has informed horse owners that genetic material may be collected for research purposes and owner agreement to this requirement is implicit by participation in the sport.

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For manuscripts reporting medical studies that involve human participants, a statement identifying the ethics committee that approved the study and confirmation that the study conforms to recognised standards is required, for example: **Declaration of Helsinki**; **US Federal Policy for the Protection of Human Subjects**; or **European Medicines Agency Guidelines for Good Clinical Practice**.

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EVJ's Antimicrobial Stewardship policy is, in part, a self-certifying process. In many clinical studies which are not seeking to document treatment outcomes, for example those describing large retrospective series relating to surgical procedures or pathological findings, it may not be necessary or appropriate to list extensive details of antimicrobials given to the horses in the study and it is often appropriate to include statements such as 'horses were given perioperative antimicrobials' without expanding further. At the time of submission, authors will be asked if Critically Important Antimicrobials have been used and, if Critically Important Antimicrobials have been used and reported, authors are expected to give specific details and to discuss and justify their use explicitly, including proposing alternative options in future if appropriate. When cephalosporins licensed for use in the horse in the country of origin of the study described are used (such as ceftiofur and cephquinome), it is not necessary to discuss this explicitly except where the specific indication differs from those licensed (i.e. off label use).

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