UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA DE BOTUCATU
CAMPUS DE BOTUCATU

PREVALÊNCIA DA MUTAÇÃO E321G NO GENE *MYH1*, ASSOCIADA A MIOSITE IMUNO-MEDIADA, EM CAVALOS QUARTO DE MILHA NO BRASIL

ANA LUÍSA HOLANDA DE ALBUQUERQUE

Botucatu - SP

Julho de 2020

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Dissertação apresentada junto ao Programa de Pós-graduação em Medicina Veterinária para obtenção do título de Mestre.

Orientador: Prof. Dr. José Paes de Oliveira Filho

Co-orientador: Prof. Dr. Diego José Zanzarini Delfiol

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Dedico à minha família, Mariana,
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RESUMO

A miosite imunomediada (IMM) causa atrofia muscular aguda e infiltração linfocítica de miofibras. Em cavalos Quarto-de-milha (QM), uma mutação missense E321G em gene *MYH1* está altamente associada à suscetibilidade ao desenvolvimento de IMM. O objetivo deste estudo foi investigar a frequência alélica da variante E321G em gene *MYH1* em QMs no Brasil. Dos 299 QMs genotipados, 44 animais (14,7%) foram identificados como heterozigotos (N/My) para a variante E321G em gene *MYH1* e 255 (85,3%) foram homozigotos para o alelo *wild type* (N/N); portanto, a frequência do alelo foi de 0,074. Cavalos da linhagem de rédeas mostraram uma frequência de heterozigotos significativamente maior do que outras linhagens. Não houve associação entre miopatia de armazenamento de polissacarídeos tipo 1 ou hipertermia maligna e a variante E321G em gene *MYH1* nos animais avaliados. Além disso, aqui, descrevemos aqui pela primeira vez no Brasil dois potros (N/My) com sinais clínicos de IMM. Este estudo destaca a importância de medidas de controle para evitar um aumento na incidência de IMM associado ao E321G em gene *MYH1* em QMs no Brasil, principalmente em cavalos de rédeas.

Palavras-chave: miosite, doença muscular, doença genética, mutação, Quarto de Milha.

ALBUQUERQUE, A. L. H**. PREVALENCE OF THE E321G *MYH1* VARIANT, ASSOCIATED WITH IMMUNE-MEDIATED MYOSITIS IN QUARTER HORSES IN BRAZIL.**

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ABSTRACT

In Quarter horses (QHs), myosin heavy chain myopathy (MYHM), characterized by nonexertional rhabdomyolysis or immune-mediated myositis (IMM) with acute mus-cle atrophy, is highly associated with a missense E321G MYH1 mutation. We identified two related QH foals in Brazil with the E321G MYH1 mutation that had clinical signs of MYHM, with the histological confirmation of IMM in one foal. This prompted an investigation into the allele frequency of the E321G MYH1 variant across performance QHs using a DNA archive in Brazil. Of 299 genotyped QHs, 44 animals (14.7%) were identified as heterozygous (My/N) for the E321G MYH1 variant, and 255 (85.3%) were homozygous for the wild-type allele (N/N), with an allele frequency of 0.074. Reining horses showed a significantly higher prevalence of heterozygosity than those in other disciplines (P=0.008). The prevalence of type 1 polysaccharide storage myopathy was 0.032, with only two E321G MYH1 heterozygotes possessing the GYS1 mutation. This study highlights the existence of MYHM and the high prevalence of the MYH1 mutation in QHs in Brazil, especially in reining QHs, underlining the importance of control measures to prevent an increase in the incidence of MYHM in QHs in Brazil.

Keywords: muscle disorders; mutation; MYH1 gene; myositis.

1. INTRODUÇÃO

Após a substituição do uso de cavalos para trabalho e transporte por máquinas e automóveis, os equinos começaram a ser utilizados para lazer e se tornaram grandes representantes deste segmento. Estima-se a existência de 1,1 milhões de equinos neste segmento no Brasil desde 2015, gerando movimentação econômica de 5,84 bilhões de reais no mesmo ano. A partir de 2004, o Quarto de Milha começou a liderar o *ranking* de comercialização no Brasil (CORRÊA & MOTA, 2007).

Durante os últimos 20 anos, algumas bases genéticas de doenças que afetam cavalos da raça Quarto de Milha foram identificadas (TRYON et al., 2009). Estas são: paralisia periódica hypercalêmica (HYPP) (RUDOLPH et al., 1992), deficiência de enzima de ramificação do glicogênio (GBED) (WARD et al., 2004), astenia cutânea hereditária equina (HERDA) (TRYON et al., 2007), hipertermia maligna (MH) (ALEMAN et al., 2008), miopatia por acúmulo do polissacarídeo tipo 1 (PSSM1) (MCCUE et al., 2008) e Trombastenia de Glanzmann (CHRISTOPHERSON et al., 2007) e miosite inflamatória imunomediada/*Immune-Mediated Myositis* (IMM) (FINNO et al., 2018; GIANINO et al., 2019; VALBERG et al., 2018). Com exceção da última, todas estas doenças já tiveram a prevalência determinada em cavalos Quarto de Milha no Brasil (BADIAL et al., 2014; DELFIOL et al., 2015; ARAÚJO et al., 2018; DELFIOL et al., 2018; LEITE et al.,2019).

Atualmente, o painel de doenças genéticas que devem ser obrigatoriamente testadas afim de registrar garanhões na American Quarter- Horse Association (AQHA) é composto por HYPP, GBED, HERDA, PSSM e MH. O teste genético para diagnóstico de HYPP também é obrigatório para registro na Associação Brasileira de Criadores de Quarto de Milha (ABQM) para descendentes do garanhão Impressive.

A IMM equina é uma enfermidade muscular associada a uma mutação genética pontual, ainda não descrita no Brasil, denominada miosite inflamatória imunomediada (IMM) (FINNO et al., 2018; GIANINO et al., 2019; VALBERG et al., 2018). Clinicamente é caracterizada por intensa atrofia muscular, principalmente por episódios agudos e recorrentes, de musculatura epaxial e glútea, fraqueza muscular, apatia e infiltração de linfócitos CD 4+, CD 8+ e CD 20+ ao redor devasos sanguíneos e infiltrados de miofibras sem evidência de vacúolos orbitais (FINNO et al., 2018; VALBERG et al., 2018). A etiologia de várias doenças

autoimunes não é completamente compreendida, porém sabe-se que estímulo do ambiente e predisposição genética são fatores relevantes (FINNO et al., 2018). Não se sabe ao certo quais fatores ambientais contribuem com o desenvolvimento da doença em cavalos, porém 39% dos casos de IMM em equinos têm histórico de infecção por *Streptococcus* spp. ou vacinação para *Streptococcus equi subsp. equi* ou herpes vírus, quatro a três semanas antes do quadro clínico (FINNO et al., 2018). Porém, os fatores desencadeantes permanecem desconhecidos em mais de 40% dos casos (VALBERG et al., 2018).

A IMM, assim como demais doenças genéticas, foi correlacionada de acordo com a linhagem de trabalho, havendo maior prevalência em linhagens de rédeas (FINNO et al., 2018). A seleção de equinos, em especial da raça Quarto de Milha, é realizada baseada no desempenho atlético, o que levou a acentuada estratificação da população (BETTLEY et al., 2012).

Atualmente, já há a disponibilidade de teste genético para IMM nos EUA, possibilitando o diagnóstico precoce e evitando a reprodução indiscriminada possibilitando o nascimento de animais afetados, porém, ainda não há a identificação da mutação responsável pela IMM ou a disponibilidade de teste genético no Brasil.

2. REVISÃO DE LITERATURA

2.1 Miosite Inflamatória Imunomediada

Miopatias inflamatórias são alterações inflamatórias ou imunomediadas caracterizadas pela presença de linfócitos na musculatura esquelética (FINNO et al., 2018). Miosite Imunomediada é uma enfermidade conhecida que leva a atrofia muscular em cavalos Quarto de Milha e raças relacionadas, por vezes associada a fatores ambientais como vacinação e histórico de doença respiratória (VALBERG et al., 2018). A susceptibilidade da IMM é atribuída a uma

mutação *missense* no gene miosina de cadeia pesada 1 (*MYH1*) codificador da cadeia pesada da proteína miosina de fibras musculares tipo 2X (GIANINO et al., 2019).

2.1.1 Etiologia

Miosite inflamatória pode ocorrer devido à infecção prévia, pré-neoplásica ou causas imunomediadas. Causas de miosite infecciosa em cavalos incluem clostriodioses, estreptococose e sarcocistose, caracterizadas por mionecrose e infiltrados polimorfonucleares em tecido muscular (LEWIS et al., 2007). A etiologia de algumas doenças imunomediadas não são totalmente conhecidas, porém estímulo ambiental associado a predisposição genética é a causa mais provável para a IMM (FINNO et al., 2018). Doenças de caráter autoimune são caracterizadas como decorrentes de uma síndrome consequente da ativação de células T e/ou B. Atualmente, acredita-se que a ocorrência de doenças autoimune dependa de um ou mais mecanismos como susceptibilidade genética, características ambientais que podem levar ao desenvolvimento da doença, desencadeando auto-reatividade de linfócitos, alterações no processo patológico ao longo da progressão da doença, e múltiplos mecanismos de lesão tecidual (MACKAY & ROSEN, 2001).

Não estão totalmente esclarecidos quais são os fatores ambientais associados à IMM, porém 39% dos animais que manifestam a doença apresentavam histórico de infecção poucas semanas antes dos primeiros sinais clínicos, principalmente por *Streptococcus* spp., ou vacinação para influenza, rinotraqueíte equina ou *Streptococcus equi subsp. equi* (LEWIS et al., 2007; HUNYADI et al., 2017).

A infecção por *S. equi* subesp. *equi* pode levar à complicações como púrpura hemorrágica, empiema de bolsa gutural, obstrução de via respiratória superior, garrotilho bastardo, pneumonia, pleurite, agalactia, abcessos periorbitais e rabdomiólise (VALBERG, 2006b) A rabdomiólise grave e aguda é uma complicação rara e fatal por infecção de vias aéreas superiores por *Streptococcus* em equinos. Sugere-se que ocorra por presença de exotoxinas ou proteases na musculatura, devido a bacteremia, além de hipotensão e hipoperfusão, acidose lática, superantígenos estreptocócicos e ação de células T (SPONSELLER et al., 2005). Ainda não é esclarecido porque a musculatura superficial é particularmente mais afetada (LEWIS et al., 2007).

A IMM foi recentemente associada à mutação E321G no gene *MYH1*, conferindo susceptibilidade à doença, potencialmente dependente da presença de outros fatores desencadeadores de doenças imunomediadas (FINNO et al., 2018). A mutação missense identificada no gene *MYH1* está localizada em uma região altamente conservada da cabeça globular da proteína miosina em sub-fragmento-1 entre a região hélice loop-hélice de Hélice J e Hélice K. Os laços helicoidais entre desempenham um papel importante ara ligação do ATP. A mutação *MYH1* E321G substitui um ácido glutâmico (E) carregado negativamente por uma glicina não polar (G) que necessita de cadeias laterais necessárias para a formação de ligações de hidrogênio, resultando em instabilidade da proteína moisina 2 X devido a maior redução de contato entre os domínios SWITCH1 e hélice 1 da cabeça globular da miosina 2X (FINNO et al., 2018).

2.1.2 Epidemiologia

Na IMM, a população de risco está relacionada a fatores ambientais que desencadeiam a doença, associada a linhagens, pedigree e frequência alélica. Altas frequências de determinadas doenças genéticas ocorrem em linhagens específicas de maneira desproporcional (GIANINO et al., 2019). Nos EUA, por exemplo, as frequências alélicas de HERDA e GBED são mais elevadas em equinos da linhagem de apartação (respectivamente, 0,142 e 0,068) e *western pleasure* (respectivamente, 0,064 e 0,132). Em subgrupos de linhagem de Conformação há maior frequência alélica para HYPP (0,299) e PSSM (0,155) e alta prevalência da Síndrome Letal do Oveiro Branco em linhagens da raça Paint Horses (TRYON et al., 2009). A IMM ocorre predominantemente em linhagens de cavalos da raça Quarto de Milha (LEWIS et al., 2007). Nos EUA frequência alélica de IMM foi encontrada em maior proporção em linhagens de rédeas (0,135), representando 24,3% dos cavalos Quarto de Milha desta linhagem, seguido por *working cow* (0,085) e conformação (0,08). Encontrada também, em linhagens de apartação (0,044) e *western pleasure* (0,021), não encontrada em linhagens de tambor e corrida (GIANINO et al., 2019). Esta enfermidade tem distribuição bimodal de idade, tanto em humanos quanto equinos, afetando jovens (cavalos abaixo de 8 anos) e idosos (cavalos acima de 17 anos) (LEWIS et al., 2007).

2.1.3 Patogênese

A musculatura consiste de um conjunto de fibras com diferentes estruturas moleculares, contráteis, com propriedades metabólicas, que contribuem para várias habilidades funcionais (MORENO-SANCHÉZ et al., 2010). A função da musculatura é determinada por sua estrutura e tipo de fibra muscular que a compõe (WANG et al., 2017). A existência de múltiplas isoformas de proteínas miofibrilares é o principal fator que leva a diversidade de tipos de fibra, e sua composição principal responsável por caracterizar a contração. A classificação de fibras mais aceita é tipo 1, de lenta contração e tipos 2A e 2X, de rápida contração (ENNION et al., 2005).

A miosina de cadeia pesada (MyHC) é a molécula motora do músculo e forma estrutura de filamentos sarcômeros. Se houver mutação nesta molécula, há diferentes expressões fenotípicas, dependendo de qual é o isoforma mutada, seu tipo e localização da mutação. Em equinos, o gene *MYH1* codifica miosina de cadeia pesada, encontrada em fibras musculares de contração rápida tipo 2X (VALBERG et al., 2018). A miosina é um motor molecular que converte energia química em força mecânica. A miosina classe II convencional é uma proteína hexamérica composta por duas subunidades de miosina de cadeia pesada (MyHC), que por sua vez, apresenta dois domínios funcionais formados por dois domínios: cabeça globular amino-terminal, na qual se ligam as correntes de luz exercendo função motora e o terminal alfa-hélix que apresenta propriedade de formação de filamentos (VALBERG et al., 2018).

A susceptibilidade a IMM é atribuída a mutação genética *missense* E321G em região altamente conservada do gene *MYH1* do cromossomo 11 (GIANINO et al., 2019). O gene *MYH1* mutado apresenta composição de aminoácidos alterada da região da cabeça globular, potencialmente impactando na estabilidade proteica uma vez que a mutação E321G na proteína substitui um ácido glutâmico (E) negativamente carregado, por uma glicina (G) não polar, que por sua vez, não possui ligação com hidrogênio, havendo redução de contato entre áreas de domínio da cabeça globular da fibra 2X (FINNO et al., 2018). Em cavalos portadores do alelo mutado, a exposição de nova isoforma de miosina ao ambiente extracelular parece iniciar infiltração linfocítica e destruição de miofibras, predominantemente do tipo 2X (GIANINO et al., 2019).

Quando ocorre IMM e intensa atrofia muscular, há menos fibras musculares 2X e presença de infiltrado linfocítico em miofibras tipo 2X.

Miopatias hereditárias associadas a miosina têm formado um importante grupo de doenças que variam em sintomatologia clínica e relação a autoimune humoral (LEWIS et al., 2007). A IMM equina é caracterizada por intensa atrofia muscular e infiltração de linfócitos CD4+, CD8+ e CD20+ ao redor de vasos sanguíneos e infiltrados de miofibras sem evidência de vacúolos orbitais (FINNO et al., 2018; VALBERG et al., 2018). O infiltrado linfocítico encontrado em amostras de musculatura de equinos com IMM consiste principalmente de células CD4+, células plasmáticas e CD8+ encontradas em áreas de intensa inflamação (VALBERG et al., 2018).

No caso de IMM decorrente de infecção estreptocócica, a proteína M liberada da superfície bacteriana forma complexos junto ao fibrinogênio que induz à liberação de proteína de ligação à heparina, elevando intensamente a permeabilidade vascular. A proteína de ligação à heparina e neutrófilos primariamente polimorfonucleares é mediada por CD11/CD18, integrina que também é expressa em monócitos e ativada por linfócitos. Essa ligação ao CD11/CD18 leva a indução de várias quinases intracelulares como tirosina- quinases, que resulta em mobilização de cálcio intracelular (PAHLMAN et al., 2006). A proteína M também é potente ativador de monócitos, sua interação com TLR2 em monócitos resulta em produção de citocinas, especialmente IL-6 (PAHLMAN et al., 2006). Além disso, Finno (2019) mostrou similaridades entre regiões de alinhamento proteico entre as proteínas M do *Streptococcus equi* e a sequênca de aminoácidos MyHC 2 X, e sugere-se por este motivo a resposta imune adaptativa poderia ser acionada por epítopos compartilhados entre bactérias (como a proteína M de *Streptococcus* sp.) e a miosina.

2.1.4 Sinais Clínicos

Miopatias imunomediadas em equinos podem levar a sintomatologia aguda, edema generalizado, infarto ou atrofia muscular (VALBERG, 2006a). Sinais clínicos de IMM incluem rápida atrofia, particularmente de musculatura epaxial e gluteal, depressão e aumento do tônus muscular (LEWIS et al., 2007). Porém, não se encontra na literatura a justificativa do porque estes grupos musculares são os mais afetados. Sinais clínicos mais comuns descritos por Hunyadi et al (2017) foram rápida, simétrica, difusa e progressiva atrofia muscular (80% dos casos), aumento do tônus muscular/enrijecimento do andar

(74%) e febre (44%). Quadro clínico de miosite imunomediada associada a recente infecção de *Streptococcus equi. subsp. equi* foi descrito como agudo, grave, com intenso infarto muscular e necrose fibronoide de vasos sanguíneos, edema subcutâneo, e sinais clínicos de abdômen agudo (BEECH, 2000). Em quatro casos, descritos por Valberg e colaboradores (1996) após exposição a *S. equi* sem sintomatologia de garrotilho, observou-se atrofia rápida e progressiva de musculatura epaxial e glútea. Beech (2000) descreve sintomatologia semelhante em um equino não exposto a *Streptococcus equi*, porém desenvolveu atrofia muscular multifocal. Barrot et al. (2004), relataram um caso de suspeita de IMM em um pônei de 16 anos que apresentava atrofia bilateral de musculatura do pescoço, o diagnóstico foi realizado por achados histológicos da musculatura, exclusão de outras enfermidades e melhora ao tratamento com dexametasona. O caso foi descrito como atrofia muscular progressiva e indolor, limitada à musculatura de região medial do pescoço, bilateral e acompanhada de fraqueza.

2.1.5 Patologia Clínica e Histopatológico

Como demais rabdomiólises, espera-se valores séricos de creatinina kinase (CK) iguais ou acima de 5.000 U/L (VALBERG et al., 2018). Equinos com IMM apresentam, em média, valores séricos de CK e AST (aspartato aminotransferase) de 9.746 (Referência de 119 – 187 U/L) e 2.880 (Referência 138 a 409 U/L), respectivamente (LEWIS et al., 2007). Além disso, leucocitose (60%) e hiperfibrinogenemia (51%) são comumente observadas em casos de IMM (HUNYADI et al., 2017).

Achados histopatológicos em miopatias imunomediadas são descritos por vasculite leucoclástica com pouco infiltrado de células mononucleares, provavelmente decorrente de deposição de imunocomplexo microvasculares (LEWIS et al., 2007). Suspeitou-se de causa imunomediada para a degeneração muscular causada pela miosite em equinos a partir de infiltrados linfocíticos em amostras de equinos com rabdomiólise, havendo predominacia de CD4+ seguido de CD8+, de maneira semelhante a outras miosites imunomediadas como miosite mastigatória do cão, dermatomiosite em humanos (ambos com predominância de CD4+), e polimiosite em cães (predominância de CD8+) (DURWARD-AKHURST et al., 2016). O achado diagnóstico clássico de IMM é infiltrado linfocítico em miócitos em variados graus, na maioria dos casos ao redor de vasos sanguíneos (DURWARD-AKHURST & VALBERG, 2018). A IMM equina é

caracterizada por infiltrados linfocíticos CD4+, CD8+ e CD20+ ao redor de vasos sanguíneos e infiltrados de miofibras sem evidencia de vacúolos (FINNO et al., 2018).

A causa imunomediada para miosite em equinos foi melhor compreendida a partir de achados de biópsia (infiltrado linfocítico) e expressão sarcolemal de MHC I e MCH II em proporção de miofibras. A expressão de MHC ocorreu em algumas miofibras específicas em equinos afetados com PSSM, na ausência de infiltrados linfocíticos (DURWARD-AKHURST et al., 2016).

A figura 1 ilustra a atrofia de musculatura glútea, assim como região biopsiada e histopatológico.

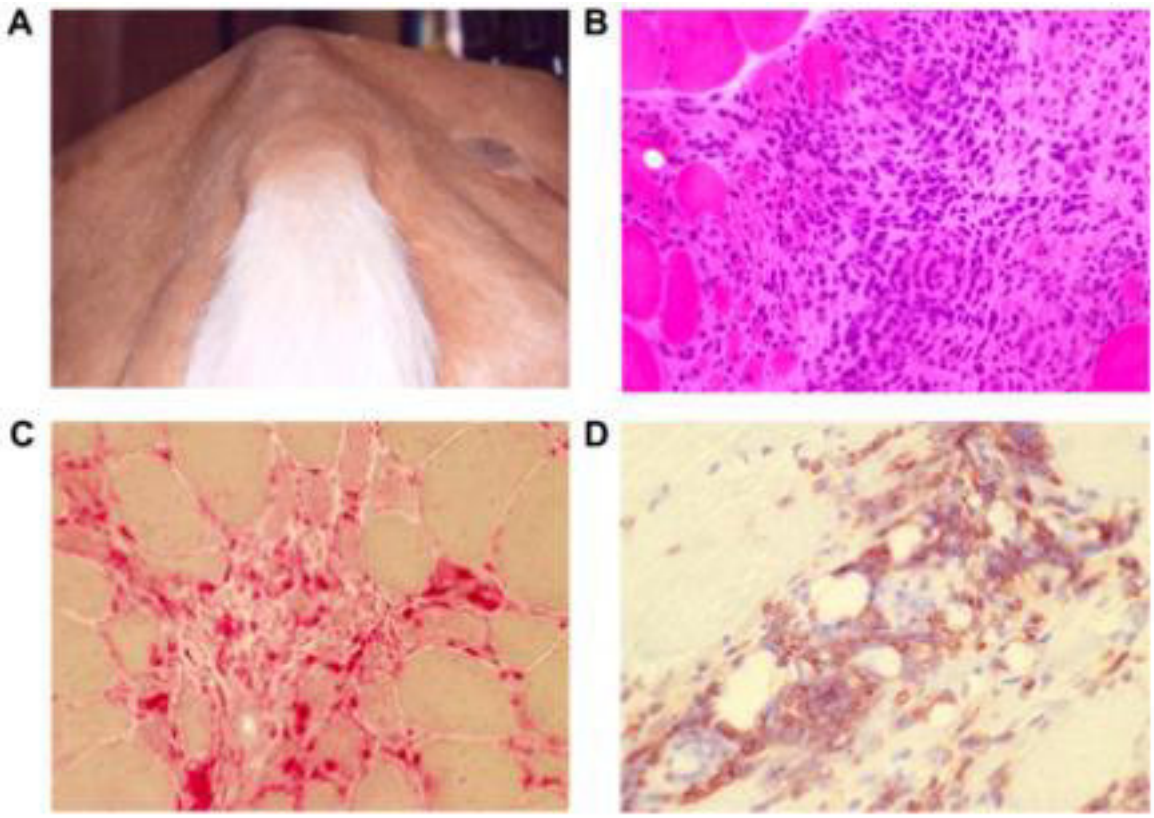

Figura 1. Aleman, M. A review of equine neuromuscular disorders. Neuromuscular disorders. v.18. p. 277–287. 2008. Miosite imunomediada (A) Cavalo doente com perda muscular profunda. Músculo glúteo médio deste cavalo a 40X (B e C). (B) Coloração H&E recém-congelada mostrando infiltração histiocítica e linfocítica endomisial, perimisial e perivascular. (C) Reação fosfatase ácida para destacar a infiltração histiocítica. (D) coloração de CD4 + destacando linfócitos T CD4.

2.1.6 Diagnóstico

O diagnóstico de doenças musculares em cavalos é composto pelo histórico, exame físico e alguns exames laboratoriais e perfil de bioquímica sérica

(CK, AST, LDH, mioglobina, concentração de vitamina E e selênio), urinálise e fração de excreção renal de eletrólitos. Com base nos achados laboratoriais pode-se determinar se os sinais musculares são primários ou secundários, se primários podem ser lesão muscular focal, rabdomiólise, fraqueza e intolerância muscular sem rabdomiólise, contração muscular anormal ou atrofia muscular (VALBERG, 2009). Exames complementares de ultrassonografia, eletromiografia, termografia, cintilografia nuclear, biópsia muscular para análise histopatológica, testes genéticos e teste de resposta ao exercício (em suspeita de rabdomiólise associada ao exercício assintomática, o exame é positivo havendo elevação de três a quatro vezes na concentração basal de CK a exercício de 15 minutos ao trote) (VALBERG, 2006b; VALBERG, 2009).

O diagnóstico de IMM em equinos é realizado a partir da sintomatologia clínica na ocorrência de rabdomiólise, seguido de teste genético e, se necessário, biópsia muscular para histopatológico (VALBERG et al., 2018). A biopsia transcutânea de músculos afetados é um dos testes mais úteis para diagnosticar equinos com IMM ativa. Amostras de Trucut fixadas em formalina ou em agulhas Bergstrom frescas-congeladas de musculatura epaxial/abaxial são geralmente suficientes para diagnóstico (DURWARD-AKHURST & VALBERG, 2018). As demais miopatias de origem genética (PSSM, HYPP e MH) têm teste diagnóstico biomolecular disponíveis. Da mesma maneira, o teste genético para a variante E321G permite o diagnóstico de IMM e rabdomiólise não associada ao exercício em equinos, permitindo a seleção de animais para a reprodução, evitando a produção de potros afetados (GIANINO et al., 2019). Apesar da mutação ser descrita em E321 pela literatura (FINNO et al., 2018; GIANINO et al., 2019; VALBERG et al., 2018), ela está identificada em E320 de acordo com o National Center for Biotechnology Information (NCBI).

2.1.7 Diagnósticos Diferenciais

Doenças que causam lesões e sintomatologia de grupos musculares em equinos são diversas e comuns. A maior parte das vezes são causadas por rabdomiólise induzida por esforço. Doença muscular da ‘segunda-feira de manhã’, azoturia, sídrome do *tying-up* e rabdomiólise associada ao exercício usualmente são ditas como sinônimos, apesar de haver algumas variações entre os termos. Azotúria é o termo para denotar grave lesão muscular, enquanto *tying up* geralmente é resultado de leve lesão muscular. Outros estudos sugerem que *tying up* ocorre após exercício físico intenso e prolongado, e que rabdomiólise

associada ao exercício é resultado de depleção de energia muscular e somente associado a exercícios aeróbicos. Porém, apesar da falta de consenso, o termo rabdomiólise associada ao exercício é o mais aceito e os demais não devem mais ser utilizados (FREESTONE & CARLSON, 1991).

Outras doenças de origem muscular que são diferenciais para IMM são atrofia por desuso, miosite por agentes infecciosos como: *sarcocystis*, *Streptococcus equi*, influenza, *Clostridium sordelli* (FREESTONE & CARLSON, 1991), *Salmonella enterica* subsp. *enterica* sorovar *Infantum* (QUIST et al., 2011) ou *Acinetobacter calcoaceticus* (DICKIE & REGNIER, 1978); além de, miodegeneração nutricional por deficiência de vitamina E e selênio, miopatia imumomediada devido a púrpura hemorrágica, miopatia mitocondrial e rabdomiólise induzida por demais etiologias (BARROT et al., 2004; ALEMAN, 2008).

As doenças musculares que levam ao quadro clínico de rabdomiólises podem ainda ser classificadas em rabdomiólises associadas ao exercício ou rabdomólises não associadas ao exercício (Tabela 1). De acordo com a tabela 1, a IMM é uma rabdomiólise não associada ao exercício, classificada como miopatatia inflamatória e imuno-mediada. Mas também, como ocorre em curso mais avançado da doença, relacionada a atrofia muscular.

Tabela 1. Classificação de miopatias em equinos. VALBERG, S.J. Skeletal Muscle and Lameness. In: ROSS, M. W. & DYSON, J. S. Diagnosis and Lameness in the Horse. Saunders, 2011. Chap. 83, p. 821.

Rabdomiólise não associada ao exercício	**Rabdomiólise associada ao exercício**
1. Miopatias Inflamatórias	1.Lesão muscular focal
1.1 Imuno-mediadas	1.1 *Tying up* esporádico
1.1a Púrpura hemorrágica	1.1a Nutricional (deficiências/desequilíbrios) vitamínicos, minerais, eletrolíticos.
1.1b Miosite Imunomediada	1.1b Excesso de exercício físico
2. Infecciosas	1.1c Síndrome da exaustão
2.a Miosite por Clostridiose	1.2 *Tying up* crônico
2.b Miosite por *Streptococcus equi*	1.2a Polysaccharide storage myopathy (PSSM) tipo 1
2.c Miosite por Anaplasma	1.2b Polysaccharide storage myopathy (PSSM) tipo 2
2.d Miosite viral	1.2c Hipertermia Maligna
2.e Miosite por Sarcocystis	1.2d Rabdomiólise associada ao exercício recorrente
3. Miopatias Nutricionais	1.2e Rabdomiólise associada ao exercício crônica idiopática
3.a Deficiência de Vitamina E e Selênio	**Miopatia associada ao exercício (sem alteração de CK)**
4. Miopatias por intoxicação	1. Miopatia Mitocondrial
4.1 Intoxicação por ionóforo	**Atrofia Muscular**
4.2 Miopatias de pastagem	1.1 Atrofia Miogênica
4.2a *Rayless goldenrod/white snakeroot*	1.1a Por disuso
4.2b *Cassia occidentalis*	1.1b Por caquexia
4.2c Mioglobinúria atípica	1.1c Por Cushing's disease
5. Miopatia traumática	1.1d Por Miosite Imunomediada (atrofia rápida)

5.1 Miopatia Anestésica Compressiva	1.1e Por Polysaccharide storage myopathy (PSSM) tipo 2
5.2 Trauma	1.1f Rabdomióse grave
6. Miopatia Metabólica	1.2 Atrofia neurogênica
6.1 Glycogen branching enzyme deficiency(GBED) em Quarto de Milha	1.2.a Mielite protozoária equina
7. Polysaccharide storage myopathy (PSSM) tipos 1 e 2	1.2.b Trauma neural local
8. Hipertermia Maligna	1.2c Doença do neurônio motor

.

Doenças de origem neurológica também são diagnósticos diferenciais para atrofia muscular, como EPM, polineurite equina, mielite verminótica, doença do neurônio motor inferior e atrofia neurogênica (BARROT et al., 2004). Perda de massa muscular devido a atrofia neurogênica pode ser descartada por biópsia, assim como a grande maioria dos diferenciais (BARROT et al., 2004). Outros diagnósticos diferenciais descritos são Intoxicações por ionóforo, cantaridina e *snakeroot* branco (QUIST et al., 2011).

Por fim, alterações musculares também podem ser de resultado de mutações genéticas, como é o caso de doenças já descritas em cavalos Quarto de Milha no Brasil, são elas HYPP, PSSM1 e MH (DELFIOL et al., 2015; DELFIOL et al., 2018), além de GBED (ALEMAN, 2008; ARAUJO et al., 2018).

Equinos com PSSM apresentam rabdomiólise sem haver infiltrado inflamatório acentuado por linfócicos. Também não possuem expressão de MHC classes I ou II em sarcolema como equinos com IMM, exceto em amostras de PSSM associado a ausência de infiltrados linfocíticos ou características de regeneração (como núcleo deslocado centralmente ou citoplasma basofílico), provavelmente devido a inflamação por citocinas causada pela presença de polissacarídeo anormal (DURWARD-AKHURST et al., 2016).

2.1.8 Tratamento

Tratamento instituído em pônei com suspeita de IMM foi 0,1 mg/kg de dexametasona reduzindo 0,04mg/kg a cada quatro dias. O curso inicial continuou por oito semanas, após este período não havia mais perda de massa muscular visível, porém o tratamento se prolongou por mais 12 semanas, com acompanhamento histopatológico de biópsias musculares e ultrassonografias da musculatura afetada (BARROT et al., 2004). Tratamento realizado por Lewis et

al. (2007) em suspeita de IMM foi dexametasona (0,07 a 0,1 mg/kg) ou prednisona/prednisolona (1 mg/kg) e antibioticoterapia caso houvesse infecção em progresso e acompanhamento dos casos com biópsias musculares.

No caso de miopatia secundária à infecção estreptocócica, alta mortalidade foi reportada em cavalos que receberam penicilina intravenosa durante tratamento para garrotilho ou miopatia, mesmo havendo alta suscetibilidade de espécies estreptocócicas à antibióticos beta-lactâmicos. Porém, a associação de penicilina à rifampicina (antimicrobiano que inibe síntese proteica) aumenta a taxa de sobrevivência em rabdomiólise estreptocócica. Nesses casos, antiinflamatórios não esteroidais ou altas doses de corticoides de ação curta podem diminuir resposta inflamatória. Infusão continua de lidocaína, detomidina ou cetamina podem diminuir ansiedade e dor. Caso haja decúbito decorrente da miopatia, os cavalos devem permanecer em baia com cama alta e alterado o decúbito a cada quatro horas ou erguidos em *sling*, caso consigam manter-se de pé sob os membros pélvicos (VALBERG, 2006b).

2.1.9 Prognóstico

Hunyadi et al. (2017) relataram 87% de proporção de sobrevivência em cavalos acometidos com IMM. Fatores como raça (animal não ser Quarto de Milha) e época do ano (inverno) parecem influenciar negativamente na taxa de sobrevivência. Assim como, animais com febre ou doença concomitante apresentam prognóstico desfavorável em relação à vida. Porém, tratamento imediato está associado a prognóstico favorável. Apesar de elevado aumento em atividade de enzimas musculares, a AST e CK não foram uteis como indicadores de prognóstico (HUNYADI et al., 2017).

2.2 Miosite Inflamatória Imunomediada em outras espécies

A miosina é uma proteína ubíqua e altamente conservada, encontrada em todas as células eucarióticas, onde fornece a função motora para diversos movimentos, como citocinese, fagocitose e contração muscular. Todas as miosinas contêm um domínio motor/terminal amino-terminal e um domínio cauda terminal-carboxi. Devido ao grande número de moléculas diferentes identificadas até o momento, as miosinas foram divididas em sete classes distintas com base nas propriedades do domínio da cabeça. Uma dessas classes, miosinas de classe II, consiste nas miosinas de duas cabeças convencionais que formam filamentos e são compostas por duas subunidades de cadeia pesada de miosi-

na (MYH) e quatro subunidades de cadeia leve de miosina. A subunidade MYH contém a atividade ATPase, fornecendo energia que é a força motriz dos processos contráteis mencionados acima, e existem numerosas isoformas MYH nos vertebrados para desempenhar essa função (WEISS &, LEINWAND, 1996).

Desta maneira, é descrita a mutação em miosinas também em outras espécies, como humanos e cães. Em humanos, mutações dominantes em genes isoformes de MyHC (*MYH3 e MYH8*) são associados a síndromes de artrogripose distal. Mutações dominantes ou recessivas afetando MyHC tipo IIA (*MYH2*) são associadas a episódio precoce de miopatias com fraqueza muscular variável em intensidade e oftalmoplegia (TAJSHARGHI & OLDFORS, 2013). Miopatia congênita relacionadas às mutações *misssense* do gene *MYH2* em humanos é uma rara doença neuromuscular, também causando oftalmoparesia/oftalmoplegia e disfagia, dispneia ao nascer (D'AMICO et al., 2013). Também, miopatias de acúmulo de miosina e miopatia distal de Laing são, em geral, enfermidades resultantes de mutação dominante no *MYH7* (TAJSHARGHI & OLDFORS, 2013). O tipo e distribuição de células inflamatórias mononucleares em histopatológicos diferem dentre miopatias (LEWIS et al., 2007).

Um grupo heterogêneo de doenças musculares imunomediadas é descrito em cães e humanos e pertencem à duas categorias: polimiosites e dermatomiosites (LEWIS et al., 2007). Shelton (2007) descreve ainda uma terceira categoria: miosite por corpos de inclusão. São ligadas a atributos inespecíficos como envolvimento difuso e simétrico da musculatura, fraqueza generalizada, responsividade a corticoides e desconhecimento de causa primária. Doenças virais e infecciosas podem levar a IMM em cães e humanos, doença de Lyme já foi reportada como causa de IMM em humanos. Em cães, a IMM pode levar a miosite de musculatura mastigatória (LEWIS et al., 2007). A IMM em humanos é caracterizada por fraqueza muscular, lesões de pele, doença pulmonar intersticial e neoplasia (ROTHWELL et al., 2013). Em humanos, polimiosite também já foi relatada em associação com doenças sistemicas, como lúpus eritematoso, esclerose progressiva sistêmica e periarterite nodosa (MEDSGER et al., 1970) e doenças imunomediadas de articulações (BENNET & KELLY, 1987).

2.3 OBJETIVOS

O objetivo deste trabalho é verificar a frequência alélica da mutação E321G no gene *MYH1* no Brasil, e nas linhagens de criação (rédeas, tambor/baliza, conformação, apartação e corrida) em cavalos Quarto de Milha no Brasil. Além de descrever caso clínico de IMM em potro Quarto de Milha heterozigoto para a mutação E321G em gene *MYH1*.

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Capítulo II

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Original Article

Prevalence of the E321G *MYH1* variant in performance subgroups of Quarter horses in Brazil

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Abstract

In Quarter horses (QHs), myosin heavy chain myopathy (MYHM), characterized by nonexertional rhabdomyolysis or immune-mediated myositis (IMM) with acute muscle atrophy, is highly associated with a missense E321G *MYH1* mutation. We identified two related QH foals in Brazil with the E321G *MYH1* mutation that had clinical signs of MYHM, with the histological confirmation of IMM in one foal. This prompted an investigation into the allele frequency of the E321G *MYH1* variant across performance QHs using a DNA archive in Brazil. Of 299 genotyped QHs, 44 animals (14.7%) were identified as heterozygous (My/N) for the E321G *MYH1* variant, and 255 (85.3%) were homozygous for the wild-type allele (N/N), with an allele frequency of 0.074. Reining horses showed a significantly higher prevalence of heterozygosity than those in other disciplines (P=0.008). The prevalence of type 1 polysaccharide storage myopathy was 0.032, with only two E321G *MYH1* heterozygotes possessing the *GYS1* mutation. This study highlights the existence of MYHM and the high prevalence of the *MYH1* mutation in QHs in Brazil, especially in reining QHs, underlining the importance of control measures to prevent an increase in the incidence of MYHM in QHs in Brazil.

Keywords: muscle disorders; mutation; MYH1 gene; myositis.

Introduction

The equestrian industry is a strong segment of Brazilian agribusiness, and sporting breeds certainly play a fundamental role in the industry. Quarter horses (QHs) stand out as one of the main breeds of sport horses in Brazil, with horses specializing in specific disciplines. Muscle disorders are among the major health concerns that affect the performance of athletic horses (Valberg, 2018). Genetic muscle disorders in this breed include hyperkalaemic periodic paralysis (HYPP), glycogen branching enzyme deficiency (GBED), type 1 polysaccharide storage myopathy (PSSM1) and malignant hyperthermia (MH), all of which are included in the American Quarter Horse Association (AQHA) breed panel test (Valberg, 2020). The R309G *GYS1* mutation causing PSSM1 is the most prevalent in QHs, with an allele frequency of 0.055 in the United States (USA) (Tryon et al., 2009).

Recently, a codominant E321G mutation in the *MYH1* gene encoding fast-twitch type 2X myosin heavy chain has been identified in QHs; this mutation causes myosin heavy chain myopathy (MYHM) (Finno et al., 2018; Valberg et al., 2018). Horses with MYHM can present with nonexertional rhabdomyolysis or immune-mediated myositis (IMM) characterized by acute muscle atrophy and lymphocytic infiltration into the myofibres (Lewis et al., 2007; Durward-Akhurst et al, 2016; Finno et al., 2018; Valberg et al., 2018). The allele frequency of the E321G *MYH1* mutation in the general QH population in the United States (US) is 0.034; however, frequencies vary among performance horse disciplines, and the frequency is much higher in reining horses (Tryon et al., 2009).

To date, clinical cases of MYHM have not yet been reported in QHs in Brazil, although reining is a very popular discipline. The purpose of the present study was to report the presence of MYHM in two QH foals in Brazil and to evaluate the allele frequency of

69 E321G *MYH1* in Brazilian QHs used in various disciplines (reining, barrel racing, hal-
70 ter, cutting and racing).
71
72 **Materials and Methods**
73 This study was approved on July 10, 2019, by the Institutional Animal Care and
74 Use Committee (approval number 110/2019-CEUA-UNESP).
75
76 **Clinical Cases:** Two potential cases of MYHM on a stud farm in Brazil were investi-
77 gated. Medical records, clinical signs, blood work and muscle histopathology were re-
78 viewed when available. The foals (foals 1 and 2) and their healthy parents (horses 3 to
79 6) were genotyped for MYHM, PSSM1 and MH, as described previously or detailed
80 below (Delfiol et al., 2018). Histopathological analysis of skeletal and cardiac muscle
81 (foal 1) was performed with formalin-fixed paraffin-embedded tissue stained with hae-
82 matoxylin and eosin, periodic acid Schiff (PAS), and amylase-PAS.
83
84 ***MYH1* Allele Frequencies:** To estimate the allele frequency of the E321G *MYH1* vari-
85 ant in Brazilian QHs, DNA samples from a total of 299 elite performance QHs were
86 obtained from the Laboratory of Molecular Biology of the Veterinary Clinic (LBMCV)
87 of the São Paulo State University (UNESP), School of Veterinary Medicine and Animal
88 Science Botucatu/Brazil database. All horses were registered with the Brazilian Associ-
89 ation of Quarter Horse Breeders (ABQM), and at the time of collection, they were com-
90 peting or had already competed in official ABQM events. The blood samples, which
91 were collected from 2010 to 2014, were from 299 horses located at 30 stud farms or
92 equine training centres (10 specializing in reining; 6 specializing in halter; 5 specializ-
93 ing in barrel racing; 5 specializing in cutting farms; and 4 specializing in racing).

Genotyping: PCR was performed with specific primers (JPOF_MYH1F1, 5'-CCAGCTAAAGGCGGAAAGAA-3', and JPOF_MYH1R1, 5'-GGGCAGAGTAGGAGTGAGTAA-3') that amplified the E321G mutation region in the *MYH1* gene (Finno et al. 2018). The PCR products (713 bp) were purified and subjected to Sanger sequencing. For validation, genotypes for 10 DNA samples from *MYH1* heterozygotes and 20 from wild-type horses were genotyped at the Veterinary Genetics Laboratory at the University of California Davis (VGL-UC Davis). The genotypes for PSSM1 and RYR1 had previously been analysed and were collated for this performance horse subset (Delfiol et al., 2018).

Pedigrees from horses with the *MYH1* variant were obtained from ABQM data and analysed using Pedigraph (Garbe and Da, 2008). Seven generations of ancestors of the affected animals were considered during the construction and analysis of the pedigrees.

Statistical analysis: The allele frequency and standard error for each competitive discipline were estimated as previously described (Delfiol et al., 2018). Chi-square tests were performed using GraphPad Prism 7 software to compare the prevalence of the E321G *MYH1* mutation stratified by sex and performance discipline. Significance was set at $P<0.05$.

Results

Clinical cases: Two QH foals with potential cases of MYHM were reported within a 14-month period on the same stud farm.

Foal 1 was a 9-month-old colt that developed acute onset stiffness, excessive sweating, muscle tremors, reluctance to move, well-defined contracted musculature, a fever of 40.5°C, acute severe atrophy of the gluteal and epaxial muscles, severe muscle stiffness and limb spasticity, decubitus and difficulty standing up. The colt's serum creatine kinase (CK) activity was increased at 8,000 U/L (reference range reference 119–287 U/L) (Kaneko et al., 2008). Neither myoglobinuria nor signs of concurrent respiratory disease were observed. The foal was treated with dexamethasone (0.1 mg/kg bodyweight), a muscle relaxant (thiocolchicoside, 0.04 mg/kg bodyweight), selenium-vitamin E, and fluid therapy. Despite treatment, foal 1 clinically deteriorated (8^{th} day), developing severe atrophy of the gluteal and epaxial muscles, severe muscle stiffness and limb spasticity, and difficulty standing up, which progressed to decubitus and, ultimately, death.

Foal 2 was a 11-month-old filly that had clinical signs compatible with nonexertional rhabdomyolysis, characterized by the acute onset of limb spasticity, muscle tremors, reluctance to move, and a fever of 40.5°C. The CK level was 6,500 U/L, with myoglobinuria. Signs of concurrent respiratory disease were not observed. The foal improved clinically after being treated in the same way as foal 1, and on day 40, the foal appeared to be clinically normal.

Samples of the semimembranosus and semitendinosus muscle of foal 1 were obtained after death. There was marked myocyte necrosis characterized by the loss of cross striations and hypereosinophilia, in addition to some fibres showing flocculant cytoplasm and some mineralization (Fig. 1A and B). Both muscle samples contained diffuse moderate-to-severe inflammatory infiltrates composed of lymphocytes and macrophages, in addition to rare neutrophils and multinucleated giant cells. (Fig. 1A and B). There was mild regeneration of the muscle fibres. PAS and amylase-PAS stains showed normal glycogen. Microscopic lesions were not observed in the heart.

143 Neither the foals nor their parents possessed the *GYS1* mutation for PSSM1 nor the
144 *RYR1* mutation for MH, however, both foals and their sires (horses 3 and 6) were heter-
145 ozygous for the E321G *MYH1* variant, while their dams (horses 4 and 5) were homozy-
146 gous wild-type (Fig. 1C). Pedigree analysis determined that the two foals were related:
147 the sire of foal 1 was the grandsire of foal 2 (Fig. 1D).
148
149 ***MYH1* allele frequency.** A total of 299 elite performance QH DNA samples obtained
150 between 2010 and 2014 were used in the present study. At the time of sampling, the
151 horses (197 females and 102 males) were healthy, with a mean age of 8.8 ± 5.7 (SD)
152 years. In total, 14.7% (44/299) were E321G *MYH1* heterozygotes (My/N), 0% (0/299)
153 were homozygotes (My/My), and 85.3% (255/299) were wild-type homozygotes (N/N).
154 The overall allele frequency of the E321G *MYH1* was 0.074 (Table 1). The allele fre-
155 quency and prevalence of the E321G *MYH1* was the highest in the horses involved in
156 reining (21.9%, 34/155), followed by those in the barrel racing (10%, 4/40), cutting
157 (6.1%, 2/33), racing (5.7%, 2/35), and halter (5.6%, 2/36) disciplines (Table 1). The
158 allele frequency of *MYH1* heterozygotes was significantly higher in reining QHs than
159 QHs in other disciplines (P=0.008). There was no significant difference in the distribu-
160 tion of the E321G *MYH1* mutation between males (17.6%, 18/102) and females (13.2%,
161 26/197), (P=0.3875). The results of the genotyping validation performed at the VGL –
162 UC Davis confirmed the genotypes obtained by LBMCV.
163 The prevalence of the PSSM1 mutation was 3.9% in reining QHs (6/155) and 36% in
164 halter QHs (13/36), with a total allele frequency of 0.032 (19/299). Out of 44 E321G
165 *MYH1* heterozygous QHs, two were heterozygous for the PSSM1 mutation, and none
166 possessed a MH mutant allele (Delfiol et al., 2018).
167

Pedigree analysis: All E321G *MYH1* heterozygous QHs with an available pedigree (30/44) could be traced back to a common sire within four to nine generations (Fig. S1). Two stallions were present in the pedigrees of 27 of the 30 QHs and another stallion was present in the pedigrees of 26 of the 30 QHs. The average inbreeding coefficient was generated after the construction and analysis of the 30 affected horses' pedigrees, and it was found to be 0.006 (smallest non-zero: 0.0001; maximum: 0.31) in the QH population.

Discussion

In the present study, the prevalence and allele frequency of the E321G *MYH1* variant was investigated in a group of 299 elite performance QHs in Brazil using DNA from the LBMCV stock. The allele frequency of the E321G *MYH1* variant in QHs in Brazil was estimated to be 0.074, which was twice as high as the frequency observed in 146 randomly selected QHs in the USA and Canada (0.034) but similar to that observed in elite performance QHs in the USA (0.049) (Gianino et al., 2018). As observed for *MYH1*, the frequencies of alleles involved in other genetic diseases, i.e., hereditary equine regional dermal asthenia (HERDA), hyperkalaemic periodic paralysis (HYPP), glycogen-branching enzyme deficiency (GBED), and PSSM1, in Brazilian QHs (Badial et al., 2014; Delfiol et al., 2015; Araujo et al., 2018; Delfiol et al., 2018) are also close to those found in American QHs (Tryon et al. 2009). The similarity in the allele frequencies of these mutations may be due to the fact that Brazilian QHs are often closely related to American QHs (Leite et al., 2019).

In the present study, the highest allele frequency was observed in reining QHs (0.110, n=155), which was similar to the result reported in the USA (reining QHs 0.135, n=37) (Gianino et al., 2018). In the Brazilian QHs, the *MYH1* mutation was also observed in

193 other QHs in other disciplines, i.e., barrel racing (0.050, n=40), cutting (0.030, n=33),
194 racing (0.029, n=35), and halter (0.028, n=63); however, the frequencies in the QHs in
195 those disciplines were significantly lower than that in reining QHs (P=0.008). Among
196 the elite performance QHs assessed in the USA, the E321G *MYH1* variant was also ob-
197 served in halter and cutting QHs but was not observed in elite barrel racing and racing
198 QH stallions (Gianino et al., 2018).
199 Gianino et al. (2018) compared the frequency of the E321G *MYH1* variant in the gen-
200 eral QH population (0.034) with the frequencies of alleles involved in important genetic
201 diseases in QHs and found that the frequency of the E321G *MYH1* variant in the general
202 QH population was slightly higher than the reported whole-breed estimates of allele
203 frequencies for HyPP (0.008) and HERDA (0.021) but lower than those for PSSM1
204 (0.055) and GBED (0.054) (Tryon et al., 2009). However, in the Brazilian QHs, the
205 frequency of the E321G *MYH1* (0.074) variant was higher than the frequencies of al-
206 leles involved in other genetic diseases, i.e., HERDA (0.029) (Badial et al., 2014), and
207 HyPP (0.021) (Delfiol et al., 2015), GBED (0.054) (Araujo et al., 2018), and PSSM1
208 (0.034) (Delfiol et al., 2018).
209 It is important to note that the sample population in Brazil was not random and that
210 more reining QHs were evaluated in the present study than in other studies. This may
211 have contributed to the higher prevalence of the *MYH1* mutation. The most likely ex-
212 planation for the higher allele frequency of E321G *MYH1* in Brazilian reining QHs than
213 in QHs in other disciplines is that there is substantial consanguinity in Brazilian QHs.
214 The use of popular sires within performance groups can result in the inadvertent con-
215 centration of deleterious genetic traits or the hitch-hiking of deleterious traits with per-
216 formance-enhancing traits. All heterozygous animals with available pedigrees (n=30) in
217 the present study could be traced back to a common sire. Three other stallions were

218 overrepresented in the pedigrees, with two stallions present in the pedigrees of 27 of the
219 30 horses and one stallion present in the pedigrees of 26 of the 30 QHs.
220 In the present study, we definitively identified IMM in one Brazilian foal through histo-
221 pathology and genetic testing, and the likelihood of the second foal on the same stud
222 farm having the same diagnosis was high, according to the clinical signs, relatedness
223 and genetic testing. These appear to be the first two cases of MYHM associated with the
224 E321G *MYH1* variant reported in QHs in Brazil. Despite the high allele frequency of
225 *MYH1,* the disease may not yet be recognized and properly diagnosed in Brazil.
226 The percentage of horses with the *MYH1* mutation that go on to develop nonexertional
227 rhabdomyolysis or muscle atrophy is unknown at this time. The samples used in our
228 study were previously collected from healthy horses by our research group to assess the
229 prevalence of other genetic mutations (Badial et al., 2014; Delfiol et al., 2015; Araujo et
230 al., 2018; Delfiol et al., 2018; Leite et al., 2019). We were, therefore, unable to associate
231 the presence of the mutated *MYH1* allele with the occurrence of MYHM. Clinical signs
232 of MYHM appear to be dependent upon both a genetic predisposition and environmen-
233 tal stimuli. In 39% of IMM cases, the affected horses had a history of infection with
234 *Streptococcus spp*. or vaccination against *Streptococcus equi* subsp. *equi* (Lewis et al.,
235 2007; Finno et al., 2018). Another study implicated *Corynebacterium pseudotuberculo-*
236 *sis, Anaplasma phagocytophilum*, equine herpes virus 1 [EHV1], and equine influenza
237 virus and other antigens contained in influenza and EHV1 vaccines as potential trigger-
238 ing factors (Hunyadi et al., 2017). In the present study, we were unable to accurately
239 identify the trigger of rhabdomyolysis in the two foals, and in these two cases, rhabdo-
240 myolysis was not associated with exercise.
241 In conclusion, there is a high prevalence of the E321G *MYH1* mutation in Brazilian
242 QHs, especially in those used for reining, and clinical cases of MYHM have now been

documented in Brazil. Breeders and owners in Brazil need to be aware of the clinical signs of MYHM, including severe nonexertional rhabdomyolysis and acute muscle atrophy, and use genetic testing not only to achieve a diagnosis but also to inform appropriate breeding strategies.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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312

313 **Figure Legends**

314

315 **Fig. 1. A** Moderate diffuse inflammatory infiltrates and myocyte necrosis in the semi-
316 membranosus muscle of foal 1 (H&E bar=200 µm). **B.**; Infiltrates of lymphocytes and
317 macrophages surrounding and within myocytes (H&E bar=50 µm). **C,** partial chroma-
318 togram showing capillary sequencing results for homozygous (top) and heterozygous
319 (bottom) alleles of the E321G *MYH1* variant in mare and foal 1, respectively. Wild-type
320 allele adenine (A, green arrow) and the respective amino acid glutamic acid (E) and
321 double peaks (adenine/guanine) are observed (red arrow); in addition, note the amino
322 acid glutamic acid (E) in heterozygous horses. Images were obtained using Geneious®
323 10.0 software. **D**, pedigree of MYHM-affected foals 1 and 2. Horses 1 to 6 were geno-
324 typed for the E321G *MYH1* variant in the present study, and the other genotype infor-
325 mation was obtained from the AQHA stud book. Circles = females, squares = males.
326 Black shape = My/My genotype. Black/White shape = My/N genotype. White shape =
327 N/N genotype. Grey shape = unknown genotype.

328

329 **Fig. S1.** Pedigree analysis of the 30 *MYH1* heterozygous (My/N) quarter horses (green
330 shape) obtained in Pedigraph (Garbe & Ya 2008). All affected horses could be traced
331 back to a common sire, A281 (red arrow), within four to nine generations. Circles =
332 females, squares =males.

333

Table 1. Genotype prevalence and allele frequency (estimated value ± SE) of the E321G *MYH1* variant in Brazilian Quarter horses.

Discipline	Sample size	Genotype			Brazil allele frequency	US allele frequency[1]
		N/N	My/N	My/My		
Reining	155	121	34	0	0.110 ± 0.018	0.135 ± 0.040
Barrel racing	40	36	4	0	0.050 ± 0.024	0
Cutting	33	31	2	0	0.030 ± 0.021	0.044 ± 0.023
Racing	35	33	2	0	0.029 ± 0.020	0
Halter	36	34	2	0	0.028 ± 0.019	0.080 ± 0.027
Total	299	255	44	0	0.074 ± 0.011	0.050 ± 0.009

[1] Data previously published by Gianino et al. 2018.

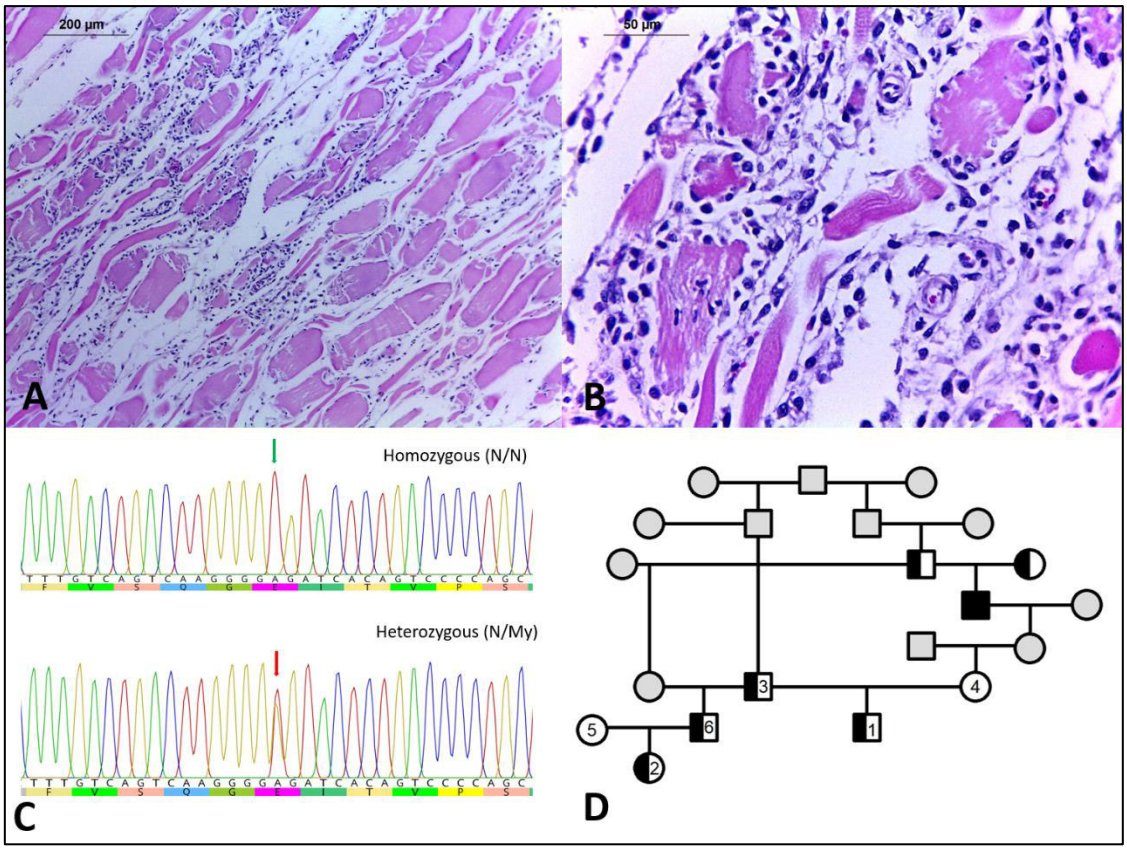

Fig. 1.

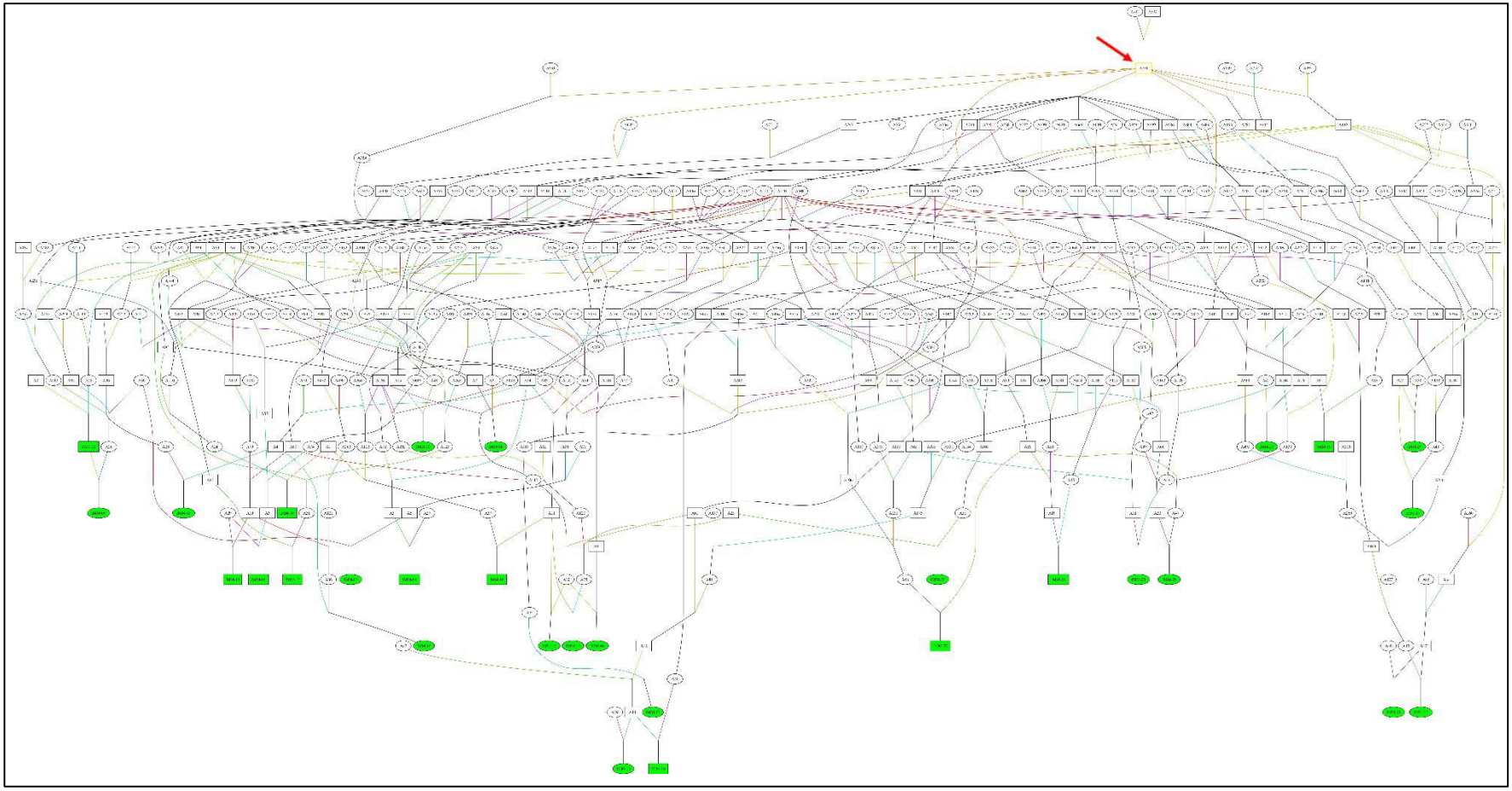

Fig S1.

Considerações Finais

Foram identificados 44 animais heterozigotos, totalizando a frequência alélica de 0,074 ± 0,011, superior à encontrada nos Estados Unidos. Diante deste resultado, concluímos que a mutação está presente no Quarto de Milha do Brasil e IMM deve ser diagnóstico diferencial na ocorrência de rabdomiólise. A maior frequência da mutação foi encontrada na linhagem de rédeas, assim como nos EUA, de onde importamos muitos animais (ABQM). Tratando-se de mutação dominante, é ainda maior a importância de diagnóstico precoce afim de identificar animais heterozigotos da reprodução equina e evitar a reprodução indiscriminada (*inbreeding*) que pode levar a animais afetados. Por conseguinte, diante das frequências alélica expostas, é interessante a disponibilidade de teste genético para IMM no Brasil.

Normas para a submissão da revista “The Veterinary Journal”

Guide for Authors

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Introduction

The Veterinary Journal (established 1875) is an international journal of veterinary research that publishes original papers and reviews on all aspects of veterinary science. Contributions reporting investigative work in the scientific disciplines involving veterinary species are particularly welcome where they make a significant contribution to the field. The Editors will be pleased to consider suggestions for Special Issues on subjects of topical importance. The journal also publishes Guest Editorials and occasionally Personal Views by invitation, but does not have a Letters section. Book Reviews are published on-line. Articles of purely regional significance and studies of non-domestic species are considered only if they clearly have broader scientific importance. Manuscripts that report novel studies with substantial importance to the profession are preferred, including analytical studies that are relevant to practising veterinarians.

Types of paper

Manuscripts may describe original work in a Full Paper (Original Article) or a Short Communication, or may form a Review of the existing state of knowledge on a particular aspect of veterinary science. Reviews should, in general, be written in support of original investigations. Case Reports are not published.

Model Article Examples

Model article formats in WORD are available (click to follow link below as appropriate):

- Original Article
- Short Communication
- Review
- Guest Editorial
- Viewpoint
- Book Review

Reporting Standards

The Editors and reviewers use several published guidelines for reporting standards (see Appendix below). Conforming to these reporting standards allows the Editors and reviewers to assess the quality and originality of submissions and offers readers sufficient information to judge the relevance of the work in an appropriate context. Omission of requirements specified in the relevant guidelines for reporting standards may lead to rejection of a manuscript. For further information, please see The Veterinary Journal (2010) 184, 249-250 (view article).

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
• E-mail address
• Full postal address

All necessary files have been uploaded:
Manuscript:
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• All figures (include relevant captions)
• All tables (including titles, description, footnotes)
• Ensure all figure and table citations in the text match the files provided
• Indicate clearly if color should be used for any figures in print
Graphical Abstracts / Highlights files (where applicable) *Supplemental files* (where applicable)

Further considerations
• Manuscript has been 'spell checked' and 'grammar checked'
• All references mentioned in the Reference List are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Internet)
• A competing interests statement is provided, even if the authors have no competing

interests to declare
• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

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Before You Begin

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

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Studies published in The Veterinary Journal must adhere to the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) humane endpoints in animal experimentation. Please see their website for further information: https://www.nc3rs.org.uk/humane-endpoints

For manuscripts reporting experimental models that induce disease, please provide full details of rescue protocols, welfare/pain/health monitoring protocols, and animal welfare end points set **a priori** for withdrawal from the study. Additionally, please provide evidence that these protocols were approved **before** the study commenced. Also, provide full details of the fate of each animal in the study, including those that were withdrawn because they reached animal welfare endpoints set *a priori*. If you are unable to provide this information, including all specific details, your manuscript will be rejected.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places: 1. A summary declaration of interest statement in the title page file (if double-blind) or the manuscript file (if single- blind). If there are no interests to declare then please state this: 'Declarations of interest: none'. This summary statement will be ultimately published if the article is accepted. 2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches. More information.

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Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

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Options will be given for Authors to select a set of classifications for their papers, as well as a category designation (Original Article, Review, Short Communication etc.), from a given list.

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Preparation

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Use of Word Processing Software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, please use a table grid, (however use only one grid for each individual table and not a grid for each row). The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: https://www.elsevier.com/guidepublication). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Authors submitting papers that are suitable for consideration but do not comply fully with this Guide will be asked to amend the text and re-submit. Model article formats in WORD are available (click to follow link below as appropriate):

- Original Article
- Short Communication
- Review
- Guest Editorial
- Personal View
- Book Review

Original Articles should be no longer than 3,000 words in length, excluding the Title page, Abstract, Acknowledgements, Tables, Figures and References. Reviews should be about 4,000 words in length and Short Communications up to 1,000 words.

Articles

Original Articles (word limit 3,000 words) should be arranged as follows: (1) Title page; (2) an Abstract of up to 250 words (with no sub-headings), which should emphasise objectives, the experimental procedure, results and conclusions; up to five alphabetically arranged Keywords in Sentence case should be supplied below the Abstract; (3) the main text must be sub-divided into Introduction, Materials and methods, Results, Discussion, and Conclusions; (4) Conflict of interest statement; (5) Acknowledgements; (6) Appendix: Supplementary material (where this is provided; see below); (7) References; (8) Tables; (9) Figure legends; (10) Figures (uploaded as separate files); (11) Highlights (uploaded as a separate file). The sections should not be numbered. Please see the model article provided (view here)

Please note:

- Insert a page break only after the Title page, after the Abstract with Keywords, after the References section, between each Table, and before the Legends to figures.
- The Results and Discussion sections must be distinct and not combined.
- Avoid sub-headings in the Discussion section.
- References must not be included within the Conclusions section.
- The first person (I, we, our) must be avoided in the Abstract, but may be used elsewhere in the paper.

Tables should be included within the article and placed sequentially after the References, but before the Figure legend(s), with one Table per page.

Figure legends should be included in the main manuscript file after any Tables. Each figure should be uploaded as a separate file (Fig. 1, Fig. 2 etc.).

Short Communications should follow the requirements for full manuscripts, but the text must not exceed 1,000 words and the paper should not be divided into conventional sections. Headings for the Abstract, Keywords, Acknowledgements, Conflict of interest statement and References should be included, but there should be no other headings or subheadings in the main text. An Abstract is required and up to five alphabetically arranged Keywords in Sentence case should be supplied below it. Please see the model article provided (view here).

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appraisals of published evidence, with appropriate reference to the work of published authors on the topic. They should be about 4,000 words in length and should follow the layout for Original Articles, but with the main text subdivided as appropriate to the subject matter, starting with an Abstract and Introduction, and incorporating a Conclusions section and a Conflict of interest statement. Sections should not be numbered. Please see the model Review Article provided(view here).

Guest Editorials are commissioned by the Editors; unsolicited Guest Editorials will not be accepted. Commissioned Guest Editorials usually have 500-1000 words of text and must follow the layout of the model article provided (view here). They usually include references (up to a maximum of 10), but should not be divided into sections and no Acknowledgements or Conflict of interest statement are required. Where a Guest Editorial is commissioned to accompany another article, it must refer to that article in the text and the reference should be provided at the end of the Editorial.

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Publishers or Authors wishing to have a book considered for review in The Veterinary Journal should first contact the Books Editor at tvj.bookseditor@gmail.com. Book reviews are published in electronic format only.

Subdivision - unnumbered sections

Divide your article into clearly defined sections. Each subsection is given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by heading as opposed to simply 'the text'.

Essential title page information

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Highlights

Highlights are optional yet highly encouraged for this journal, as they increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: example Highlights.

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

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Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.
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Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the

references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Prior presentation of data

Prior presentation of results, in the form of abstracts, posters or oral presentations at conferences, must be mentioned clearly in the Acknowledgements, with wording along the lines of: "Preliminary results were presented as an Abstract at the 9th International Congress of Veterinary Virology, Madrid, 4-7 September 2012."

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Nomenclature and Units

Units. Metric units must be used. If other units need to be given, they must be placed in brackets after the Metric equivalent. Units, symbols and abbreviations of units should conform to the International System of Units as defined in Baron, D.N., McKenzie-Clarke, H., 2008, Units, Symbols and Abbreviations: A Guide for Authors and Editors in Medicine and Related Sciences, 6th Ed., The Royal Society of Medicine, London (available here). All other abbreviations should be unambiguous and should be clearly explained where they are first mentioned in the Abstract and text. Do not list abbreviations separately.

Note that litre is abbreviated to 'L', millilitre 'mL', (also mmol/L etc.); probability is given as *P* (upper case italics), as in $P<0.05$; also note 'Student's *t* test' and Mann-Whitney *U* test; correlation coefficient r as in $r = 0.92$, coefficient of determination, r^2 as in $r^2 = 0.72$; standard deviation and standard error should be abbreviated to SD and SE, respectively, but defined when first used; hour, minute and second are abbreviated to h, min and s; day, week and year are given in full. For drug dose frequency use e.g. 'three times daily' or '8-hourly' rather than Latin terms such as t.i.d. or q 8 h. Where centrifugation has been performed, use *g* values not rpm. Other common abbreviations include 'IV' for intravenous or intravenously, 'IM' for

intramuscular or intramuscularly, 'SC' for subcutaneous and subcutaneously, 'PO' for per os or orally; 'vs.' can be used for 'versus'. Use the abbreviation G for gauge of needle. The symbol for degrees Celsius should be written in the format '°C', with the value separated from the unit by a space, e.g. '37 °C'. Use the WORD symbols for ±, Greek letters etc. Percentages should be referred to as, for example, '15%' or 'Fifteen per cent' when starting a sentence. Note also 'post-mortem' and 'ante-mortem'.

When a number is followed by a unit use the digits as in '10 mL' unless starting a sentence in which case write in full 'Ten microlitres'. When the number describes a quantity of items write the number in full up to nine as 'four sheep' or 'nine tubes' then in digital form thereafter as '24 horses' or '200 blood samples'. Avoid the symbol # or abbreviation 'No.' for 'number'.

Single ('...') quotation marks should be used for specific extracts, as in: "A PubMed search utilising the search terms 'canine castration local anaesthesia' returned three publications." Where a reference is cited or a quote given, use single quotation marks and place the text in italic font as: "However, in the 'Recommended Guideline for the Conduct and Evaluation of Prognostic Studies in Veterinary Oncology' developed recently by the American College of Veterinary Pathologists ..." Double ("...") quotation marks should be avoided.

Anatomical terminology. Terminology should comply with the World Association of Veterinary Anatomists Nomina Anatomica Veterinaria (2005) and terms should be given in English where possible, unless the paper is a specialist anatomy paper (see: http://www.wava-amav.org/Downloads/nav_2005.pdf).

Currencies. A footnote should be inserted at first use if a currency is given in the text, as in 'UK£5001' and conversion rates provided using the following three currencies US$, UK£ and Euros (€). The footnote should read as appropriate, for example: '£1 = approx. US$1.60, €1.24 at 2 December 2012.' Rates can be updated by the Author at proof stage if necessary. An easy to use currency converter is available here: http://uk.reuters.com/business/currencies.

Manufacturers. Manufacturers and suppliers should be indicated within the text after the name of the product. For example: 'diazepam (Valium, Roche)' or 'using an infusion pump (Medfusion 2010, Medex)'. Addresses/locations of manufacturers should not be given and the use of ® or ™ should be avoided. Note: proprietary names must not appear in the title or Abstract.

Applying capital letters to directions. Compass directions such as North, South, East and West, as well as their derivatives, such as North-East, North-West, South-East and South-West, should be capitalised when they are used to designate defined or recognised geographical regions, or when they are an integral part of a proper name. Examples include "Eastern Europe", "Southern France", "North-East England", "in the North", "down South", "the West Coast" and "the Eastern Seaboard", "the Western Region of Kazakhstan", "Southern California". Compass directions should not be written with capital letters when they indicate general locations or directions without a specific location. Examples include "bluetongue virus initially spread in a north- westerly direction on air currents", "the northern boundary of the quarantine zone",

"cases were clustered in the east of the region", "westerly winds". The first letter of each word of a Compass direction should be capitalised when used to refer to people in a region, particularly in social, cultural or political contexts. Examples include "wildebeest are hunted by the Southern tribes", "horses have been an integral part of Western civilisation since the Middle Ages". Words such as northern, southern, eastern, and western that precede a place name usually are not capitalised, since they indicate a general location within a region. When these words are an integral part of the place name, they should be capitalised. For example, write "northern Connecticut", but "Northern Ireland" and "Western Australia".

Nucleotide sequences

-Submission of a manuscript implies that primary nucleotide sequence data will be deposited with an internationally available repository. Sequence reference numbers should be provided, where appropriate, in the main text, Tables, Figures or as an e-only supplementary file.

Controls for immunohistochemistry/immunocytochemistry - Please confirm that proper negative controls are used - See: The Histochemical Society's standards: http://jhc.sagepub.com/content/early/2014/07/31/0022155414545224.full.pdf

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Figures. The quality of all Figures submitted must be high. The Editors will reject Figures of an unacceptable standard or ask the Authors to replace them. Figures should be referred to sequentially in the text as Fig. 1, Fig. 2, Figs. 3a,b and 4, etc. A Legend must be provided for each Figure and placed after any Tables in the main manuscript file. Do not write legends on the figures themselves. Scale bars must be provided on all photomicrographs and electron micrographs.

In preparing figures, Authors should note the following:

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- Save text in figures as "graphics" or enclose the font.
- Only use the following fonts in your figures: Times New Roman, Arial, Courier, Helvetica, Symbol.
- Number the figures according to their sequence in the text.
- Use a logical naming convention for your artwork files.
- Provide all figures as separate files.
- Produce images near to the desired size of the printed version.
- Ensure that all units and wording in the figures conform to TVJ style (see Units above).

Please note that each figure must be uploaded to the journal website separately and not included in the main manuscript.

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General points

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• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the published version.
• Submit each illustration as a separate file.
• Ensure that color images are accessible to all, including those with impaired color vision.

A detailed guide on electronic artwork is available.
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats
If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format. Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings, embed all used fonts.
TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
• Supply files that are too low in resolution;
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Book reference - Cunningham, J.C., Klein, B.G., 2007. Endocrinology. In: Textbook of Veterinary Physiology, Fourth Edn. Saunders Elsevier, St. Louis, MO, USA, pp. 439-448.

Proceedings - Elbers, A.R., Mintiens, K., Staubach, C., Gerbier, G., Meiswinkel, R., Hendrinckx, G., Backx, A., Conraths, F.J., Meroc, E., Ducheyne, E., et al., 2007. Bluetongue virus serotype 8 epidemic in North-western Europe in 2006: Preliminary findings. Proceedings of the Annual Meeting of the Society for Veterinary Epidemiology and Preventive Medicine, Dipoli, Finland, 28th-30th March 2007 pp. 231-245.

Theses - Duz, M. 2009. Assessment of a methodology for determination of H^2O^2 concentration and pH in exhaled breath condensate in horses with and without lower airway inflammation. Thesis, Master of Veterinary Medicine, University of Glasgow, United Kingdom.

Web addresses - FAOSTAT, 2008. Food and Agricultural Organization Statistical Database: Live Animals. http://faostat.fao.org (accessed 15 July 2010).

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Appendix Reporting Guidelines

Reporting guidelines are available for a broad range of study designs and allow research to be critically evaluated. These guidelines have been designed by international scientific teams to promote the quality of research reporting and to ensure there is a transparent, accurate and complete account of the research. The guidelines are freely available and include the following:

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2. Standards for the reporting of observational studies in epidemiology (STROBE) http://www.strobe-statement.org
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5. Systematic reviews and meta-analyses (PRISMA) http://www.prisma-statement.org
6. Randomised control trials for livestock and food safety (REFLECT) http://www.reflect-statement.org/statement
7. Enhancing the quality and transparency of health research (including good

publication practice for pharmaceutical companies), economic evaluations and qualitative research (EQUATOR) http://www.equator-network.org

For further information see The Veterinary Journal (2010) 184, 249-250 (view article).