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MARIANA MARCANTONIO CONEGLIAN

INFLUÊNCIA DA SAÚDE BUCAL NA EXPRESSÃO FACIAL E NO EXAME CLÍNICO POR ACUPUNTURA EM EQUINOS

Influence of buccal health on facial expression and clinical examination of acupuncture in equine.

CURITIBA
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Tese apresentada ao Programa de Pós-Graduação em Ciência Animal, área de concentração Saúde, Tecnologia e Produção Animal, da Escola de Ciências da Vida da Pontifícia Universidade Católica do Paraná, para obtenção do título de Doutora em Saúde, Tecnologia e Produção Animal Integrada.

Orientador: Prof. Dr. Pedro Vicente Michelotto Jr.

Coorientador: Prof. Dr. Saulo Henrique Weber

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TERMO DE APROVAÇÃO

Pontifícia Universidade Católica do Paraná
Programa de Pós-Graduação em Ciência Animal
Câmpus Curitiba

PUCPR
GRUPO MARISTA

ATA Nº 0005 E PARECER FINAL DA DEFESA DE TESE DE DOUTORADO EM CIÊNCIA ANIMAL DA ALUNA MARIANA MARCANTONIO CONEGLIAN

Aos vinte e dois dias do mês de agosto do ano de dois mil e dezenove, às 14h, realizou-se na sala de vídeo 02, térreo do Bloco Verde, da Pontifícia Universidade Católica do Paraná, localizada no Campus de Curitiba, Rua Imaculada Conceição, nº 1155, Prado Velho – Curitiba – PR, a sessão pública de defesa de tese da doutoranda **Mariana Marcantonio Coneglian**, intitulada: "INFLUÊNCIA DA SAÚDE BUCAL NA EXPRESSÃO FACIAL E NO EXAME CLÍNICO POR ACUPUNTURA EM EQUINOS". A doutoranda concluiu os créditos exigidos para obtenção do título de Doutor em Ciência Animal, segundo os registros constantes na secretaria do Programa. Os trabalhos foram conduzidos pelo Professor orientador e Presidente da banca, Dr. Pedro Vicente Michelotto Junior (PUCPR), auxiliado pelos Professores Doutores Luiz Guilherme Achcar Capriglione (PUCPR), Rodrigo Romero Corrêa (USP), e as Doutoras Tâmara Duarte Borges e Fabiane Cassou. Procedeu-se à exposição da tese, seguida de sua argüição pública e defesa. Encerrada a fase, os examinadores expediram o parecer final sobre a tese, que foi considerada APROVADA

MEMBROS	ASSINATURA
Prof Dr Pedro Vicente Michelotto Junior - Orientador	
Prof Dr Luiz Guilherme Achcar Capriglione (PUCPR)	
Prof Dr Rodrigo Romero Corrêa (USP)	
Dra Tâmara Duarte Borges	
Dra Fabiane Cassou	

Proclamado o resultado, o Presidente da Banca Examinadora encerrou os trabalhos, e para que tudo conste, eu Caroline Nocera Bertton, confiro e assino a presente ata juntamente com os membros da Banca Examinadora.

Curitiba, 22 de agosto de 2019.

Caroline Nocera Bertton
Caroline Nocera Bertton
Secretária do Programa de Pós-Graduação em Ciência Animal

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SUMÁRIO

Dedicatória

> “Eu asseguro que, se vocês tiverem fé do tamanho de um grão de mostarda, nada será impossível para vocês.”
>
> Mateus 17:20

À minha avó Ruth Nogueira Coneglian. Dedico.

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Mãe, Pai, Tatá, Sabrina, Lorena e Matheus, mais uma conquista
para NOSSA conta!

FORMATO DA TESE

A presente tese é composta por capítulos. O capítulo 1 apresenta uma introdução geral, a contextualização do tema e os objetivos do estudo. O capítulo 2 trata-se do artigo científico completo, intitulado: Use of the Horse Grimace Scale to identify and quantify pain due to dental disorders in horses. Contendo referências e formatado nas normas da revista Applied Animal Behaviour Science, para o qual foi submetido. O capítulo 3 trata-se do artigo científico completo, intitulado: Influence of buccal health on the facial expression and on the acupuncture examination in equine; contendo referências, e formatado nas normas da revista Applied Animal Behaviour Science, para a qual será submetido. O capítulo 4 finaliza esta tese com as considerações finais deste trabalho. As referências do capítulo 1 encontram-se ao final da tese.

RESUMO GERAL

As alterações odontológicas são frequentes nos equinos e podem causar desconforto e dor crônica, afetando o bem-estar e o desempenho atlético dos cavalos. No entanto, estas condições podem não se manifestar com sinais clínicos óbvios, levando ao diagnóstico e cuidados tardios. Tal fato predispõe os equinos portadores de desordens odontológicas a desenvolverem outros distúrbios, como doenças gastrointestinais ou condições dolorosas no resto do corpo. O presente estudo teve como objetivo avaliar a influência da saúde bucal na expressão de dor e no exame por acupuntura de equinos. A dor foi avaliada através da observação da expressão facial equina (HGS, *Horse Grimace Scale*). Para isso, seis características faciais foram avaliadas (posição das orelhas, tensão acima da área dos olhos, aperto orbital, tensão na musculatura mastigatória, boca tensa com queixo pronunciado e narinas tensas com achatamento do perfil). Foram estudados 87 equinos adultos, de diferentes raças, machos e fêmeas, que estavam regularmente envolvidos em atividades esportivas ou de trabalho durante todo o período do estudo. A dor foi pontuada em presença física direta (*in loco*) pelo investigador, e ao mesmo tempo a face dos cavalos foi fotografada para análise posterior. Para os primeiros 33 animais a avaliação fotográfica foi realizada por sete examinadores que desconheciam o estudo e a condição de cada animal, sendo três treinados no uso da HGS e quatro veterinários de equinos, com experiência clínica, mas não treinados no uso da HGS. Para os 54 equinos restantes, a avaliação fotográfica foi realizada por apenas um avaliador treinado para o uso da HGS, o qual não tinha conhecimento sobre a condição dos animais. O exame de acupuntura foi realizado sempre pelo mesmo examinador. Esse foi iniciado com pressão do dedo sobre a articulação temporomandibular e regiões adjacentes, seguida de teste de sensibilidade dos pontos de acupuntura nos caminhos dos meridianos. Os animais foram avaliados antes (M0) e 15 dias após (M1) a avaliação e tratamento odontológico. Todos os 87 equinos estudados apresentavam alguma desordem odontológica e, apesar destas, todos apresentavam parâmetros de exame físico geral dentro da normalidade para a espécie e permaneciam no esporte ou atividade de trabalho durante todo o estudo. Houve concordância significativa nos escores de dor entre todos os avaliadores treinados para a utilização da HGS (presencial e fotográfico), com um forte e significativo coeficiente de correlação intraclasse (ICC = 0.86). No entanto, não houve concordância completa entre os avaliadores veterinários não treinados, e entre esses e os avaliadores que utilizaram a HGS. Após o tratamento odontológico observou-se diminuição significativa tanto no número de pontos de acupuntura reativos (11,2 ± 5,6 vs. 4 ± 2,9 pontos) quanto no escore de dor. Este passou de e 3 (0-10), para um escore mediano 1 (0-6). Além disso, observou-se redução das queixas dos proprietários em relação ao processo mastigatório e à qualidade do trabalho dos animais. Alguns pontos de acupuntura reativos em animais com alterações dentárias apresentaram redução significativa após o tratamento odontológico. Com ênfase para o Ponto do Dente (44 vs. 4), Estômago (E)-7 (31 vs. 3), Triplo Aquecedor (TA)-17 (27 vs. 4) e TA-16 (22 vs. 4), mostrando que o exame de acupuntura pode auxiliar no diagnóstico das alterações dentárias e, principalmente, auxiliar na determinação do sucesso do tratamento odontológico. Notou-se, ainda, relação positiva entre a presença de úlceras na mucosa bucal e o escore de dor, o número de pontos de acupuntura reativos e a qualidade do trabalho. A presença de ganchos e rampas influenciou a qualidade

mastigatória dos animais, já a presença dos dentes de lobo não influenciou em nenhum dos parâmetros estudados. Por fim, observou-se que em M0, apesar de não apresentarem claudicação, um número significativo de equinos (n=49/87) apresentou pontos de acupuntura sensíveis indicando síndromes dolorosas descritas no diagnóstico por acupuntura e relacionadas à coluna toracolombar ou membros posteriores, o que reduziu em M1(n=16/87). Em conclusão, o tratamento odontológico resultou em redução da dor, modificando a expressão facial dos cavalos e reduzindo o número de pontos de acupuntura sensíveis a palpação ou pressão. A integração do exame pela palpação/pressão de pontos de acupuntura e a avaliação da dor utilizando a HGS, no exame de rotina dos equinos, pode ser útil na avaliação clínica geral e específica odontológica, e especialmente para o acompanhamento após o tratamento odontológico, bem como auxiliou para demonstrar a influência de alterações na cavidade oral sobre o sistema musculoesquelético.

Palavras-chave: Acupuntura; Bem-estar; Desordens odontológicas; Equinos; Expressão facial.

ABSTRACT

Dental disorders are frequent in equines and can cause discomfort and chronic pain, affecting horses' welfare and athletic performance. However, these disorders may not be manifested with recognizable clinical signs, leading to late diagnosis and care. This fact predisposes horses with dental disorders to develop other disorders, such as gastrointestinal diseases or painful conditions in the rest of the body. The present study aimed to evaluate the influence of oral health on pain expression and equine acupuncture examination. Pain was assessed by the equine facial expression using the horse grimace scale (HGS). Were evaluated 87 adult horses, both males and females, that were regularly involved in sports or working activities. Initially, pain was scored in direct physical presence (in loco) by the investigator. At the same time, the horses' faces were photographed for further analysis. For the first 33 animals the photographic evaluation was performed by seven evaluators, three trained in the use of HGS, and unaware of the study and condition of each animal and four equine veterinarians not trained in the use of the scale. For the remaining 54 horses the photographic evaluation was performed by only one evaluator trained in the use of HGS, who unaware of the study and condition of each animal. The acupuncture test was always performed by the same evaluator, who evaluated the palpation sensitivity of the acupoints. This was initiated with finger pressure on the temporomandibular joint and adjacent regions, followed by sensitivity test of the acupuncture points in the meridian paths. The animals were evaluated before (T0) and 15 days after the evaluation and dental treatment (T1). All 87 equines studied had some dental disorders and, despite dental alterations, all presented parameters of general physical examination within normal for the species and remained in the sports or work activity throughout the study. A significant agreement in the pain scores was observed among all the evaluators trained in the used of HGS (direct physical examination and from horses' photographs), with a strong and significant intra-class correlation coefficient (ICC = 0.86). However, there was no complete agreement among untrained veterinary evaluators, and between these and the evaluators who

used the HGS. After dental treatment, there was a significant decrease in both the number of reactive acupuncture points (11,2 ± 5,6 vs. 4 ± 2,9 points) and pain score. This went from 3 (0-10) to a median score of 1 (0-6). In addition, there was a reduction of the owners' complaints regarding the masticatory process and the quality of work. Some reactive acupuncture points in animals with dental disorders significantly reduced after treatment. With emphasis on Dental point (44 vs. 4), Stomach (ST) -7 (31 vs. 3), Triple Heater (TH) -17 (27 vs. 4) and TH-16 (22 vs. 4), showing that the acupuncture exam can help in the diagnosis of dental alterations and, mainly, help in determining the success of dental treatment. It was noted a positive relationship between the presence of ulcers in the oral mucosa and pain scores, the number of reactive acupuncture points and the quality of work. The presence of hooks/ramps influenced masticatory quality of animals and the presence of wolf teeth did not affect any of the studied parameters. Finally, it was observed that in T0, although they did not present lameness, a significant number of horses (n=49/87) presented sensitive acupuncture points that indicated painful syndromes in the thoracolumbar spine or hind limbs, which reduced in T1 (16/87). In conclusion, the dental treatment resulted in reduction of pain, changing the facial expression of the horses and reduced the number of acupuncture points sensitive to palpation or pressure. The integration of acupuncture point palpation/pressure examination and pain assessment using HGS in equine routine examination may be useful in general and specific dental clinical evaluation, and especially for follow-up after dental treatment, as well as assisting to demonstrate the influence of changes in the oral cavity on the musculoskeletal system.

Keywords: Acupuncture; Dental disorders; Equine; Facial expression; Welfare.

CAPÍTULO 1 - INTRODUÇÃO E CONTEXTUALIZAÇÃO

A equideocultura é um setor de grande importância econômica no Brasil. A atividade movimenta anualmente cerca de R$ 16,15 bilhões e gera aproximadamente 610 mil empregos diretos e 2.230 mil empregos indiretos. O país possui uma tropa superior a 5 milhões de cavalos (MAPA, 2016). O cavalo é um animal que se destaca tanto em atividades de trabalho quanto esporte e lazer, e os investimentos nestes animais podem atingir altos valores (Lima et al., 2006). Consequentemente a seleção, a domesticação e o confinamento destes animais passaram a ser realizados de forma cada vez mais intensa, acarretando em modificações dos padrões alimentares e comportamentais, comprometendo assim a formação dentária e culminando em uma série de afecções odontológicas (Dixon et al., 2007; Easley et al, 2011; Amaya et al., 2012). Tais afecções são consideradas a terceira causa mais comum de distúrbios dentro da clínica equina nos EUA e estão habitualmente associadas a alterações do padrão mastigatório, disfagia, perda de peso e distúrbios gastrointestinais (Galloway e Easley, 2008; Ardila e Montoya, 2009; Amaya et al.,2012; Dixon, 2017). Nos animais atletas pode ocorrer ainda incomodo com a embocadura e relutância com agressividade durante a doma (Easley et al.,2011; Jiménez e Díaz, 2011).

De acordo com a medicina veterinária ocidental, o diagnóstico das alterações odontológicas se dá através da realização de um exame detalhado que começa antes da avaliação direta da cavidade oral do cavalo, com a inspeção e palpação da cabeça, além da verificação da mobilidade da mandíbula (Speirs, 1999; Pence, 2002; Easley et al., 2011).

Através deste exame é possível diagnosticar e tratar as principais alterações odontológicas dos equinos. No entanto nem todos os equinos apresentam sinais de desconforto na cavidade oral de forma clara, e a odontologia equina é ainda muitas vezes negligenciada, levando muitos cavalos a sofrerem sem um diagnóstico preciso (Ashley et al., 2005; Brigham e Duncanson, 2010a, 2010b; Amaya et al., 2012; Alencar-Araripe et al., 2013). Animais que não são precocemente diagnosticados com alterações odontológicas podem inclusive apresentar alterações mais graves em outros sistemas, incluindo o sistema musculoesquelético, resultando em queda de

desempenho ou até claudicações (Stashak e Hill, 2006; Du Toit et al., 2008; Galloway e Easley, 2008; May, 2008; Easley et al., 2011; Cain, 2015).

Neste contexto, a medicina veterinária tradicional chinesa (MVTC) surge como mais uma opção, tanto para o diagnóstico quanto para o tratamento das doenças em equinos, além de servir como parâmetro para avaliação geral do bem-estar animal (Fleming, 2006; Shmalberg e Xie, 2009; Michelotto et al., 2014; Pellegrini et al., 2018).

Dentre as diferentes técnicas diagnósticas da MVTC, a verificação de sensibilidade em pontos de acupuntura tem sido proposta como método prático e eficiente, podendo auxiliar no diagnóstico de diferentes afecções nos equinos (Fleming, 2006; Shmalberg e Xie, 2009; Cain, 2015).

Este exame tem como base a observação da resposta dos animais à palpação dos meridianos e à pressão em pontos de acupuntura específicos (McCormick, 1997; McCormick, 1998; Schoen, 2000; Fleming, 2006; Shmalberg et al. Xie, 2009). A combinação de um conjunto de acupontos sensíveis a essa pressão permite determinar o diagnóstico de algumas condições dolorosas ou síndromes (Angeli et al., 2007; Michelotto et al., 2007, Angeli et al., 2008; Xie e Preast, 2011; Alfaro, 2014; Cain, 2015).

Alguns pontos de acupuntura são citados como pontos diagnósticos para alterações da articulação temporomandibular (ATM) ou dor na área da cabeça e boca (Fleming, 2006; Xie e Preast, 2011; Cain, 2015), entretanto, não há estudos direcionados especificamente para o diagnóstico das afecções odontológicas de equinos e suas complicações.

O correto e precoce reconhecimento da dor é outro fator que pode auxiliar na avaliação da saúde e bem estar dos equinos, o que, no entanto, nem sempre é claro uma vez que na presença de possíveis predadores, no caso os seres humanos, os cavalos podem suprimir os sinais óbvios de dor (Ashley, 2005; Dalla Costa et al., 2014).

Atualmente estão sendo descritas algumas formas de reconhecimento da dor em equinos além da rotineira avaliação dos sinais vitais, entre elas destacam-se as observações de discretas alterações na expressão facial como as descritas por Dalla Costa et al. (2014) na *Horse Grimace Scale* (HGS). No entanto, escores pré e pós-tratamento odontológico para a expressão facial em diferentes alterações dentárias não foram relatados.

Neste contexto, consideramos a hipótese de que as alterações odontológicas influenciam o bem-estar equino, e isso pode ser avaliado pelo escore de dor determinado pela análise da expressão facial e pela resposta ao exame diagnósticos de pressão de acupontos. Além disso, este estudo teve como objetivo determinar se há concordância na determinação da dor entre avaliação face a face e avaliação utilizando fotografias da face dos cavalos com alterações odontológicas. Por fim, avaliou-se a confiabilidade da acupuntura como método diagnóstico para afecções odontológicas e suas possíveis complicações.

1.1. Principais alterações odontológicas de equinos.

As afecções da cavidade oral podem ser de origem genética ou adquirida. Dentre as principais afecções odontológicas adquiridas dos equinos pode-se citar inicialmente a presença de pontas excessivas de esmalte dentário (PEED) (Easley et al., 2011; Dixon, 2017). A presença de PEED é considerada a alteração odontológica mais frequente em equinos, com uma prevalência de 70 a 90% em equinos domesticados (Dixon et al., 2000). Estas alterações acometem mais frequentemente equinos de até 9 anos de idade devido a rizogênese dos dentes permanentes (Dixon e Dacre, 2005; Pagliosa et al., 2006). As PEED ocorrem quando a excursão lateral da mandíbula está diminuída, fazendo com que o ciclo da mastigação não se complete e não atinja a extremidade lateral da superfície oclusal dos pré-molares e molares de um dos lados. Isto pode ocorrer devido a diferentes fatores como dor, tipo de alimentação, ou por fatores anatômicos, quando, por exemplo, a mandíbula é muito estreita. Como a erupção dentária em equinos é continua, esta situação resulta na formação das PEED nos locais onde o desgaste não está ocorrendo (Johnson e Porter, 2006b). Na arcada superior estas pontas apresentam-se em direção à face bucal e na arcada inferior ocorrem em direção à face lingual (Easley et al., 2011; Dixon, 2017).

A presença das PEED geralmente é relatada como responsável por dor ou desconforto durante a mastigação ou o trabalho. Além disso, pode levar à ulceração da mucosa vestibular ou lingual (localizada ou difusa, aumentando a dor na cavidade oral e resultando em perda de peso e problemas de treinamento (Dixon et al., 1999; Dixon e Dacre, 2005; Easley et al., 2011). As

PEED podem causar ainda mudança na biomecânica mastigatória tornando-a deficiente, com trituração insuficiente dos alimentos e consequentemente diminuição da produção de saliva, o que pode afetar a digestibilidade dos alimentos (Johnson e Porter, 2006b; Pagliosa et al., 2006).

Desta forma, a remoção das PEED é sempre recomendada (Easley et al., 2011), se negligenciadas, estas podem eventualmente envolver toda a superfície oclusal (boca de cisalhamento), condição relatada como extremamente dolorosa (Dixon, 2000; Dixon e Dacre, 2005).

A distrofia Eruptiva do Primeiro pré-molar é outra condição frequente nos equinos, mais conhecido por presença do dente de lobo. A literatura disponível sugere que os dentes de lobo maxilar são prevalentes (13-80% de prevalência dependendo da raça estudada) e os dentes de lobo mandibular são muito raros (Brigham e Duncanson, 2000a, 2000b; Dixon e Dacre, 2005; Ozanam et al., 2005; Filho et al., 2014). Seu tamanho e forma são bastante variáveis e geralmente são facilmente visíveis, porém, quando inclusos (permanecem na submucosa) pode haver a necessidade de uso de radiografia para localizá-los (Dixon e Dacre, 2005; Velazquez, 2009; Filho et al., 2014). Os dentes de lobo geralmente se posicionam 1-2 cm rostralmente ao segundo pré-molar, localização esta onde devem se posicionar os freios ou bridões, o que leva a predisposição de traumas podendo ocasionar desconforto e dor ao animal, que consequentemente reagirá com alterações comportamentais (Dixon e Dacre, 2005; Scrutchfield, 2006; Dixon, 2017). A extração é frequentemente indicada nessa condição (Dixon e Dacre, 2005; Ozanam et al., 2005; Easley et al, 2011; Filho et al., 2014).

Ganchos são alterações resultantes do desgaste irregular dos dentes que culminam na formação de projeções dentárias terminais que ultrapassam mais de 1/3 da superfície oclusal e que podem ferir a língua e a mucosa das bochechas, principalmente durante a mastigação (Easley et al., 2011; Dixon, 2017). Os ganchos projetam-se além desta superfície, sendo quase que exclusivamente uma morbidade que atinge o segundo pré-molar da arcada superior e o terceiro molar da arcada inferior, os ganchos podem apresentar grande projeções e geralmente são bastante aguçados. As rampas em contrapartida são declives além da superfície oclusal que acometem os dentes

molares mandibulares, geralmente no segundo pré-molar e costumam ser mais progressivas (Peters et al., 2006; Carmalt e Rach, 2013).

Os degraus, também são resultantes de falhas de desgaste, neste caso a falha gera uma variação de comprimento em dentes adjacentes numa mesma arcada. O crescimento deficiente, extração, perda ou quebra de um dente molar ou pré-molar, predispõe a um sobrecrescimento do dente correspondente na arcada oposta. Esta anormalidade progride levando à restrição do “esmagamento” dos alimentos, causando dor e prejudicando a mecânica da mastigação (Dixon e Dacre, 2005; Dixon 2017).

Já as ondas ocorrem geralmente quando a erupção dos dentes ocorre em velocidades diferentes. A erupção depende da saúde da membrana periodental, ou seja, uma membrana infeccionada promove uma erupção retardada dos dentes, sendo este um dos principais causadores das ondas na superfície oclusal (Dixon e Dacre, 2005). Na arcada inferior, as ondas se caracterizam pela descida na zona do terceiro e quarto pré-molares, seguida da elevação novamente na zona do primeiro molar, e então novamente pela descida na área do segundo molar, subindo por último ao nível do terceiro molar. Esta ondulação verifica-se também na arcada superior, sendo oposta à descrita para a arcada inferior (Dixon e Dacre, 2005). Tal alteração gera dificuldade mastigatória e consequentemente dor aos animais (Dixon e Dacre, 2005; Johnson e Porter, 2006b).

Dentre as desordens odontológicas dos equinos pode-se citar ainda a má oclusão dos incisivos, as quais podem ser de origem genética ou adquirida. O braquignatismo e o prognatismo são má oclusões congênitas onde na primeira os incisivos superiores se sobrepõem aos inferiores e na segunda os incisivos inferiores ultrapassam os superiores (Johnson e Porter, 2006a; Easley et al 2011). Outros desalinhamentos dos incisivos são as curvaturas, que podem ser ventral, dorsal ou mordida em diagonal, estas segundo Johnson e Porter (2006a), podem ter origem congênita ou adquirida. Quando adquiridas estão relacionadas à alimentação e aos hábitos do animal. A curvatura ventral se define pelo maior desenvolvimento dos incisivos laterais (cantos) da arcada mandibular, do que seus correspondentes da arcada maxilar. Já na curvatura dorsal os incisivos laterais superiores se desenvolvem mais que os da mandíbula (Johnson e Porter, 2006a; Easley et al, 2011). Em caso de mordida

em diagonal, um dos lados da arcada maxilar junto do lado contralateral da arcada mandibular, apresentam os incisivos bem maiores que os outros, dando uma superfície oclusal em diagonal (Peters et al., 2006).

Independente da má oclusão presente, estes desalinhamentos estão habitualmente associados a alterações do padrão mastigatório de outros dentes, o que pode gerar graves desconfortos e, portanto aumenta a importância dos cuidados odontológicos (Johnson e Porter, 2006b; Dixon, 2017).

Para finalizar é importante falar da periodontite ou doença periodotal. O periodonto inclui a gengiva, o ligamento periodontal, o osso alveolar e o cemento. A doença periodontal é uma doença crônica de origem bacteriana que acomete estes tecidos, provocando a sua destruição e resultando provavelmente na perda dos dentes afetados (Easley et al., 2011; Dixon, 2017). A sua prevalência é maior na idade adulta, apresentando uma incidência de 60% em equinos acima dos 15 anos (Dixon e Dacre, 2005). O movimento de mastigação contribui para a manutenção de um periodonto saudável. Situações que limitem a mastigação, como as já citada anteriormente, podem resultar em doença periodontal secundária e destruição progressiva do ligamento periodontal e osso alveolar, que resultam numa eventual perda do dente e graves consequências para a saúde e bem estar deste cavalo (Dixon e Dacre, 2005).

De forma geral todas estas alterações odontológicas podem gerar sinais clínicos semelhantes e estes podem se apresentar de forma mais ou menos clara. Alguns cavalos apresentam sinais leves, como lentidão na mastigação, acúmulo de alimentos na bochecha ou deixam cair alimentos parcialmente mastigados da boca. Outros apresentam balanço excessivo da cabeça durante a mastigação, halitose e disfagia; perda de peso e distúrbios gastrointestinais podem ser observados em casos mais graves. Nos animais atletas ou mais exigidos no trabalho existem ainda sinais de incômodo com a embocadura, com balanço excessivo da cabeça durante o exercício, relutância com agressividade durante a doma e resistência à ação das rédeas. Estes incômodos podem alterar o padrão de locomoção dos animais e consequentemente afetar o sistema musculo esquelético e o desempenho atlético dos animais (Thomassian, 2005; Stashak e Hill, 2006; Jiménez e Díaz,

2011; Amay et al., 2012). Segundo a visão médica tradicional isso ocorre uma vez que ao sentir o desconforto na boca, o equino pode balançar excessivamente a cabeça, atira-la para trás ou trabalhar com sua cabeça e pescoço em uma posição não natural, o que afeta o movimento. Inicialmente isso pode ser confundido com uma claudicação e futuramente pode realmente gerar uma alteração osteomuscular (Bennett, 2006; Stashak e Hill 2006). Nestes casos, apesar da claudicação realmente existir, é fundamental também diagnosticar e tratar a desordem odontológica.

Apesar destes fatos, a odontologia equina é ainda muitas vezes negligenciada na clínica equina e muitos cavalos sofrem sem diagnóstico relacionado com alterações dentárias (Easley et al., 2011; Dixon, 2017). Para evitar tal cena faz-se necessário o exame rotineiro e detalhado da cavidade oral dos equinos, sendo que o sucesso para uma boa avaliação odontológica baseia-se inicialmente no conhecimento sobre os aspectos fundamentais da anatomia e da morfologia oral, assim como da fisiologia da mastigação dos cavalos. Desta forma há uma melhor compreensão dos processos patológicos que podem acometer a cavidade oral dos equinos, o que permite então a realização de um exame odontológico confiável (Ardila e Montoya, 2009; Easley et al, 2011).

1.2. Exame odontológico de equinos.

A realização de um bom exame odontológico deve, assim como o exame clínico, seguir uma sequência lógica, começando pela anamnese. Em seguida recomenda-se que a cabeça do animal seja inspecionada e palpada, antes de qualquer tentativa de exame da cavidade oral o qual deve ser realizado com auxílio de especulo oral e com o animal sedado (Speirs, 1999; Pence, 2002; Dixon e Dacre, 2005).

A anamnese deve ser a mais completa possível. Primeiramente, deve-se recolher todos os dados do animal, seguido das informações sobre o alimento que ele ingere incluindo o tipo, quantidade, local de fornecimento e até se derruba o alimento na hora de comer, se está emagrecendo ou não atinge condição corporal satisfatória. Questionar a casuística de cólicas, e também o comportamento do cavalo, principalmente durante o exercício, como balançar

da cabeça ou resistência ao freio. Episódios de claudicação também devem ser investigados (Pence, 2002; Dixon e Dacre, 2005; Velazquez, 2009).

Durante a inspeção deve-se observar a simetria, conformação e formato da cabeça. Observar, por exemplo, a presença de inflamação, abscessos e/ou corrimentos nasais. Feito isto deve-se então passar para a palpação pela bochecha, analisar toda a região da boca, da mandíbula, da maxila, dos pré-molares e molares na busca de zonas dolorosas. Os seios paranasais também devem ser avaliados através de percussão e o teste de desvio lateral da mandíbula deve ser realizado (Dixon e Dacre, 2005; Velazquez, 2009; Easley et al., 2011).

Para a inspeção da cavidade oral, deve-se utilizar um espéculo oral e uma fonte luminosa, porém, se o animal não estiver sedado, o procedimento se torna difícil e perigoso (Dixon, 2003; Dixon e Dacre, 2005; Velazquez, 2009). Portanto, indica-se a realização da sedação, com uso de um alfa-2 agonistas, como xilazina 10% ou detomidina 1% e em algumas ocasiões pode realizar a associação destes com um opióide, como butorfanol (Pence, 2002; Dixon, 2003; Dixon e Dacre, 2005). Neste momento, deve-se examinar além dos dentes, a gengiva, a língua e a mucosa vestibular, na busca de lesões e ulcerações. Deve-se verificar o número de dentes presentes, se são decíduos ou permanentes, mobilidade, sincronia das trocas dentárias, simetria dos incisivos ou quaisquer outras alterações perceptíveis nos mesmos (Pence, 2002; Dixon, 2003; Dixon e Dacre, 2005; Velazquez, 2009).

Avalia-se também a presença e quantidade de dentes de lobo, a presença de pontas excessivas de esmalte dentário nos pré-molares e molares, além de outras alterações que possam estar presentes (Pence, 2002; Dixon, 2003; Dixon e Dacre, 2005).

Uma vez identificadas todas as alterações, estas são anotadas em um odontograma e então se procede à correção parcial ou total dos problemas.

1.3. Acupuntura - Introdução e sua utilização como método diagnóstico em equinos.

A acupuntura é uma técnica desenvolvida há mais de 5.000 anos no oriente e que faz parte da medicina tradicional chinesa. O termo acupuntura deriva dos radicais latinos *acuse pungere*, que significam agulha e puncionar.

Na medicina veterinária a acupuntura é descrita há aproximadamente 3.000 anos, sendo que por volta de 650 anos a.C. foi escrito o Cânon de Medicina Veterinária, por um general chamado Sun-Yang, o qual ficou conhecido devido seu notável trabalho com equinos, como “pai” da Medicina Veterinária Chinesa. Nesta época os cavalos recebiam mais atenção em relação aos outros animais, já que estes eram de grande utilidade nas guerras e na agricultura. (Jagger e Robinson, 2006; Shmalberg e Xie, 2009). Fora da Ásia, entretanto, poucos são os relatos de acupuntura veterinária até a década de 1960, quando três veterinários europeus começaram a estudar e trabalhar neste campo de forma independente um dos outros, sendo considerados os promotores do renascimento da acupuntura veterinária no Ocidente. Desde então, a acupuntura tem sido utilizada na medicina veterinária para tratar uma variedade de problemas (Jagger e Robinson, 2006). Esta técnica consiste, portanto, na estimulação de pontos específicos do corpo chamados de “acupontos” ou "pontos de acupuntura" com objetivo de atingir um efeito terapêutico ou homeostático (Jagger e Robinson, 2006; Schwartz, 2008; Scognamillo-Szabó e Bechara, 2010).

Vale ressaltar, entretanto, que a aplicabilidade da acupuntura não se dá apenas durante a fase de tratamento, mas também tem papel importante no diagnóstico das diferentes afecções que podem acometer os equinos (Schoen, 2006). Para realizar avaliação diagnóstica através da acupuntura os seguintes métodos podem ser utilizados: o interrogatório, a audição, a observação e a palpação (Fleming, 2006; Schwartz, 2008; Shmalberg e Xie, 2009).

Os equinos respondem de forma bastante confiável ao exame de palpação dos meridianos e seus respectivos pontos de acupuntura (Fleming, 2006; Shmalberg e Xie, 2009). Este exame baseia-se na observação da resposta dos animais à palpação dos trajetos dos meridianos e pressão dos pontos de assentimento e costuma utilizar a teoria dos meridianos para interpretar os achados e desta forma chegar aos possíveis diagnósticos (McCormick, 1997; McCormicK, 1998; Schoen, 2006; Shmalberg e Xie, 2009). Meridianos que apresentam em seu trajeto pontos de acupuntura anormalmente reativos à pressão são considerados desequilibrados, e a combinação de um conjunto de acupontos sensíveis a esta pressão permite determinar o diagnóstico de algumas condições específicas ou síndromes

(Angelil et al., 2007; Micheloto et al., 2007; Angeli et al., 2008; Michelotto et al.,2014; Cain, 2015).

Diferentes pesquisadores estudaram a relação entre os pontos de acupuntura sensíveis à palpação em equinos e as diferentes afecções apresentadas por estes. McCornick (1997) avaliou, através do exame de palpação de pontos de acupuntura, 189 cavalos já comprovadamente diagnosticados como claudicantes devido alterações distais dos membros anteriores. Nestes animais ele conseguiu definir um padrão de pontos reativos que tinha correlação com as claudicações de membros anteriores com origem na articulação interfalageana proximal.

Em um estudo de caso-controle para detecção de infecção por herpesvírus equino tipo 1, recente ou reativado (EHV-1) em cavalos com desempenho diminuído, Chvala et. al (2004) demonstrou que todos os cavalos do grupo infectado eram sensíveis à manipulação de todos os pontos de acupuntura que se acredita estarem associados a infecções por EHV, o que não ocorreu nos animais do grupo controle.

Michelotto e colaboradores (2014), estudando 810 cavalos de diferentes modalidades atléticas conseguiram mostrar que o exame de acupuntura é realmente útil no diagnóstico das síndromes envolvendo a articulação da soldra.

Cain (2015) em seu livro Acupuncture Diagnosis and treatment of the Equine descreve uma série de síndromes clínicas relacionadas a conjuntos de pontos de acupuntura reativos à palpação.

Entretanto, estas pesquisas dentro da odontologia equina e o que isso representa na saúde do cavalo como um todo, ainda são escassas. A literatura cita que animais com alterações odontológicas tendem a apresentar reatividade em alguns acupontos localizados na região da cabeça (Figura 1).

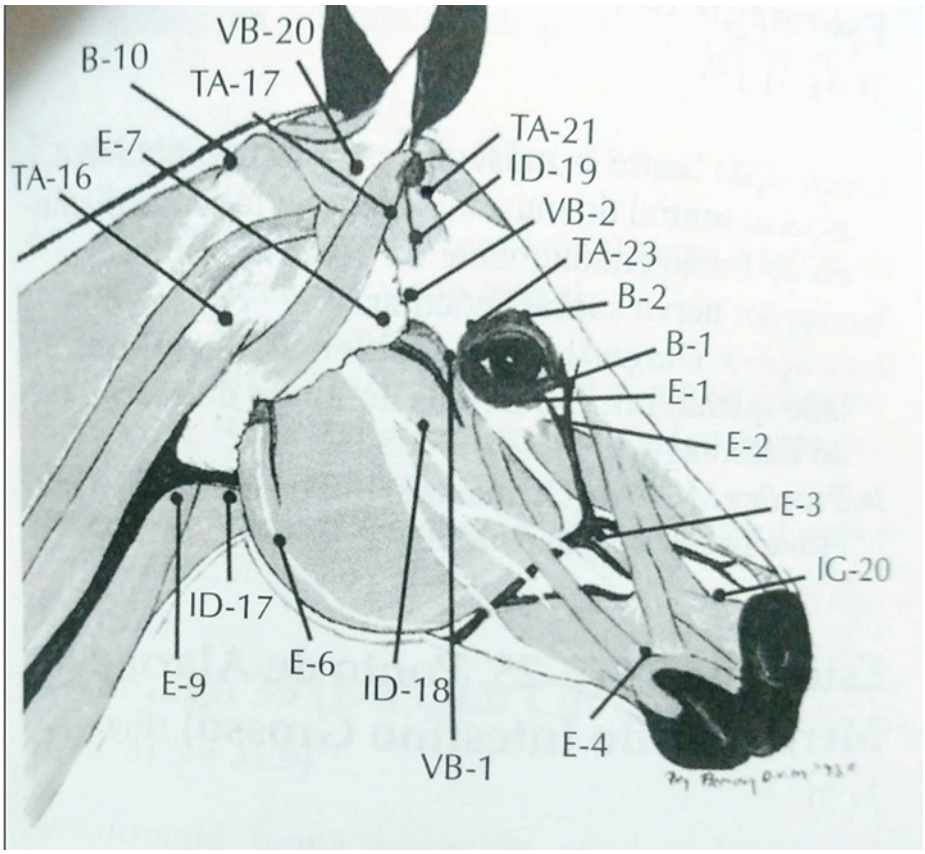

Figura 1: Acupontos localizados na região da cabeça e pescoço de equinos (Schoen, 2006).

Equinos com alterações na articulação temporomandibular (ATM), por exemplo, tendem a apresentar desequilíbrio e dor nos pontos Estômago (E)-7, Vesicula Biliar (VB)-1, Triplo aquecedor (TA)-16 e 17 e Intestino Delgado (ID)-10. Já para a presença de pontas excessivas de esmalte espera-se reação nos pontos VB-20 e Bexiga (B)-10 (Schoen, 2006; May,2008; Shmalberg e Xie, 2009). Além disso, Cain (2015) cita que sensibilidade em um ponto localizado no tendão entre o atlas e o osso occiptal, logo atrás da base da orelha, seja indicativa de problemas odontológicos de forma geral (Figura 2).

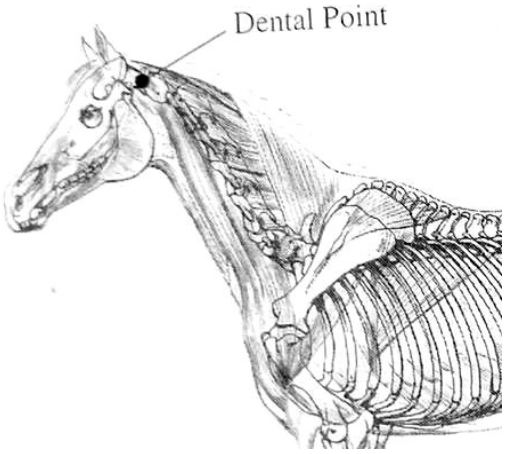

Figura 2: Ponto descrito por Cain (2015) para diagnóstico de desordens odontológicas.

Sabendo-se também que todos os meridianos da cabeça e pescoço são meridianos Yang e que passam na região da maxila, mandíbula ou ATM (Schoen, 2006; Shmalberg e Xie, 2009), há de se esperar que animais com alterações odontológicas possam apresentar, ao longo do tempo, problemas no trajeto destes meridianos (Cain, 2015).

Desta forma, assim como na medicina ocidental, a MVTC reconhece que a saúde da cavidade oral, com especial atenção para a articulação temporomandibular e alterações dentárias, tem um importante papel na saúde do equino como um todo (Fleming, 2006; May, 2008).

Atualmente sabe-se que o tratamento dentário melhora a postura da cabeça durante o trabalho o que influencia de forma consistente na biomecânica e consequentemente no sistema músculo esquelético e no desempenho de um cavalo atleta (Bennett, 2006; Fleming, 2006; Stashak e Hill, 2006, May, 2008). Cain (2015) ainda cita ter observado que muitos animais com claudicação originada na região de soldra e jarrete apresentaram melhora significativa após receberem tratamento odontológico. Desta forma, o reconhecimento e alívio da dor na cavidade oral são fundamentais tanto para o bem-estar quanto para o melhor desempenho dos animais.

Neste contexto, a palpação diagnóstica de acupontos pode ser utilizada de forma integrada ao exame rotineiro dos equinos, este uso combinado é denominado medicina integrativa. Acredita-se que através da medicina

integrativa é possível diagnosticar alterações sutis ou em estágio inicial que seriam dificilmente diagnosticadas apenas pela medicina ocidental (Schoen, 2006; Michelotto et al., 2007; May, 2008).

1.4. Identificações da dor em equinos.

A dor é uma parte essencial da vida cotidiana e é considerada inicialmente necessária e útil nas diferentes espécies para que estas possam sobreviver em um ambiente potencialmente hostil (de Grauw e van Loon, 2016; Guedes, 2017). Os seres humanos são capazes de relatar objetivamente se a dor está presente ou não, o que é facilmente entendido por outro humano verbalmente capaz. Este pode ainda oferecer detalhes da magnitude, duração e características da experiência da dor (Guedes, 2017).

Na prática veterinária, entretanto, a identificação da dor é um desafio, e quando não identificada e tratada, gera estresse e compromete o desempenho e bem-estar dos animais (de Grauw e van Loon, 2016; Guedes, 2017).

O reconhecimento e a quantificação precisos da dor nos cavalos são fundamentais tanto para o diagnóstico quanto para o estabelecimento de tratamento adequado, mas isso nem sempre é simples, uma vez que os cavalos tendem a demonstrar a dor de forma mais discreta como uma atitude de preservação da espécie (Ashley et al., 2005; Dalla Costa et al., 2014; de Grauw e Van Loon, 2016).

Observações de comportamento/postura, juntamente com a avaliação de parâmetros fisiológicos, são a base para avaliação da dor na espécie equina (Guedes, 2017). Contudo, é importante reconhecer que receitas exatas para avaliação da dor não existem. de Grauw e Van Loon (2016), em uma análise dos diferentes estudos envolvendo a identificação da dor em equinos, notaram que uma ferramenta ou sistema de avaliação de dor pode não funcionar tão bem para os diferentes tipos de dor (dor visceral ou somática, aguda ou crônica, dor nociceptiva, inflamatória ou dor neuropática).

Atualmente não há um "padrão ouro" para avaliação da dor em cavalos, mas uma gama de modelos de identificação sistemática da dor foram descritos para uma variedade de condições dolorosas. Dentre os métodos para a avaliação sistemática da dor em cavalos estão: avaliação dos parâmetros vitais (frequência cardíaca, frequência respiratória) (Price et al., 2003; Pritchett et al.,

2003; Rietmann et al., 2004), avaliação comportamental (Rietmann et al., 2004; Bussières et al., 2008), medidas endócrinas (Pritchett et al., 2003; Rietmann et al., 2004) e avaliação da expressão facial (Dalla Costa et al., 2014; Gleerup et al. , 2015; Wathan et al., 2015; de Grauw e van Loon, 2016; van Dierendonck e van Loon, 2016; van Loon e van Dierendonck, 2017).

Nos últimos anos, escalas de dor com base nas alterações da expressão facial foram desenvolvidas para humanos e outras espécies, ganhando destaque na medicina equina, uma vez que provou ser uma ferramenta sensível, de fácil aplicação com pouco investimento de tempo e treinamento e alta confiabilidade dentro e entre observadores (de Grauw e van Loon, 2016).

Para a elaboração da escalas de dor baseadas na expressão facial, como é o caso da “Horse Grimace scale”, várias características faciais consistentemente associadas à dor foram validadas (de Grauw e van Loon, 2016; Dalla Costa et al., 2014). Dala Costa e colaboradores (2014) utilizam seis características faciais para determinar o grau de dor nos equinos, sendo elas: o posicionamento das orelhas, compressão dos olhos, tensão muscular acima da área dos olhos, tensão dos músculos faciais, retração dos lábios e tensão ao redor das narinas. Cada característica deve ser classificada como ausente (0), levemente aparente (1) ou evidentemente presente (2). Desta forma, o animal poderá receber um escore de dor que pode variar de zero (ausência de dor) a doze (dor evidente).

Este método mostrou-se eficaz para a identificação da dor em equinos após o procedimento de castração (Dalla Costa et al., 2014). Gleerupe et al. (2015) avaliaram a expressão facial de equinos após a indução de dor aguda por dois estímulos nocivos, um torniquete no ante-braço e aplicação tópica de capsaicina. Em seu estudo, uma “face dolorosa” foi observada durante a indução de dor aguda, que incluiu orelhas “baixas” e/ou “assimétricas”, uma aparência angulosa dos olhos, um olhar retraído e/ou tenso, narinas dilatadas, tensão dos lábios e queixo e tensão dos músculos faciais. O estudo de van Dierendonck e van Loon (2016) avaliou a dor em equinos com cólica aguda tanto através da expressão facial quanto das alterações comportamentais. Os autores verificaram que ambas as formas de avaliação mostraram diferenças estatisticamente significativas entre equinos com dor abdominal e os controles saudáveis e entre equinos com cólicas que poderiam ser tratados de forma

conservadora e aqueles que necessitavam de tratamento cirúrgico ou eram sacrificados. Além disso, a avaliação da expressão facial também foi confiável para a determinação da dor em cavalos com dor aguda e pós-operatória oriunda de cirurgias localizadas na cabeça, incluindo dor dentária, dor ocular ou dor decorrente de traumatismo craniano (van Loon e van Dierendonck, 2017). No entanto, nenhum estudo relatou a eficácia do uso da HGS para dor originada especificamente de alterações odontológicas em equinos ou pré e pós-tratamento odontológico.

2. OBJETIVOS

2.1. Objetivo Geral

Estudar as modificações resultantes dos problemas odontológicos e do tratamento odontológico no bem-estar de equinos através de avaliação de dor e da avaliação diagnóstica por acupuntura.

2.2. Objetivos Específicos

- Avaliar a influência dos problemas odontológicos no bem-estar dos equinos.
- Avaliar a sensibilidade e a especificidade da avaliação diagnóstica por acupuntura para afecções odontológicas em equinos.
- Avaliar a influência da saúde da cavidade oral sobre a saúde do sistema musculoesquelético de equinos através do exame por acupuntura.
- Avaliar a aplicabilidade do método de avaliação de dor pela observação da expressão facial em equinos com desordens odontológicas.

3. HIPÓTESES

H0: Alterações da cavidade oral não interferem no bem-estar ou na saúde do sistema musculoesquelético em equinos, não modificando a expressão facial deste ou o exame clínico por acupuntura.

H1: Cavalos que apresentam alterações da cavidade oral apresentam maior escore de dor determinado pela avaliação da expressão facial.

H2: Alterações da cavidade oral influenciam o exame clínico por acupuntura, predispondo os animais a desenvolverem distúrbios no sistema musculoesquelético

CAPÍTULO 2

(Artigo científico submetido para publicação/ Applied Animal Behaviour Science, B1,Q1)

Use of the Horse Grimace Scale to identify and quantify pain due to dental disorders in horses

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ABSTRACT

Dental disorders can cause discomfort and chronic pain, affecting the athletic performance and welfare of the horses. However, dental disorders may not be manifested with recognizable clinical signs and may, therefore, lead to late diagnosis or care. The present study aimed to evaluate the effect of dental disorders on equine welfare by analyzing the equine facial expression using the Horse Grimace Scale (HGS). Six different facial characteristics (ears held stiffly backwards, tension above the eye area, orbital tightening, prominently strained chewing muscle, mouth strained with pronounced chin, and strained nostrils with flattening of the profile) were evaluated in 33 adult horses, both males and females, that were regularly involved in sports or working activities. The animals had not received dental treatment for at least 6 months and were investigated using HGS, minutes before diagnosis and 15 days after the odontological treatment. Initially, pain was scored in direct physical presence (*in loco*) by the investigator. At the same time, the horses' faces were photographed for further analysis by three evaluators trained in the use of horse grimace scale, and unaware of the study and condition of each animal. Additionally, four other equine veterinarians, not trained in the use of HGS, evaluated the same photographs subjectively, scoring 0 for no pain, 1 for mild pain, 2 for moderate pain, and 3 for severe pain, based on their professional experience. A significant agreement in the pain scores ($p < 0.05$) (Kappa test) was observed among all the evaluators who used the HGS (direct physical examination and from horses' photographs), with a strong and significant intra-class correlation coefficient (0.86; $p < 0.05$). However, there was no agreement among the veterinarian evaluators who used the subjective scale. In comparison with that before treatment, the pain score significantly decreased after dental treatment, as evaluated by the trained veterinarians ($p < 0.05$). While two of the subjective evaluators identified an improvement, the other two did not. In conclusion, dental disorders result in discomfort or pain and modify the facial expression of the horses. The HGS is reliable for the identification and quantification of pain associated with dental disorders either by face-to-face evaluation or by evaluation of photographs. However, the same photographs are not suitable for subjective evaluation.

Keyword

Dental disorders; Equine; Horse Grimace Scale; Welfare.

1. Introduction

Accurate recognition and adequate quantification of pain in horses is essential for the correct diagnosis and management of different painful conditions (de Grauw and van Loon, 2016). Diverse models of systematic identification of pain have been described for many conditions in horses using vital parameters (heart rate, respiratory rate) (Price et al., 2003; Pritchett et al., 2003; Rietmann et al., 2004), behavioral evaluation (Rietmann et al., 2004; Bussières et al., 2008), endocrine measurements (Pritchett et al., 2003; Rietmann et al., 2004), and facial expression (Dalla Costa et al., 2014; Gleerup et al., 2015; Wathan et al., 2015; de Grauw and van Loon, 2016; van Dierendonck and van Loon, 2016; van Loon and van Dierendonck, 2017). Pain scales based on facial expression changes have been developed for humans and other species, gaining prominence in equine medicine since it proved to be a sensitive tool that is easy to apply with a little investment of time and training, and high reliability within and between observers (de Grauw and van Loon, 2016).

The Horse Grimace Scale (HGS) was described as a pain coding system evaluating six different characteristics of equine facial expression (ears held stiffly backwards, tension above the eye area, orbital tightening, prominent and strained chewing muscle, mouth strained with pronounced chin, and strained nostrils with flattening of the profile). This method proved to be effective for the identification of pain in horses after the castration procedure (Dalla Costa et al., 2014). Gleerupe et al. (2015) evaluated pain behaviour in horses after the induction of acute pain by two noxious stimuli, a tourniquet on the antebrachium and topical application of capsaicin. In their study they decribed in detail the existence of an equine pain face. The "painful face" was observed during the induction of acute pain, and included "low" and/or "asymmetrical" ears, an angulated appearance of the eyes, a withdrawn and/or tense stare, mediolaterally dilated nostrils, and tension of the lips, chin, and certain facial muscles. The study by van Dierendonck and van Loon (2016) validated the use of pain assessment by facial expression and by behavioral evaluation for identification and quantification of pain in horses with acute colic. The authors found that both forms of evaluation showed statistically significant differences between horses with colic and healthy control horses and between horses with colic

that could be treated conservatively and those that required surgical treatment or were euthanized. In addition, the evaluation of facial expression was also reliable for pain determination in horses with acute and postoperative pain originating from the head, including dental pain, ocular pain, or pain due to trauma to the skull (van Loon and van Dierendonck, 2017). However, no study has reported the efficacy of the use of the facial pain scale for pain originating specifically from dental disorders in horses.

The presence of cheek teeth enamel overgrowth or excessive enamel points (EEP) is a common odontological condition in horses that can lead to pain or discomfort during mastication or work. The EEP is considered to be the most frequent dental disorder in horses with a prevalence of 70–90% in domesticated horses (Dixon et al., 2000). Moreover, it may lead to oral (localized or diffuse) soft tissue ulceration, oral pain leading to quidding and weight loss, and training problems (Dixon et al., 1999; Dixon, 2000; Dixon and Dacre, 2005; Pagliosa et al., 2006; Easley and Dixon, 2010). Removal of the dental overgrowths is recommended (Dixon, 2000). If neglected, they can eventually involve the entire occlusal surface (shear mouth) leading to increased oral pain (Dixon, 2000; Dixon and Dacre, 2005). Another very common dental disorder is the presence of the first premolar (wolf's teeth). The available literature suggests that maxillary wolf teeth are prevalent (13–80% prevalence depending on the breed studied) and mandibular wolf teeth are very rare (Brigham and Duncanson, 2010a, 2010b; Dixon and Dacre, 2005; Filho et al., 2014; Hole, 2016; Ozanam et al., 2005). The wolf's teeth, when present, can occur bilaterally or unilaterally, and is blamed for the oral discomfort and behavioral and training problems due to bite interference. Extraction is often indicated in this condition (Dixon and Dacre, 2005; Ozanam et al., 2005; Easley and Dixon, 2010; Filho et al., 2014). The retention of deciduous teeth (incisor or cheek teeth), disparity in the length of the cheek teeth rows, diastema, periodontitis, as well as changes resulting from focal dental overgrowths (colloquially termed ‘‘hooks’’, ‘‘steps’’, and ‘‘ramps’’) can also result

in oral discomfort, and must be corrected (Dixon and Dacre, 2005; Easley and Dixon, 2010; Cruz Amaya et al., 2012).

Even though it is accepted that these dental disorders may lead to different levels of discomfort and pain, they may not be manifested with apparent clinical signs, and horses could experience dental disorders without receiving an early diagnosis or accurate care (Dixon and Dacre, 2005; Brigham and Duncanson, 2010a, 2010b; Cruz Amaya et al., 2012; Alencar-Araripe et al., 2013). Furthermore, the pre and post-treatment scores for the facial expression in different dental disorders have not been reported. In this context, we considered the hypothesis that dental disorders influence the equine well-being, and this can be evaluated using the pain score determined by facial expression analysis, with reproducible and reliable results being obtained for different observers. Thus, this study aimed to investigate the changes in the horses' facial expressions due to dental disorders, determine whether there is an agreement in pain determination between face-to-face evaluation and evaluation using photographs of the horses' face, and to compare the two types of facial expression evaluators, namely, evaluators with HGS training and those performing subjective evaluation.

2. Material and methods

2.1 Type of study and ethical aspects

The present study is a cohort study and was approved by the Ethics Committee on the Animal Use of the Pontifícia Universidade Católica do Paraná - PUCPR under the registration number 01083/2016.

2.2. Animals and study design

A total of 33 adult horses (16 Quater Horse, 16 Crioullo, and one Arabian) aged between 2.5 and 14.0 years (4.9 ± 3.3), from Guarapuava city, located at center-south region of the Paraná State, Brazil, were used. There were 13 males and 20 females, regularly involved in sports activities, like barrel racing or calf roping (n = 28), working cow horse (n = 4), or use in equine therapy (n = 1). A general physical examination was performed assessing vital

parameters (heart rate and respiratory rate) and owners and trainers responsible for the horses were asked about any complaints related to health problems in their horses. Horses under any clinical care, those who develop any clinical condition throughout the study period, those who received dental treatment in the previous six months, and those who could not be reevaluated for the study purpose, resulting in a sample size of 33 horses investigated in the present study.

The animals were investigated in their habitual environment, and without changes in their routines. For the HGS evaluation on the day of evaluation, the researcher entered the animal's stable, remained quiet for approximately 10 minutes for setting up, and only then did the evaluation in physical presence (P) alone. During the examination, the researcher stood approximately 1 m away from the horse, in silence, not facing them, and behaving and as neutrally as possible. The horses were then monitored for signs of pain using the HGS scale (Dalla Costa et al., 2014) for a period of one minute. This evaluation was performed by the same researcher for all the horses throughout the study. Six facial characteristics were investigated: ears held stiffly backwards, orbital tightening, tension above the eye area, prominently strained chewing muscles, mouth strained with pronounced chin, and strained nostrils with flattening of the profile. Each facial characteristic was scored using a 3-point scale (0= not present, 1= moderately present, 2= obviously present), resulting in the maximum possible HGS score of 12 (Figure 1).

After direct observation, the horses' faces were photographed in the lateral position under natural light, at an approximate distance of 1.0 m from the animal. A 12-megapixel camera of the Motorola Moto Z3 Play mobile phone was used to obtain the photos, resulting in photos with a resolution of 4032 x 3024 pixels. These photographs were used for the analysis performed by other evaluators who were unaware of the condition of each animal.

A general physical examination was performed to evaluate the heart and respiratory rates, followed by a specific examination of the oral cavity by the same presential (P) investigator (veterinarian with experience in equine dentistry).

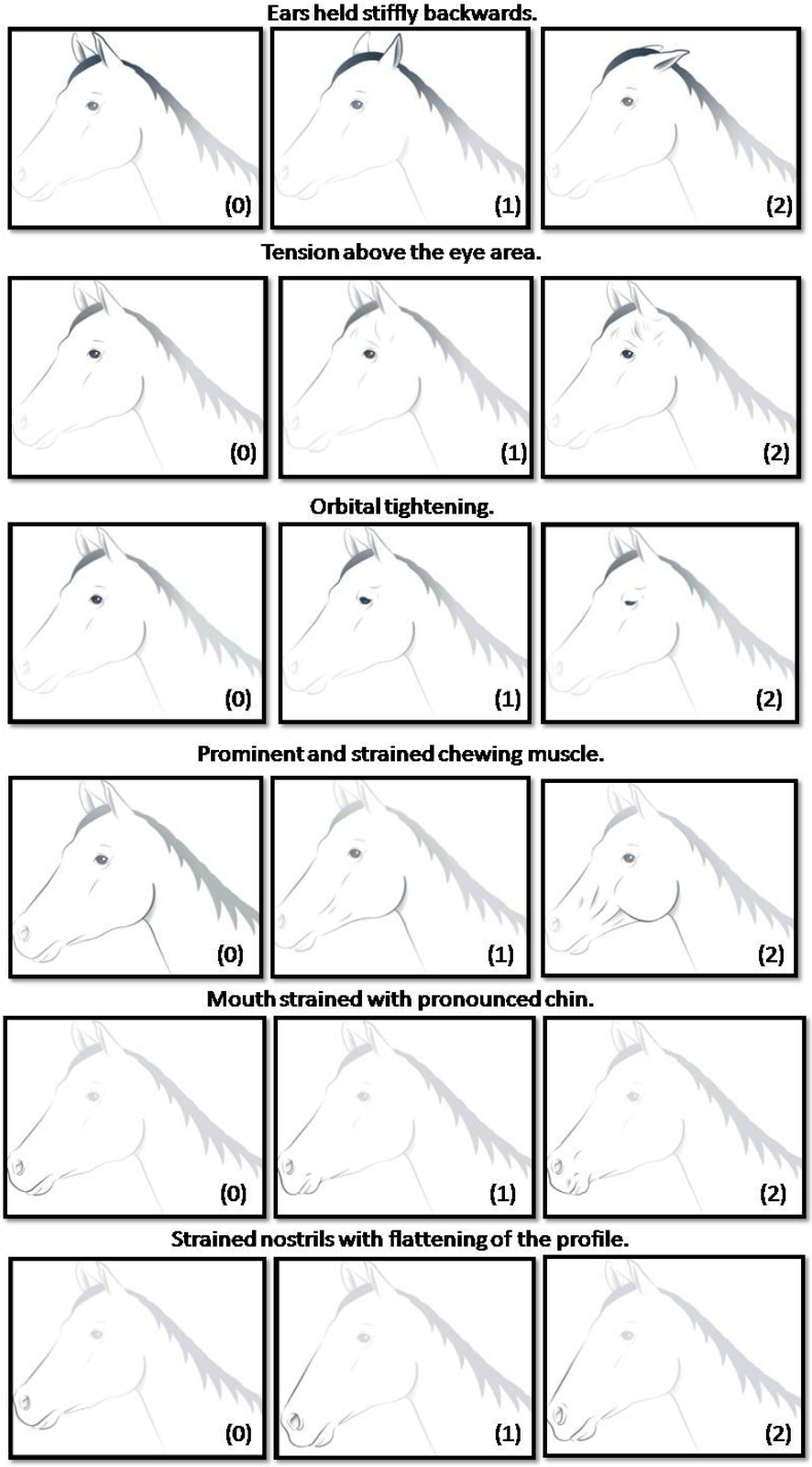

Figure 1. Horse Grimace Pain Scale with pictures for each of the six facial characteristics. Each characteristics is scored according to whether it is not present (score of 0),

moderately present (score of 1) and obliviously present (score of 2) Adapted from the HGS mobile app.

A complete dental examination was performed with detailed observation and external and internal palpation of the oral cavity, including palpation of both hard (teeth and supporting bones) and soft tissues (lips, cheeks, tongue, palate, gingiva, oral mucosa, salivary glands and ducts, and muscles of mastication) (Easley and Dixon, 2010). The condition of the oral cavity was recorded in an odontogram adapted from Easley and Dixon (2010). The animals then received appropriate dental treatment directed towards each alteration detected. For this purpose, the horses were sedated with detomidine (20 to 30 µg/kg IV, Syntec, Santana de Parnaíba, SP, Brazil), and a full mouth speculum was used (full mouth speculum, Ortovet, São Paulo, SP, Brazil). Fifteen days after the treatment, the horses were re-evaluated in the same environment by the same veterinarian to determine the new pain score using the same HGS methodology, and photographs of the facial expressions were taken.

2.3. Facial expression pain assessed from the photographs

The horses' photographs were analyzed by three additional veterinarians trained in the routine use of HGS for at least one year (E1, E2, and E3), but not aware of the study and the condition of the animals. The photographs were randomly arranged, and the evaluators did not have any information regarding the oral clinical findings of the animals or the situation when the images were obtained (pre or post-treatment).

In addition, four other equine veterinarians (V1, V2, V3, and V4) with professional experience of at least 10 years, but not trained for HGS use, subjectively evaluated the same images and scored the animals: 0 for no pain, 1 for mild pain, 2 for moderate pain, and 3 for severe pain, analyzing the horses' facial expression from the photographs, taking into account their own experience in equine medicine.

The total time in minutes taken by the evaluators to evaluate the 66 photos (33 pre and 33 post treatment photos) was registered, both by HGS trained and veterinarian evaluators, in order to find the average evaluation time for each photo.

2.4. Statistical analysis

To compare the agreement between the evaluators, a *Kappa* test was used, and inter-observer reliability was assessed using intra-class correlation coefficients (ICC). To obtain the differences in pain score between before and after dental treatment, a (Wilcoxon test) was used. These analyses were performed using the software IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp, New York, USA). For all the analyses, $p < 0.05$ was considered significant.

3. Results

3.1 Animals

Even though all the 33 investigated horses were active, all of them presented at least one type of dental disorder during the initial evaluation, with a maximum of five different disorders in the same horse. Ninety-three percent of them (n = 31) presented with EEP, while 41.9% (n = 13) of the horses with EEP also revealed evidence of oral mucosal ulceration. The second most frequent disorder was the presence of the first premolar, which was observed in 42.4% of the investigated animals (n = 14) (Table 1).

Table 1- Animals(An) described according to Breed (B) (Q: quarter horse; C: crioulo; A: Arabian horse), age (year), oral disorders (OD) (EEP: excessive enamel tips; UM: ulceration of the buccal mucosa; WT: wolf´s teeth; RD: retention of deciduous teeth; H: hook; R: Ramp; T: tartar; P: periodontitis), and median pain score (Horse Grimace Scale, varying the score possibilities between 0 to 12) of HGS trained veterinarians [(performed in the direct physical presence (P) and from horses' photographs (E1, E2, E3,)], before and 15 days after the treatment of the disorders.

An	B	Age	OD	Pre-treatment HGS score	Post-treatment HGS score
1	Q	14.0	R/ H/ T	2.0 (1-7)	1.0 (0-3)
2	Q	10.0	EEP/ H/ R/ T	6.5 (3-10)	2.0 (2-8)
3	C	7.0	EEP/ WT/ UM	4.0 (4-4)	2.0 (1-5)
4	Q	7.0	EEP	4.0 (3-4)	0.0 (0-4)
5	Q	3.0	EEP/ WT/ UM	5.5 (5-6)	2.5 (0-3)
6	Q	3.0	EEP/ UM	2.0 (0-5)	0.0 (0-3)
7	C	4.0	EEP/ WT/ UM	7.5 (7-8)	3.0 (1-3)
8	C	5.0	EEP/ UM/ RD/ P	6.5 (2-10)	0.5 (0-2)
9	Q	8.0	EEP	4.5 (1-7)	4.0 (2-6)
10	Q	5.0	EEP/ UM	2.0 (2-5)	1.0 (1-3)
11	Q	3.0	EEP/ UM	5.0 (3-6)	1.5 (1-3)
12	Q	4.5	EEP/ UM/ WT/ RD/ P	6.5 (6-8)	2.5 (1-4)
13	Q	5.0	EEP	5.0 (1-6)	0.5 (0-2)
14	Q	2.5	EEP/ UM/ WT/ RD	2.0 (0-3)	2.0 (0-4)
15	Q	2.5	WT/ RD	1.5 (0-2)	0.0 (0-1)
16	Q	5.0	EEP/ WT	2.5 (1-3)	0.5 (0-3)
17	Q	3.5	EEP/ RD	3.5 (2-5)	2.5 (1-5)
18	C	3.0	EEP/ UM/ WT	1.5 (1-7)	1.0 (0-1)
19	C	6.0	EEP/ UM	8.0 (5-10)	3.5 (2-5)
20	Q	5.0	EEP	5.5 (2-5)	3.0 (1-7)
21	Q	6.0	EEP/ UM	4.0 (3-6)	0.0 (0-1)
22	C	2.5	EEP/ WT/ RD	2.5 (1-4)	0.5 (0-3)
23	C	3.0	EEP/ UM	4.5 (1-7)	1.0 (0-1)
24	C	2.5	WT/ RD	3.0 (1-4)	2.0 (0-3)
25	A	16.0	EEP/ H/ R/ T	5.5 (0-9)	1.5 (0-4)
26	C	4.0	EEP	4.0 (4-4)	2.0 (0-3)
27	C	3.0	EEP/ WT/ RD	4.0 (1-5)	2.5 (2-4)
28	C	2.0	EEP/ WT/ H	2.5 (2-5)	1.5 (1-4)
29	C	2.0	EEP/ WT	2.0 (0-5)	1.5 (0-3)
30	C	2.0	EEP	2.0 (2-2)	0.0 (0-1)
31	C	2.0	EEP/ WT	1.5 (0-3)	0.0 (0-0)
32	C	7.0	EEP/ UM	2.0 (2-3)	0.5 (0-2)
33	C	2.5	EEP/ WT/ RD	5.0 (4-7)	1.0 (0-1)

*Median HSG pre and post-treatment scores were calculated for each animal for the observations by P, E1, E2, and E3.

3.2. Pain evaluation

The *in loco* evaluation of pain using the HGS took 11 minutes on average, considering the time for setting up (10 minutes) and the time for observation itself (1 minute). For the evaluation of the photographs by HGS, the evaluators on an average took 1.7 ±0.03 minutes per photograph, and the subjective evaluation took 0.7 ±0.08 minutes on an average.

Positive agreement was found among all the evaluators who used the HGS, either *in loco* or using the photographic evaluation ($p < 0.043$). Also, there was a strong and significant inter-

observer reliability, with the intraclass correlation coefficient (ICC) of 0.86. The individual features evaluated using the HGS also showed high ICC values, as follows: 0.78 for ears held stiffly backwards, 0.75 for orbital tightening, 0.72 for tension above the eye area, 0.75 for prominently strained chewing muscles, 0.75 for mouth strained with pronounced chin, and 0.75 for strained nostrils with flattening of the profile.

However, it is worth highlighting that the evaluation done *in loco* showed pain scores that were always lower than the scores attributed by the photographic evaluators.

With regard to the subjective pain assessment, there was no complete agreement among themselves nor with the evaluators who used HGS (P and E) ($p > 0.05$).

Table 2- Median pain score [minimum (min) and maximum (max) values] of 33 horses, evaluated before and after dental treatment, using the Horse Grimace Scale (HGS), performed in direct physical presence (P) and from the horses' photographs by veterinarians trained in the routine use of HGS (E1, E2, E3) and by equine veterinarians with professional experience of at least 10 years, but not trained for HGS (V1, V2, V3 and V4).

Evaluators	Pre- treatment HGS score	Post- treatment HGS score	p-value
P	2 (0-7)	0 (0-2)	<0.0001
E 1	4 (1-10)	1 (0-6)	<0.0001
E 2	4 (0-8)	2 (0-7)	<0.0001
E 3	4 (0-10)	3 (1-8)	<0.0001
V1	1 (0-3)	1 (0-3)	0.0400
V2	1 (0-3)	1 (0-3)	0.5850
V3	2 (0-3)	2 (0-3)	0.6000
V4	1 (0-3)	1 (0-2)	0.0010

The p values relate to pre and post treatment comparison (Wilcoxon test) for each evaluator.

The pain score observed on evaluation of the animals using the HGS was significantly different before and after dental treatment for all evaluators (P and E) ($p < 0.05$) (Figure 2 and Table 2), with the animals presenting a lower post-treatment pain score than that presented at the initial evaluation (Figure 3). In the subjective evaluation, two evaluators identified reduction of pain post-treatment, and the other two did not.

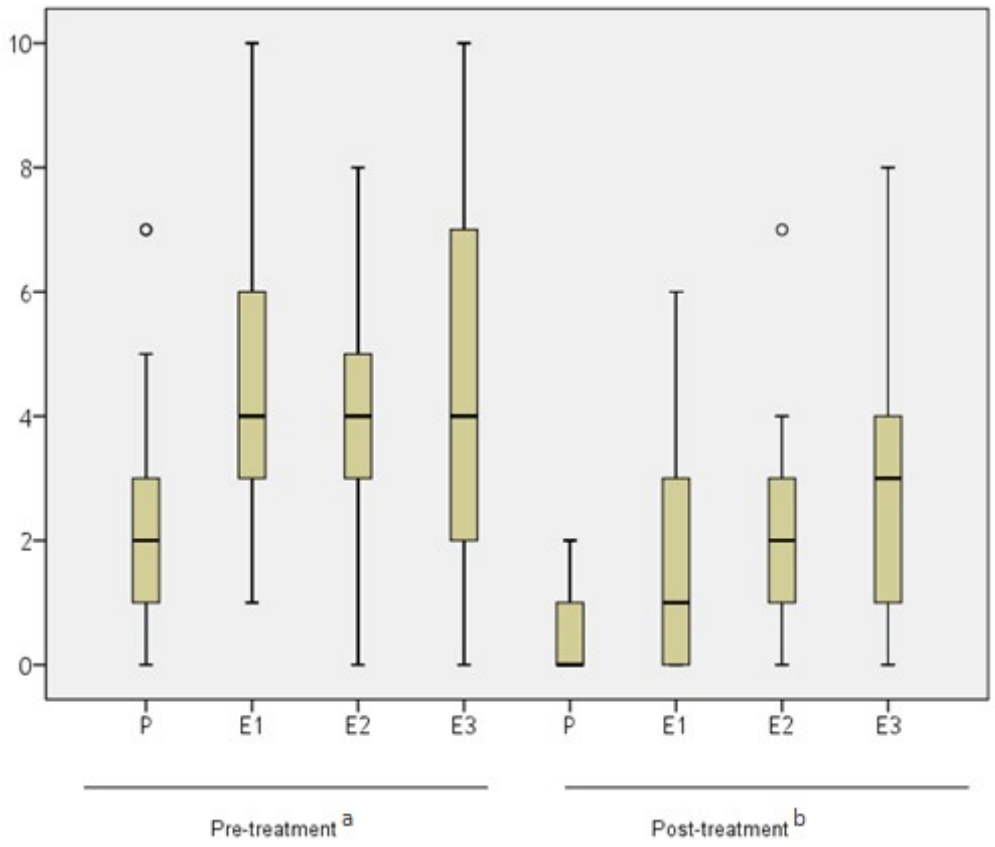

Figure 2: Pre and post-dental treatment median pain scores for 33 horses assigned by each evaluator according to the Horse Grimace Scale (HGS) [(in direct physical presence (P) and from photographs (E1, E2, E3)]. Different letters between treatments represent statistical difference ($p<0.0001$).

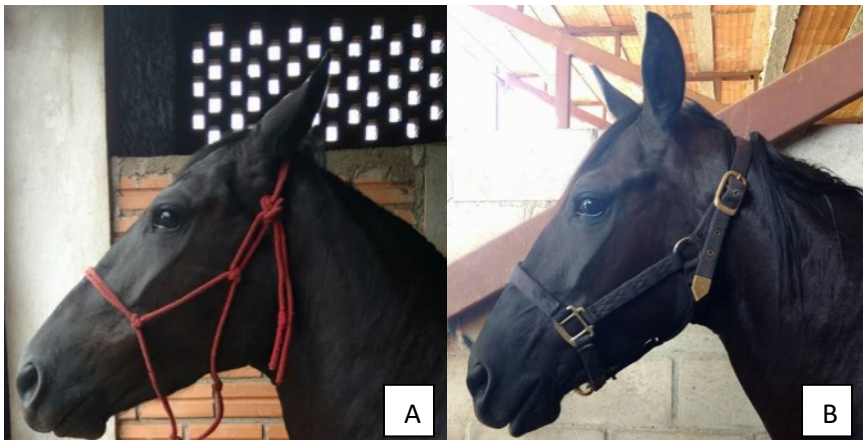

Figure 3: Photograph of the same horse before dental treatment (A) and 15 days after treatment. In image A, the animal had ears moderately held stiffly backwards, slightly stretched

mouth with pronounced chin, and moderately stretched nostrils. In image B the animal has a relaxed facial expression.

4. Discussion

In the present study, the objective of evaluating the feasibility of the HGS to identify pain due to dental disorders was accomplished, thereby demonstrating that HGS is a reliable and reproducible tool for this evaluation, as well as to follow-up on the improvement from treatment. Moreover, using the HGS shows that dental disorders result in different levels of pain or discomfort for horses, and this has not been clearly demonstrated before.

All the investigated animals were involved in some kind of sport or work activity, which demonstrates that even those horses affected by dental disorders can continue to perform athletic or work activities, and discomfort is not always perceived by owners or riders. In this way, the necessity for more detailed observation of the animals during clinical examination is demonstrated. The technique of HGS enables a better understanding of the more obvious modifications of facial expression caused by dental disorders, and probably could be applied for client education purposes for the identification of early signs of pain, in order to alert the veterinarian for early diagnosis and treatment. This still must be investigated for validation.

The HGS has already been validated as an important and sensitive tool for clinical use in pain identification, but had not been described for use in the case of dental disorders so far. In the present study, the use of HGS, during examination of the horses and after horse-examiner adaptation, demonstrated pain scores from 1.5 to 8 out of 12, demonstrating that different levels of pain or discomfort can be identified by this method. Possibly, horses can adapt to different problems in the oral cavity, thereby not clearly demonstrating signs of pain, however the use of HGS was able to identify pain or discomfort as well as the improvement after treatment.

This study was conducted in a horse population in real life; hence, we encountered certain difficulties. To overcome the bias in the present study due to the evaluator performing the evaluation in direct physical presence, treatment, and reevaluation, we recruited blinded HGS-trained evaluators and asked them to evaluate the photographs. Even though images

cannot accurately reflect the potentially changeable nature of facial expressions, this has been used in previous similar studies. Costa et al. (2016) reported a positive agreement in analyzing the pain score in horses with laminitis using videos and images obtained from the videos, showing that it is possible to analyze the level of pain of the patients using facial expressions recorded in photographs. Special care is taken to record characteristics, like ears held stiffly backwards and orbital tightening, during the acquisition of images. In the present study, there was a positive agreement between the face–to-face evaluation and photographic evaluation. We found that when HGS was used, an appropriate photograph can be reliably used for the evaluation of facial expressions for pain determination.

The pain score attributed to the animals by the physically present evaluator was always lower than that attributed by the evaluators assessing photographs. Dalla Costa et al. (2014) and Costa et al. (2016) raised this point by citing that orbit compression can be considered erroneous if the animal as it 'blinks', at the time of image acquisition. The same can occur during the interpretation of positioning of the ears, as a feature of extreme importance, since all species evaluated held their ears backwards when in pain, as observed by Guesgen et al. (2016). However, if this positioning changes in the same moment as the image is captured, the photographs may be erroneously interpreted. For this reason, Gleerup et al. (2015) cited the observation of the pattern of movement of the ears as a parameter that must be analyzed face-to-face, if possible, or by video analysis, but not by photographic analysis. In this study, special attention was accorded to the photographs to ensure that they reflected exactly what was observed on face-to-face analysis; hence, photographs with animal blinking or those in which the animals were moving were avoided. Additionally, the presence of the horse halter sometimes made it difficult to evaluate other characteristics, such as prominently strained chewing muscles. Then, it might be interesting to further investigate which of the evaluated characteristics could weight more on the facial expression evaluation. Moreover, involvement with pre and post treatment live evaluation, as well as with buccal evaluation and treatment

could have influenced for lower HGS scores; this is a limitation of the present study, however, the correlation with the other HGS evaluators confirmed its veracity.

In the methodology described by Dalla Costa et al. (2014), photographs of the horses were obtained by capturing images of the animals in twenty-minute videos that were produced by cameras previously positioned in strategic points of the stall, so that the presence of the observer did not influence recording of the pain score. In the present study, the animals were observed face-to-face and the P himself recorded the photograph, taking care not to influence the horses' movements. In this case, the observer's time for setting up is of fundamental importance since the horse could change its facial expression in the presence of something or someone who it considers a threat (Costa et al., 2016). However, Gleerup et al., (2015) realized that the horses did not seem to suppress the changes in facial expressions in the presence of the observer, although they were less pronounced whenever the horses tried to interact with the observer. For this reason, it is very important that the observer must not face the horse, and he must behave as neutrally as possible.

However, subjective evaluation of the animals by veterinarians was not completely effective, revealing that despite the professional experience of the veterinarians in working with equines and their ability to identify an animal in distress, a subjective classification might not be totally reliable for the quantification of pain. This highlights the importance of using a more precise method, such as the HGS-based assessment.

Finally, the significant difference found in the horses evaluated using the HGS pre and post-treatment indicated that, even in the absence of evident clinical signs, the odontological alterations caused some degree of discomfort in the studied horses since they began to present a more relaxed facial expression after the correction of the condition. It is noteworthy that, although the pain score decreased after dental treatment, it was not zero in many animals. Dalla Costa et al. (2014) and Gleerup et al. (2015) reported that even in a non-painful state, horses may exhibit one or two facial features occasionally altered at a low level. In addition, reevaluation of the oral cavity of the horses was not performed 15 days after treatment,

so that some animals may not have complete healing of their lesions at this time, which could still be reflected in the pain score.

In general, the present study adds to the importance of routine clinical evaluation of the buccal cavity and dental care of horses, even in the absence of classic signs of discomfort. The reduction in the pain score after treatment may reflect in the well-being of these animals.

5. Conclusions

It was concluded that different dental disorders result in pain or discomfort in horses, and that this can be reliably evaluated and quantified using the HGS in direct physical presence or by using photographic evaluation. This can prove to be clinically important in the routine evaluation of horses, and the general equine practitioner can add the HGS information to the suspicious cases of painful oral conditions. It is noteworthy that well-determined parameters (demonstrating each facial structure to be observed) were relevant for the accuracy of pain assessment.

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Conflicts of interests

The authors declare that they have no conflicts of interests.

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References

Alencar-Araripe, M.G. de, Costa, A.C.H., Costa, B.O., Castelo-Branco, D. de S.C.M., Nunes-Pinheiro, D.C.S., 2013. Oral health: comfort and ponderal gain in horses after dental

correction. Rev. Bras. Hig. e Sanidade Anim. 7, 288–300. https://doi.org/10.5935/1981-2965.20130025

Brigham, E.J., Duncanson, G.R., 2010a. An equine postmortem dental study: 50 cases. Equine Vet. Educ. 12, 59–62. https://doi.org/10.1111/j.2042-3292.2000.tb01765.x

Brigham, E.J., Duncanson, G.R., 2010b. Case study of 100 horses presented to an equine dental technician in the UK. Equine Vet. Educ. 12, 63–67. https://doi.org/10.1111/j.2042-3292.2000.tb01767.x

Bussières, G., Jacques, C., Lainay, O., Beauchamp, G., Leblond, A., Cadoré, J.L., Desmaizières, L.M., Cuvelliez, S.G., Troncy, E., 2008. Development of a composite orthopaedic pain scale in horses. Res. Vet. Sci. 85, 294–306. https://doi.org/10.1016/j.rvsc.2007.10.011

Costa, E.D., Stucke, D., Dai, F., Minero, M., Leach, M.C., Lebelt, D., 2016. Using the horse grimace scale (HGS) to assess pain associated with acute laminitis in horses (Equus caballus). Animals 6, 47. https://doi.org/10.3390/ani6080047

Cruz Amaya, J.M., Sánchez V., J.A., Vera Hernández, L.G., 2012. Caracterización y prevalencia de las enfermedades orales en el caballo criollo, departamento de Caldas, Colombia. Rev. Med. Vet. (Bogota). 39. https://doi.org/10.19052/mv.73

Dalla Costa, E., Minero, M., Lebelt, D., Stucke, D., Canali, E., Leach, M.C., 2014. Development of the Horse Grimace Scale (HGS) as a pain assessment tool in horses undergoing routine castration. PLoS One 9, 1–10. https://doi.org/10.1371/journal.pone.0092281

de Grauw, J.C., van Loon, J.P.A.M., 2016. Systematic pain assessment in horses. Vet. J. 209, 14–22. https://doi.org/10.1016/j.tvjl.2015.07.030

Dixon, P., 2000. Removal of equine dental overgrowths. Equine Vet. Educ. 12, 68–81. https://doi.org/10.2746/095777308X380346

Dixon, P.M., Dacre, I., 2005. A review of equine dental disorders. Vet. J. 169, 165–187. https://doi.org/10.1016/j.tvjl.2004.03.022

Dixon, P.M., Treimane, W.H., Pickles, K., Kuhns, L., Hawe, C., McCann, J., McGorum, B., Railton, D.I., Brammer, S., 1999. Equine dental disease Part 1: A long-term study of 400

cases: disorders of incisor, canine and first premolar teeth. Equine Vet. J. 31, 369–377. https://doi.org/10.1111/j.2042-3306.1999.tb03835.x

Dixon, P.M., Tremaine, W.H., Pickles, K., Kuhns, L., Hawe, C., Mccann, J., Mcgorum, B.C., Railton, D.I., Brammer, S., 2000. Equine dental disease Part 3: A long-term study of 400 cases: Disorders of wear, traumatic damage and idiopathic fractures, tumours and miscellaneous disorders of the cheek teeth. Equine Vet. J. 32, 9–18. https://doi.org/10.2746/042516400777612099

Easley, J., Dixon, P.M., 2010. Equine Dentistry, Third Ed. Edited by Elsevier Saunders, St. Louis, MO, USA.

Filho, A.F., Borelli, V., Gilberto, J., Pereira, L., Fernandes, M., Castro, D.S., 2014. Prevalência do primeiro pré-molar (dente-de-lobo) em equinos puro sangue inglês. J Heal. Sci Inst. 32, 198–202.

Gleerup, K.B., Forkman, B., Lindegaard, C., Andersen, P.H., 2015. An equine pain face. Vet. Anaesth. Analg. 42, 103–114. https://doi.org/10.1111/vaa.12212

Guesgen, M.J., Beausoleil, N.J., Minot, E.O., Stewart, M., Stafford, K.J., Morel, P.C.H., 2016. Lambs show changes in ear posture when experiencing pain. Anim. Welf. 25, 171–177. https://doi.org/10.7120/09627286.25.2.171

Hole, S.L., 2016. Wolf teeth and their extraction. Equine Vet. Educ. 28, 344–351. https://doi.org/10.1111/eve.12360

Ozanam, F., Silva, C.E., Severino, R.S., 2005. Erupção do dente Lupino em cavalos PSI e campolina. Biosci. J. 30, 420–423.

Pagliosa, G.M., Alves, G.E.S., Faleiros, R.R., Saliba, E.O.S., Sampaio, I.B.M., Gomes, T.L.S., Gobesso, A.A.O., Fantini, P., 2006. Influence of excessive enamel points on nutrients digestibility in diets of horses. Arq. Bras. Med. Veterinária e Zootec. 58, 94–98. https://doi.org/10.1590/S0102-09352006000100014

Price, J., Catriona, S., Welsh, E.M., Waran, N.K., 2003. Preliminary evaluation of a behaviour-based system for assessment of post-operative pain in horses following arthroscopic surgery. Vet. Anaesth. Analg. 30, 124–137. https://doi.org/10.1046/j.1467-

2995.2003.00139.x

Pritchett, L.C., Ulibarri, C., Roberts, M.C., Schneider, R.K., Sellon, D.C., 2003. Identification of potential physiological and behavioral indicators of postoperative pain in horses after exploratory celiotomy for colic. Appl. Anim. Behav. Sci. 80, 31–43. https://doi.org/10.1016/S0168-1591(02)00205-8

Rietmann, T.R., Stauffacher, M., Bernasconi, P., Auer, J.A., Weishaupt, M.A., 2004. The association between heart rate, heart rate variability, endocrine and behavioural pain measures in horses suffering from laminitis. J. Vet. Med. Ser. A Physiol. Pathol. Clin. Med. 51, 218–225. https://doi.org/10.1111/j.1439-0442.2004.00627.x

van Loon, J.P.A.M., Van Dierendonck, M.C., 2017. Monitoring equine head-related pain with the Equine Utrecht University scale for facial assessment of pain (EQUUS-FAP). Vet. J. 220, 88–90. https://doi.org/10.1016/j.tvjl.2017.01.006

VanDierendonck, M.C., van Loon, J.P.A.M., 2016. Monitoring acute equine visceral pain with the Equine Utrecht University Scale for Composite Pain Assessment (EQUUS-COMPASS) and the Equine Utrecht University Scale for Facial Assessment of Pain (EQUUS-FAP): A validation study. Vet. J. 216, 175–177. https://doi.org/10.1016/j.tvjl.2016.08.004

Wathan, J., Burrows, A.M., Waller, B.M., McComb, K., 2015. EquiFACS: The equine facial action coding system. PLoS One 10, 1–35. https://doi.org/10.1371/journal.pone.0131738

CAPÍTULO 3

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Influence of oral health on equine facial expression and on the acupuncture examination in equine.

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ABSTRACT

Dental disorders are frequent in equines and can cause discomfort and chronic pain, affecting horses' welfare and athletic performance. However, these disorders may not be manifested with recognizable clinical signs, leading to late diagnosis and care. This fact predisposes these horses to develop other disorders, such as gastrointestinal diseases or painful conditions in the rest of the body. The present study aimed to evaluate the influence of buccal health on the facial expression and the acupuncture examination. Were evaluated 87 adult horses, both males and females, that were regularly involved in sports or working activities. Initially, pain was scored in direct physical presence (*in loco*) by the investigator. At the same time, the horses' faces were photographed for further analysis. The photographic evaluation was performed by a evaluator who unaware of the study and condition of each animal. The acupuncture test was performed always by the same evaluator, who investigated acupoints' sensitivity to palpation or pressure. The animals were evaluated before (T0) and 15 days (T1) after odontologic examination and treatment. All of the 87 horses studied presented some dental disorders. A significant decrease was observed in both pain score 3 (0-10) vs. 1 (0- 6) and number of reactive acupuncture points (11.2 ± 5.6 vs. 4 ± 2.9 points) in T1 in comparison with T0, together with the reduction in complaints by the owners regarding the masticatory process and quality of work. In addition, it was observed that some reactive acupuncture points in animals with dental disorders significantly reduced after treatment. With emphasis on Dental point (44 vs. 4), Stomach (ST)-7 (31 vs. 3), Triple Heater (TH)-17 (27 vs. 4) and TH-16 (22 vs. 4), showing that the acupuncture exam can help in the diagnosis of dental alterations and, mainly, help in determining the success of dental treatment. It was possible to establish the relationship between the presence of ulcers in the buccal mucosa and the pain score, number of reactive acupuncture points and the quality of the work. The presence of hooks / ramps had an influence on the masticatory quality of the animals, and the presence of the wolf teeth did not influence any of the studied parameters. Finally, it was observed that in T0, although they did not present lameness, a significant number of horses (n = 49/87) presented

sensitive acupuncture points that indicated painful syndromes in the thoracolumbar spine or hind limbs, which reduced in T1 (n = 16/87).). In conclusion, the dental treatment resulted in reduction of pain, changing the facial expression of the horses and reduced the number of acupuncture points sensitive to palpation or pressure. The integration of acupuncture point palpation/pressure examination and pain assessment using HGS in equine routine examination may be useful in general and specific dental clinical evaluation, and especially for follow-up after dental treatment, as well as assisting to demonstrate the influence of changes in the oral cavity on the musculoskeletal system.

Keywords: Acupuncture; Dental disorders; Equine; Horse facial expression; Welfare.

1. Introduction

Dental disorders are the third most common cause of equine diseases in North America and despite this affections have great importance in the clinical practice of horses, there is a great demand for more information in the equine dentistry field (Galloway and Easley, 2008; Dixon, 2017). Dental conditions are usually associated with changes in the masticatory pattern, dysphagia, weight loss and gastrointestinal disorders (Ashley et al., 2005; Ardila and Montoya, 2009; Amaya et al., 2012; Dixon, 2017).The presence of excessive enamel points (EEP) is the most frequent dental disorder in horses with a prevalence of 70–90% in domesticated horses (Dixon et al., 2000). The EEP is normally related to pain or discomfort during mastication or work. Moreover, it may lead to ulceration of buccal mucosa, oral pain leading to weight loss, and training problems (Dixon et al., 1999; Dixon, 2000; Pagliosa et al., 2006; Easley et al., 2011). Another very common dental disorder is the presence of the first premolar (wolf teeth). Which can be present in 13- to 80% of the horses, depending on the race studied (Brigham and Duncanson, 2000b; Dixon and Dacre, 2005; Ozanam et al., 2005; Filho et al., 2014). The Wolf theeth are usually blamed for oral discomfort and behavioral and training problems due to interference with the bit. The extraction of this tooth is often indicated (Dixon and Dacre, 2005, Ozanam et al., 2005, Easley et al., 2011, Filho et al., 2014). Other dental disorders such as retention of deciduous teeth, diastema, periodontitis, ‘‘hooks’’, ‘‘steps’’, and ‘‘ramps’’ can also result in oral discomfort, and must be corrected (Dixon and Dacre, 2005; Easley et al., 2011; Amaya et al., 2012).

Even though it is accepted that these dental disorders may lead to different levels of discomfort and pain, they may not be manifested with apparent clinical signs, and horses could experience dental disorders without receiving an early diagnosis or accurate care (Brigham and Duncanson, 2000a, 2000b; Dixon and Dacre, 2005; Amaya et al., 2012; Alencar-Araripe et al., 2013).The lack of an early diagnosis can lead the animal to develop other disorders, such as gastrointestinal diseases or painful conditions in the rest of the body resulting in performance decrease or lamenesses (Pence, 2002; Stashak and Hill 2006; Du Toit et al., 2008;Galloway and Easley, 2008; May, 2008; Cain, 2015).

In this context, acupuncture diagnosis appears as an option to aid in the early diagnosis of different pathological conditions in horses, besides serving as a parameter for general evaluation of animal welfare (Fleming, 2006; Shmalberg and Xie, 2009; Michelotto et al., 2014; Pellegrini et al.,2018). Regardless of the fact that the use of acupuncture in veterinary medicine has been documented for thousands of years, Western scientific research on its efficacy is much more recent (Ramey, 2005; Scognamillo-SzabóI and Bechara,2010; Le Jeune and Jones, 2014) including on diagnosis of horses' musculoskeletal conditions (Michelotto et al., 2014).

The use of acupoint pressure is a diagnostic method in the equine physical examination, that has been proposed as a practical, efficient method capable of diagnosing different types of imbalances earlier (Schoen, 2000; Shmalberg and Xie, 2009; Alfaro, 2014; Cain, 2015; Pellegrini et al.,2018).This examination is based on observation of the animals' response to palpation of the meridian paths and acupuncture point pressure (McCormick, 1997; McCormick, 1998;Schoen, 2000; Fleming, 2006; Shmalberg et al. Xie, 2009). The combination of a set of acupoints sensitive to this pressure allows to determine the diagnosis of some painful conditions in horses or syndromes (Angeli et al., 2007; Michelotto et al., 2007; Angeli et al., 2008; Xie and Preast, 2011; Alfaro, 2014; Cain, 2015).

Some acupuncture points are cited as diagnostic points for TMJ or head and mouth pain (Dental point, ST7, TH17, TH16, GB1, GB20, ID10) (Fleming, 2006;Xie and Preast, 2011; Cain, 2015), however, few studies are specifically aimed at the diagnosis of odontological affections of horses.

Still thinking about early and correct diagnosis and management of different painful conditions an accurate recognition and adequate quantification of pain is also essential. Horses tend to demonstrate pain more discreetly as a life-saving attitude (Ashley et al., 2005).

In this context pain scales based on facial expression changes is proved to be a sensitive tool to determine painful situations in equine medicine (Gleerup et al.,2015; de Grauw and van Loon, 2016).The Horse Grimace Scale (HGS) is one of these techniques and describe a pain coding system evaluating six different characteristics of equine facial expression (ears held stiffly backwards, tension above the eye area, orbital tightening, prominent and strained

chewing muscle, mouth strained with pronounced chin, and strained nostrils with flattening of the profile) (Dalla Costa et al., 2014).

All these issues are especially important when talking about dental disorders as not all horses with dental diseases show signs of discomfort clearly, and many animals may suffer from dental disorders without showing obvious signs of pain (Brigham e Duncanson,2000a; Ashley et al., 2005; Amaya et al., 2012).

The present study had as objective to evaluate the benefits of dental treatment in the welfare by acupuncture point sensitivity assessment and pain score evaluation utilizing the HGS of the healthy horses, before dental treatment and 15 days after the same. It was also aimed at to evaluate the reliability of acupuncture as a diagnostic method for dental disorders and its possible complications, and to determine the possible benefits of acupuncture associated with the traditional Western examination in the earlier diagnosis of dental disorders.

2. Material and methods

2.1 Type of study and ethical aspects

The present study is a cohort study to investigate the response of the dental treatment on horses clinically examined using the HGS and pressure of acupoints. The study was approved by the local Ethics Committee on the Animal Use.

2.2 Animals and study design

A total of 87 adult horses were embraced (33 Thoroughbred, 26 Criollo horse, 12 Quarter Horse, 9 Brazilian Mangalarga Marchador, six Cross breed and one Arabian), 49 females and 38 males, aged between 1.8 and 24.0 years (4.48 ± 3.75), from the cities of Guarapuava and Curitiba, Paraná State, south region of Brazil. The horses were considered healthy based on physical examination and due to the regular involvement in sports activities, like racehorses (n = 33); barrel racing or calf roping (n = 41), working cow horse (n = 3), pace (n = 9) or use in equine therapy (n = 1). Horses under any clinical care, those who developed any clinical condition throughout the study period, those who received dental treatment in the

previous six months, and those who could not be reevaluated for the study purpose were excluded.

The evaluations were performed at the horse's place of residence, and preferentially without changes in their routines.

In the first evaluation (T0), the horses were examined with five different objectives; firstly they were evaluated with the intention of determining their pain scores. After then, a general physical examination was performed to evaluate the heart and respiratory rates. Then the acupuncture points reactivity was evaluated, and to determinate all possible dental disorders present in each animal, the oral cavity of the animals was evaluated. Finally, anamnesis was carried out with regard to the perception of the person responsible for the horses about the quality of chewing and working. Fifteen days after the treatment (T1), horses were re-evaluated in the same environment by the same veterinarian to determine the new pain score using the same HGS methodology, a new examination for diagnostic acupuncture using acupoints palpation was also performed and a new anamnesis.

2.3 Pain Score evaluation

For the evaluation of the pain score, the researcher (the same researcher for all the horses throughout the study) entered the animal's stable, remained quiet for approximately 10 minutes for setting up, and after this time, the horses were then monitored for a period of one minute for signs of pain using the HGS scale (Dalla Costa et al., 2014). Six facial characteristics were investigated: ears held stiffly backwards, orbital tightening, tension above the eye area, prominently strained chewing muscles, mouth strained with pronounced chin, and strained nostrils with flattening of the profile. Each facial characteristic was scored using a 0 to 2 points scale, resulting in the maximum possible HGS score of 12 (Figure 1).

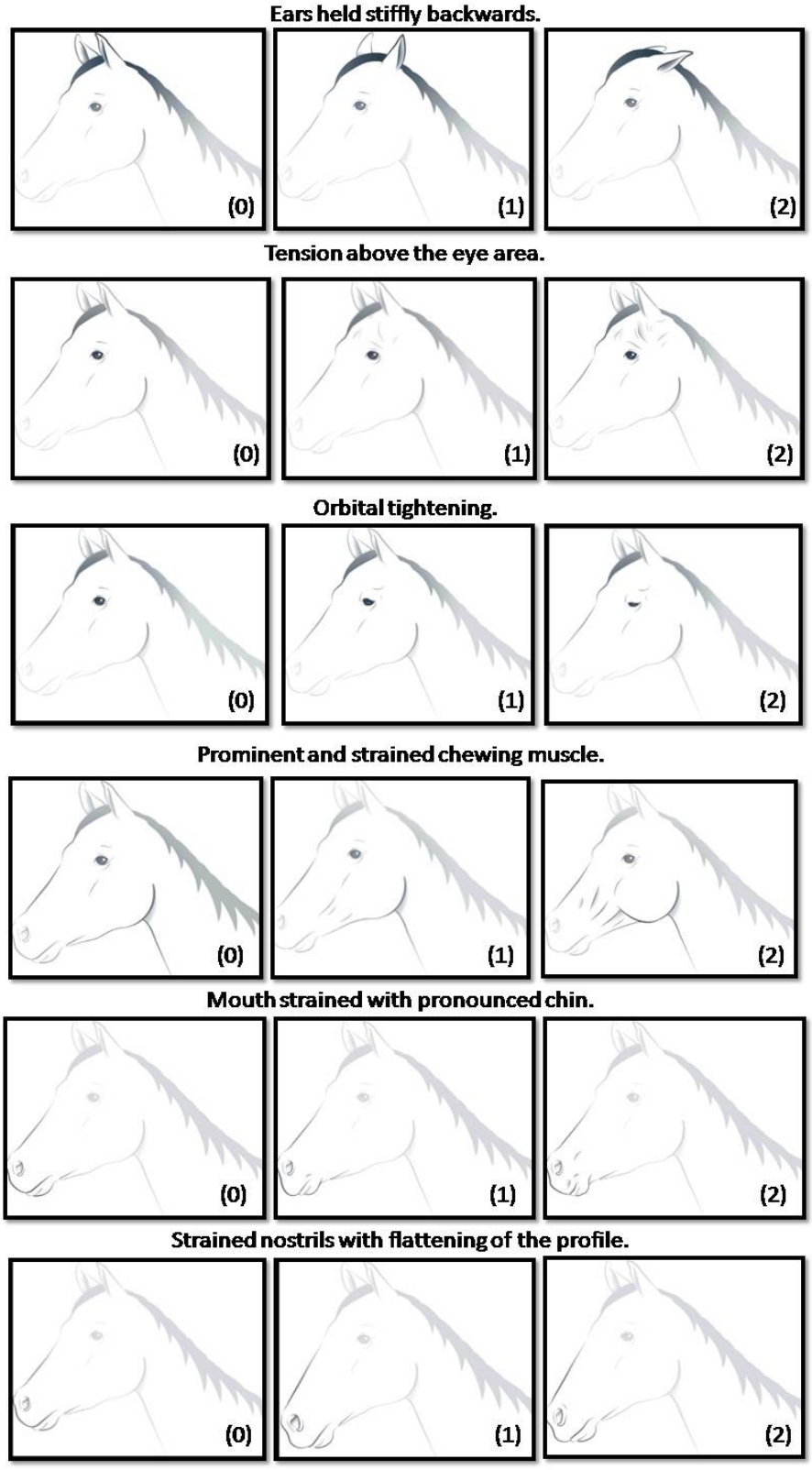

Figure 1. Figure 1. Horse Grimace Pain Scale with pictures for each of the six facial characteristics. Each characteristics is scored according to whether it is not present (score of 0),

moderately present (score of 1) and obliviously present (score of 2) Adapted from the HGS mobile app.

During the examination, the researcher stood approximately 1 meter away from the horse in silence, not facing them, and behaving and as neutrally as possible.

After direct observation, the horses' faces were photographed in the lateral position under natural light, at an approximate distance of 1.0 m from the animal. A 12 megapixel camera of the Motorola Moto Z3 Play mobile phone was used to obtain the photos, resulting in photos with a resolution of 4032x3024 pixels. The photographs were randomly arranged and were used for the analysis performed by other evaluator, also using the HGS but who were unaware of the condition of each animal.

2.4 Acupuncture diagnosis

The same trained examiner, who specializes in the field, performed the acupuncture diagnosis and the same semiologic protocol based on using the evaluation of sensibility in the acupoints was used in all the investigated horses. This examination initiated only after the horse was comfortable with the examiner's presence, being calm and still with relaxed eyes and ears. The diagnostic acupuncture was initiated with finger pressure over the temporomandibular joint and adjacents regions, followed to test of sensibility of the acupoints in the meridian paths using a rounded plastic needle cover as a probe (Shmalbergand Xie, 2009; Michelotto et al.,2014; Cain, 2015).

The acupuncture diagnosis was carried out with continuous and uninterrupted pressure initially superficial followed by a deeper pressure exerted with the needle covert at the meridian path and acupuncture points looking for reactive (painful). The examination started on the neck (stomach, large intestine, triple heater, small intestine, gall bladder, and bladder channels) and progressing along the chest (lung-1 and kidney-27),back and abdomen (back Shu points of the bladder channel), and sacral and hind leg regions (bladder, stomach, and gall

bladder channels). Finally, front Mu alarm points were investigated (Chvala et al., 2004; Michelotto et al.,2014; Cain, 2015).

Painful points were identified when the horse become uncomfortable and irritated, presented muscular contraction, moving from the examiner or even when he presented behavioral changes, with irritation or attempt to bite or kick (McCormick, 1996; Michelotto et al., 2014; Cain, 2015). All sensitive points were recorded in a separate file for further analysis and interpretation and for this the points were evaluated in isolation form, as well as the combination of these indicating imbalances in some meridian or a specific syndrome (Cain, 2015).

2.5 Examination of the oral cavity

Initially, the anamnesis was conducted with the owners and / or responsible for the animal, using a standard questionnaire for dental care, where complaints were discussed, possible changes in chewing, swallowing or during training, as well as observations regarding athletic or work performance. According to the complaints reported by those responsible for the animals, each horse was classified in a given category according to its masticatory quality, being able to receive scores from 0 to 2 (Table 1) and the quality of its performance in relation to work with reins, also receiving scores ranging from 0 to 2 (Table 2).

Table 1. Evaluation of the masticatory quality of horses, considering the subjective observation of their handlers.

Escore	*Definition*
0	Animal without difficulty to feed, does not leave leftover food in the lame, nor throw down food already chewed.
1	Animal that leaves food in the lame or overturns already chewed food.

2	Obvious difficulty in chewing, overbalancing the head during feeding, dropping already chewed foods, history of weight loss and / or colic.

A general physical examination was performed to evaluate the heart and respiratory rates, followed by a specific examination of the oral cavity by the investigator (veterinarian with experience in equine dentistry). For this purpose, the horses were sedated with detomidine (20 to 30 μg/kg IV, Syntec, Santana de Parnaíba, SP, Brazil), and a full mouth speculum was used (full mouth speculum, Ortovet, São Paulo, SP, Brazil). A detailed observation and palpation of the oral cavity was made, including palpation of both hard (teeth and supporting bones) and soft tissues (lips, cheeks, tongue, palate, gingiva, buccal mucosa, salivary glands and ducts, and muscles of mastication) (Easley et al., 2011). The condition of the oral cavity was recorded in an odontogram adapted from Easley et al (2011).

Table 2. Evaluation by the trainers as to the ease of conduction and performance of exercises and / or work with reins in the horses studied.

Escore	*Definition*
0	There are no complaints of difficulty driving the animal during exercise or work.
1	Animal shows reluctance, or reaction to the mouth during some exercise or specific work.
2	Animal has visibly difficult driving, heavy animal on the reins and / or works constantly with the head high or swinging excessively. Animal that works with the mouth open or that does not perform certain type of maneuver.

The animals then received appropriate dental treatment directed towards each alteration detected.

2.5 Statistical analysis

To obtain the differences in the number of reactive acupuncture points and pain score between T0 and T1, the Wilcoxon test was used. It was performed the sensibility and specificity test to evaluate the predictive values of the acupuncture diagnosis. To determinate the agreement between the HGS obtained presentially and the photographic evaluating, the Kappa test was used, and inter-observer reliability was assessed using intra-class correlation coefficients (ICC).

Correlations between the number of reactive acupuncture points, pain scores of the animals and the owners' complaints regarding to masticatory quality and problems during the training were evaluated using the correlation test of Spearman. For the analysis of the relationship between dental disorders and the number of reactive acupuncture points, pain score and complaints of the owners, the Mann-Whitney test was used.

Finally the pain score and the number of reactive acupuncture points in the different races and ages (animals up to 5 years old and over 5 years old) were evaluated by analysis of variance.

These analyses were performed using the software IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp, New York, USA). For all the analyses, $p < 0.05$ was considered significant.

3. Results

All of the 87 horses studied presented some dental disorders, with most animals suffering from more than one affection at a time 80.5% (70/87).Those who suffered five or more diseases at the same time correspond to the least numerous group 8% (7/87) but there is a large proportion of animals suffering 2 to 4 diseases 72.4% (63/87), and only 19.5% (17/87) had only one disease (excessive enamel points) (Figure 2).

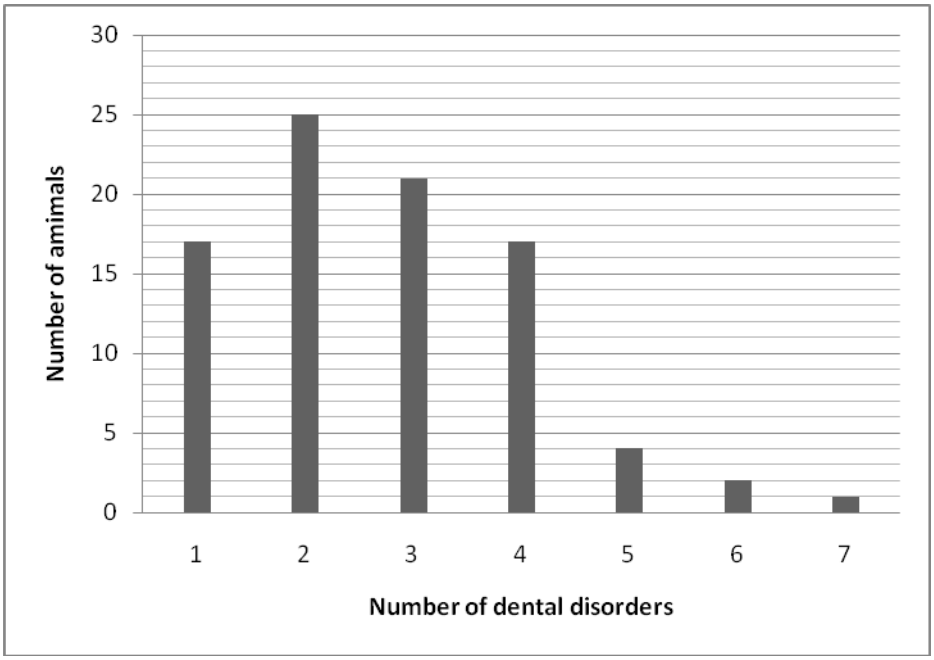

Figure 2. Number of dental disorders per animal observed in a group 87 working and athlete horses.

Among the affections found it is possible to emphasize the presence of excessive enamel points, n = 84/87 (96.6%), followed by ulcerations of the buccal mucosa, n = 38/87 (43.7%) and presence of wolf teeth n = 30/87 (34.4%) (Table 3).

Despite the dental alterations, all 87 animals evaluated presented presented cardiac rate of 32 ± 3 beats per minute and respiratory frequency of 11± 2 movements per minute and in sports or work activity throughout the study. After treatment these parameters did not change.

In the T0 acupuncture examination only one animal was found to be free of reactive acupuncture points. The remaining 86 animals showed sensitivity in one or more acupuncture points (11.2 ± 5.6 points). In the revaluation (T1) the animals presented a significant reduction in the number of reactive points in relation to T0 (4.0 ± 2.9 reactive points, p = 0.001).

Table 3. Dental disorders found in equines studied and their percentage of occurrence.

Dental disorders	*Number of affected animals (n)*	*Percentage of occurrences (%)*
Excessive enamel points	84	96.6
Ulcerations of the buccal mucosa	38	43.7
Wolf teeth	30	34.4
Hooks / Ramps	19	21.8
Retained deciduous	14	16.1
Stepmouth	8	9.2
Periodontal Disease	6	6.9
Diastema	5	5.7
Others	11	12.6

Evaluating the acupuncture points in isolation (table 4), it was observed that the most prevalent points were: the dental point that appeared in 50.5% (44/87) of the animals in the T0 and after dental treatment this number significantly reduced to 4.5% (4/87) of the evaluated ones ($p = 0.001$). Point ST7 also had a high prevalence in animals without dental treatment 35.6% (31/87), after treatment this number dropped to 3.4% (3/87) ($p = 0.001$). Evaluating the TH17 point, it was observed that in T0 it occurred in 31% (27/87) of the animals, and in T1 this value dropped to 4.5% (4/87) ($p = 0.001$). Finally, the TH16 point was found in 25.8% (22/87) of the animals in T0 and 4.5% (4/87) of them in T1 ($p = 0.001$) (Figure 3).

The sensitivity and specificity of these points were also evaluated for diagnoses related to three of the main dental conditions found, the result is shown in table 5.

Table 4. Number of animals showing pain sensitivity in points: Dental point, Stomach (ST) -7, Triple Heater (TH) -17 and TH-16 in pre-treatment (T0) and and 15 days after the dental treatment (T1).

	T0[a]	T1[b]
Dental point	44/87	4/87
ST 7	31/87	3/87
TH17	27/87	4/87
TH16	22/87	4/87

*Different letters between columns represent statistical difference (p = 0.001).

Table 5. Sensitivity values (S) and specificity (SP) of the points, Dental point, Stomach (ST) -7, Triple Heater (TH) -17 and TH-16 for the presence of ulcerations of the buccal mucosa, presence of wolf teeth and hooks and ramps.

	Ulcerations of the buccal mucosa		Presence of a wolf teeth		Presence of hooks and ramps	
	S	SP	S	SP	S	SP
Dental point	56%	69%	40%	72%	11%	67%
ST7	55%	62%	42%	69%	41%	89%
TH17	55%	61%	22%	60%	51%	91%
TH 16	72%	66%	50%	70%	13%	75%

Figure 3. Number of animals showing pain sensitivity in points: Dental point, located on the tendon between the atlas and occipital bone, at the posterior base of the ear; Stomach (ST) -7 , located in the temporomandibular joint, ventrally to the zygomatic arch; Triple Heater (TH) -17, located in a depression between the jaw and the mastoid process, caudoventrally the ear.and TH-16 located caudoventrally to the mastoid process, between the first and second cervical vertebrae, over the caudal border of the brachiocephalic muscle, .in pre-treatment and and 15 days after the dental treatment.

Considering the meridians as a way to determine the imbalances of the animals, in T0 it was found that the alteration in the gallbladder meridian was found in 42.5% (n = 37/87) of the animals, and after treatment only 3.4% (n = 3/87) remained with this changed meridian. Another alteration that attracted attention was the meridian of the stomach, present in 20.6% (n = 18/87) of the animals in T0 and reducing to 5.7% (n = 5/87) of them in T1.

In another approach to analysis of the reactive points, it was found that 56.3% (n = 49/87) of the animals presented, according to the diagnosis of acupuncture, a sensitivity pattern for painful syndromes in the posterior part of the musculoskeletal system like thoracolumbar pain, pain in the coxofemoral region, stifle or hock. This number fell to 18.3% (n = 16/87) of

them after dental treatment and without any other clinical intervention or change of management.

Regarding the analysis of the pain score evaluated by the Horse Grimace Scale, it was initially evaluated the agreement between the analyzes performed *in loco* and using the photographs of the face of the animals ($p = 0.001$) with an excellent intra-class agreement index (ICC = 0.854). In the evaluation of the animals in T0 and T1, there was a significant difference in the pain score ($p = 0.001$), both in the direct evaluation and in the photographic analysis (Figure 4), with the animals presenting pain score in the T0 of three with minimum and maximum values of 0 and 10 respectively and median pain score in T1of one, with minimum and maximum values of 0 and 6 respectively.

Still in relation to the pain score, a positive correlation was observed between this and the number of reactive points in the acupuncture test ($p = 0.0001$); with an excellent correlation coefficient ($r = 0.543$), showing that animals with a greater number of reactive points also presented facial pain expression more evident.

There was no statistical difference between pain score ($p = 0.990$) and number of reactive acupuncture points ($p = 0.327$) in the different races studied. Regarding the age of the animals, a higher pain score ($p = 0.041$) and number of reactive acupuncture points (14.25 ± 5.55) were observed in animals over 5 years old when compared to the younger ones (10.53 ± 5.55) ($p = 0.01$).

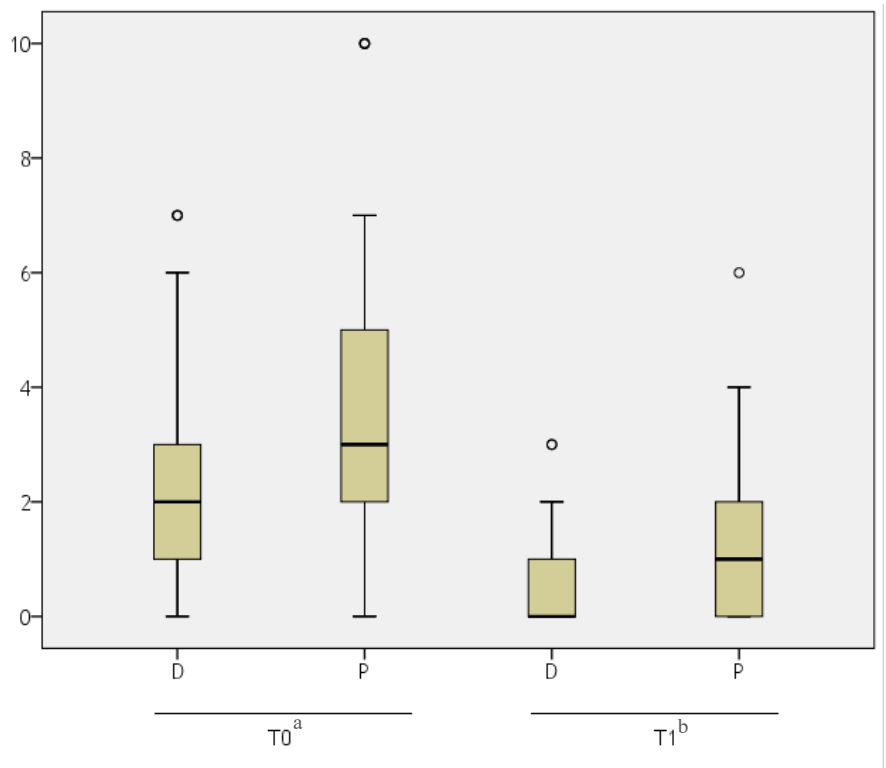

Figure 4. Median and interquartiles of pain scores for 87 horses assigned by each evaluator according to the Horse Grimace Scale (HGS) [(in direct physical presence (D) and from photographs (P)], in pre treatment (T0) and 15 days after dental treatment (T1) (p = 0.0001). Different letters between time points represent statistical difference.

When questioned about the masticatory quality of the animals, 73.5% (64/87) of the owners had no complaint or did not know how to report, but of the 23 animals complaining of masticatory quality, 95.6% (22/23) presented an improvement in the masticatory pattern after dental treatment according to the owners' remark. Regarding the quality of work, 68.9% (60/87) of the animals presented some difficulty in working with reins according to the perception of their trainers and after dental treatment 96.6% (58/60) of these coaches noticed improvement in this area.

The correlation between the number of reactive dots in the acupuncture exam and the pain score in relation to the owners' complaints was also evaluated. There was a positive correlation, with a slight to moderate correlation factor (r) between the number of reactive acupuncture points and the owners' complaints, both regarding the quality of mastication (p =

0.016; r = 0.2587) and the quality of work (p = 0.0001; r = 0.435). The same occurred between the pain score and the food difficulty complaints (p = 0.001; r = 0.336) and during work (p = 0.001; r = 0.435).

It was observed that the presence of ulcerations in the buccal or lingual mucosa had an effect on the pain score (p = 0.001) and the number of points reactive acupuncture (p = 0.001), as well as having an effect on the owners 'perception regarding the difficulty of working with reins (p = 0.001), but had no effect on the owners' perception regarding the masticatory quality of the animals (p = 0.341).

There was no significant difference between the animals with or without wolf teeth, in the pain score (p = 0.872); number of reactive acupuncture points (p = 0.425); the quality of the work (p = 0.518) and the masticatory quality (p = 0.179).

Finally, it was observed that the presence of hooks and ramps had no effect on the pain score (p = 0.098), on the total reactive acupuncture points (p= 0.130) and on the quality of the work (p = 0.954), but had an influence on the masticatory quality of these animals (p = 0.004).

4. Discussion

In the present study, the objective of evaluating the influence of odontological treatment on the general welfare of horses was accomplished, thereby demonstrating that after dental treatment the animals presented reduction in the pain score and the number of reactive acupuncture points.

All 87 equines investigated showed some dental disorders, with a large number of animals presenting more than one affections at the same time, with the majority of animals presenting from 2 to 4 dental disorders as demonstrated by Amaya et al. (2012).

In this study, the presence of excessive enamel points was the most prevalent condition (96.6%), which is in accordance with the literature that cites this condition as the most frequent in equine dentistry (Pence, 2002; Dixon and Dacre, 2005; Easley et al., 2011; Amaya et al., 2012). In sequence the animals studied present a higher prevalence of ulcerations in the buccal mucosa that are usually a consequence of the excessive enamel points (Dixon and Dacre, 2005;

Easley et al., 2011). Presence of wolf teeth was the third more frequent affection. It is noteworthy that the presence of wolf teeth may be underestimated. According Dixon (2017) some animals may lose this tooth during the exchange of the second premolar. In addition, some of the horses studied had received dental treatment at some time during their lifetime, at which point the tooth may have been extracted.

Although it is accepted that these dental disorders generate different levels of discomfort and pain (Ashley et al., 2005; Easley et al., 2011), it can be observed here that the horses with these affections may not clearly manifest clinical signs, thus remaining in their work or sports activities, and presented values of cardiac and respiratory rate within the parameters considered normal for species (Speirs, 1999). This represents a welfare problem, as the level of discomfort or pain due to oral conditions has not been quantified, horses endure working even with dental problems, which makes the early diagnosis difficult. Amaya et al. (2012) when studying 400 horses, where 76.5% presented dental affections also reported that most of the animals did not present symptoms that would call the attention of their owners. Recognition of dental pain is complicated by the hidden nature of disorders which often go unnoticed until the later stages, when more obvious discomfort signs are expressed associated with changes in the masticatory pattern, dysphagia, weight loss and gastrointestinal disorders. In this time point the affections and their consequences for the animals usually are already more serious and more difficult to be corrected (Ashley et al., 2005; Dixon et al., 2007; Ardila and Montoya, 2009; Amaya et al., 2012). The fact that horses with dental disorders do not receive early diagnosis or precise care predisposes them to the appearance of other problems, such as gastrointestinal diseases or painful conditions in the rest of the body (Brigham and Duncanson, 2000a, 2000b; Dixon and Dacre, 2005; Amaya et al., 2012; Alencar-Araripe et al., 2013).

This was observed in this study, since pre-treatment animals had more severe alterations in the acupuncture examination and higher pain scores than after dental treatment. Among the reactive acupuncture points in T0 we can highlight the points: dental point, ST-7, TH-17 and TH-16. These points are cited in the literature as diagnostic points for TMJ conditions, as well

as in the region of the oral cavity as a whole (Fleming, 2006; Xie and Trevisanello, 2007; May, 2008; Cain, 2015).

The dental point is an ASHI point located on the tendon between the atlas and occipital bone at the posterior base of the ear (Cain, 2015). Forty-four animals showed a palpation reaction at this point at initial exam, and after dental treatment only 4 of these animals remained with this sensitivity. This is according to Cain (2015) who says that according with experience of specialists in equine dentistry after dental treatment the acute reaction of this ASHI point tends to disappear.

The ST-7 sensitivity could be related either to a problem with the TMJ itself, masseter myofascial pain or both (Fleming, 2006; May, 2008; Xie and Preast, 2011). This point was reactive in 33 horses in first exam and only three animals remained with this reactive point after treatment. As one of the causes of TMJ pain are dental disorders (Easley et al., 2011), sensitivity at ST 7 could lead the veterinary to further examine the horse's mouth and dental status.

The point TH-17 is a crossing point of the TH and GB channels and is cited as a diagnostic point for painful processes in TMJ, dental disorders, cheek swelling and also cervical instability syndromes (Fleming, 2006; Xie and Preast, 2011). Dental treatment resulted in a significant reduction of this point in the animals studied. May (2008) cites that the TH 17 point is normally reactive in animals with dental changes especially when these are associated with chiropractic changes. In equines studied this point evidenced good specificity (91%) for the presence of hooks and ramps. These fact can be explained since these changes end up limiting the rostral movement during chewing (Easley et al., 2011; Paiva et al., 2018) which predisposes chiropractic problems in the area of head.

Finally, the TH 16 point is also cited as the diagnostic point for alterations in the face and head, deafness and pain in the TMJ (Fleming, 2006; Xie and Preast, 2011). This point was reactive in 22 animals in T0 and only four in T1.

In general, the points studied (Dental Point, S7, TH17 and TH16), presented higher specificity than sensitivity for the different dental affections. This information could then be

useful since the elimination of these reactive points present before the procedure would determine that the dental treatment was successfully accomplished.

Interpreting the findings of acupuncture in relation to changes in the meridians, we see that six different acupuncture channels converge on or close to the TMJ, three travel to the front legs (LI, SI, and TH), and three to the hind legs (GB , ST, and BL) (Fleming, 2006). The two meridians most frequently affected in the studied animals were the GB and ST both Yang meridians that have theirs pathway through the TMJ area (Fleming, 2006). This fact may be responsible for the greater involvement of these channels in animals with dental disorders, as these are the most common cause of TMJ pain (Easley et al., 2011). In addition, it is known that imbalances in the wood element (gallbladder) can lead to changes in the earth element (stomach) through the Cheng cycle. It is also noteworthy that the stomach meridian is directly related to the oral cavity, as it opens in the mouth (Fleming, 2006; Xie and Preast, 2011).

The meridian of the GB begins in an area just beside the TMJ and the meridian of the ST, has its initial course in the face region, passing through the mandible and temporomandibular joint region, both following to the hind legs (Fleming, 2006).

The understanding of this dynamics according to the Traditional Chinese Veterinary Medicine (TCVM), explains the understanding of, although most of the investigated horses (49 of them) did not show signs of lameness, presented a set of reactive points that according to Cain (2015) indicated involvement of the sacral, coxofemoral, stifle or hock regions.This would explain, in TCVM why a dental affection would create musculoskeletal conditions and lameness (Fleming, 2006; Cain, 2015). Cain (2015) further stresses that all the meridians of the head and neck are Yang and reviewing their passage through the maxilla and mandible, it becomes evident that a dental disorder would create problems of the stifle or hock.

After dental treatment and without any other alteration of management or therapeutic measure, 33 of these 49 animals presented a reduction of these patterns. This fact allows us to see how the musculoskeletal system can be affected by dental affections, since TMJ is connected through the meridian system to other parts of the body (Fleming, 2006; Moon, and Lee, 2011). Besides that Le June and Jones (2014) studying the correlation of acupuncture exam

would with a conventional lameness examination, found that horses that have sensitivity to palpation of acupuncture points are more likely to be lame and should undergo a full lameness examination and other diagnostic testing. In another paper, Michelotto et al. (2014), studying the stifle syndrome diagnosis in equines, noted that most of the equines with stifle syndromes, even though they did not present evidence obvious of lameness could be identified by acupuncture diagnosis.

In this way it is possible to understand the importance of dental treatment in the prevention of locomotor problems in horses and the benefits of including the acupuncture exam in the routine examination of horses.

In addition to acupuncture point sensitivity, the significant difference found in horses evaluated by pre and post-treatment HGS confirms that even in the absence of obvious clinical signs, odontological changes caused a certain degree of discomfort in the horses studied, since the animals in T0 presented a significantly higher pain score than in T1 (after dental treatment). Still regarding to the pain score, a positive correlation was found between this and the number of reactive acupuncture points, which highlights the importance of including oral cavity evaluation as a routine examination for horses. This measure makes possible the early diagnosis and treatment of any dental disorders, thus avoiding further complications and improving masticatory function and the use of animals in their activities, since a positive correlation was found between the number of reactive acupuncture points, the score of pain and complaints of owners regarding the quality of chewing and working of animals, with improvement of all parameters and reduction of complaints after dental treatment.

In a more focused analysis for the main dental disorders found in the studied animals, the animals that presented ulcerations of the buccal mucosa showed a greater number of reactive acupuncture points, score of pain and complaints in relation to the work quality. The ulcerations of the buccal mucosa are usually caused by sharp and prominent points on the lateral and medial aspects of the occlusal surface as a result from dental excessive enamel points (Brigham and Duncanson, 2000a; Easley et al., 2011; Dixon, 2017). Identify and reducing such dental excessive enamel points at an early stage is the key to prevention of the ulcerations of the buccal

mucosa. According Dixon (2017), one of the most important role in equine dental care is to prevent large dental overgrowths from developing by their early detection during routine oral examinations and their effective removal. This is possible with regular (annual or biannual) dental inspections and appropriate treatments.

For the presence of the wolf teeth there was no significant difference in any of the studied parameters. The necessity for removing wolf teeth is controversial, even though its extraction may be recommended, considering no wolf teeth does any good and may cause discomfort (Scrutchfield, 2006; Dixon, 2017). However, in the present study the presence of the wolf teeth caused no harm as might be popularly believed, agreeing with other authors that these teeth generally do not cause problems unless they are large, sharp, poorly positioned or included (Brigham and Duncanson, 2000b; Dixon and Dacre, 2005; Easley et al., 2011). These data are important as many horsemen believe that wolf teeth are primarily responsible for oral discomfort, bit problems and abnormal behaviour of horses at work (Dixon and Dacre, 2005; Scrutchfield, 2006; Dixon, 2017). It is noteworthy that in relation to the pain score it may have been underestimated in animals with wolf teeth, since animals that present discomfort due to its presence will do so during exercise and not during rest, at which time the analyzes were performed.

Considering hooks / ramps their occurrence had a significant effect on the masticatory quality of the animals. This can be explain, since the horse must be able to move his jaw from side to side (lateral excursion), forward and back (anterior/posterior movement) and up and down to reinforce the grind in order to chew food properly. Ramp is pathological exaggeration of distal upward slope of mandibular cheek teeth and hook is a sharp narrow overgrowth developed on a tooth through abnormal wear, if large these overgrowths may reduce or restrict the rostral movement of the mandible during chewing (Easley et al., 2011).This was demonstrated in practice by Paiva et al. (2018), that studying the influence of occlusal adjustment by tooth wear in masticatory biomechanics of horses showed that the occlusal adjustment increases the range of mandibular movements in horses.

It is noteworthy that the owners of the investigated horses did not report any other complaints for their horses during the entire study period.

In humans has already been established that dental occlusion/ temporomandibular joint status and general body health have organic and functional relationships (Moon and Lee, 2011). In equines it is also possible to understood that if there is anything that painfully affects a horse's head during work or chewing, this will affect your well being and the body as a whole, with special attention to the posterior region of the musculoskeletal system.

The integration of acupuncture points palpation and pain assessment using HGS in equine routine examination may be useful for diagnosis of dental disorders and their consequences. Besides offering the possibility of broadening the understanding of the interconnections between the masticatory system and the rest of the body. Consequently, resulting in greater success in the diagnosis and treatment of dental disorders.

5. Conclusion

It can be concluded that dental problems negatively influenced the general welfare of the horses, demonstrated by the reduction in pain score and the number of reactive acupuncture points after dental treatment. In addition, the acupuncture test based on the sensitivity of points: dental point, ST-10, TH- 17 and TH-16 proved to be effective in aiding the diagnosis of dental disorders and in assessing the success of dental treatment.

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Conflicts of interests

The authors declare that they have no conflicts of interests.

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References

Alencar-Araripe, M.G., Castelo-Branco, D.S.C.M., Nunes-Pinheiro, D.C.S., 2013.Alterações anatomopatológicas na cavidade oral equina. Acta Veterinaria Brasilica. 7(3), 184-192. https://doi.org/10.21708/avb.2013.7.3.3530

Alfaro, A.,2014. Correlation of Acupuncture Point Sensitivity and Lesion Location in 259 Horses. American Journal of Traditional Chinese Veterinary Medicine. 9(1):83-7.

Angeli, A.L., Joaquim, J.G.F., Luna, S.P.L., 2007. Acupuntura aplicada à medicina esportiva equina. Revista acadêmica de Curitiba. 5, 325-333. http://dx.doi.org/10.7213/cienciaanimal.v5i3.10146

Angeli, A.L., Luna, S.P.L., Garotti, J.R., 2008. Aquapuntura no tratamento das síndromes equinas: há efeito sobre os valores hematológicos e de cortisol? ARS Veterinária. 24, 153-160. http://dx.doi.org/10.15361/2175-0106.2008v24n3p153-160

Amaya, J.M.C., Sánchez V.J.A., Vera Hernández, L.G., 2012. Caracterización y prevalencia de las enfermedades orales en el caballo criollo, departamento de Caldas, Colombia. Rev. Med. Vet. (Bogota). 23, 39-50. https://doi.org/10.19052/mv.73

Ardila, M. C., Montoya,L., 2009. Desórdenes bucales eqüinos. Revista Salude Animal. 31(3), 143-151. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0253-570X2009000300002&lng=es&tlng=es.

Ashley, F.H., Waterman-Pearson, A.E., Whay, H.R., 2005. Behavioural assessment of pain in horses and donkeys: Application to clinical practice and future studies. Equine Veterinary Journal. 37, 565–575. https://doi.org/10.2746/042516405775314826

Bennett, D.G., 2006. An overview of bits and bitting. - Annual convention, focus meeting & annual resort symposium, equine dentistry - AAEP Focus Meeting. Indianapolis, USA. www.ivis.org/proceedings/aaepfocus/2006/bennett1.pdf (accesed 25 march 2019)

Brigham, E.J., Duncanson, G.R., 2000a. An equine postmortem dental study: 50 cases. Equine Vet. Educ. 12, 59–62. https://doi.org/10.1111/j.2042-3292.2000.tb01765.x

Brigham, E.J., Duncanson, G.R., 2000b. Case study of 100 horses presented to an equine dental technician in the UK. Equine Vet. Educ. 12, 63–67. https://doi.org/10.1111/j.2042-3292.2000.tb01767.x

Cain M., 2015. Acupuncture Diagnosis and treatment of the Equine, fifth ed., Bioscan Inc, Lexgton.

Chvala, S., Nowotny, N., Kotzab, E., Cain, M., van den Hovenet, R., 2004. Use of the meridian test for the detection of equine herpesvirus type 1 infection in horses with decreased performance. Journal of American Veterinary Medical Association. 225(4),554-559. https://doi.org/10.2460/javma.2004.225.554

de Grauw, J.C., van Loon, J.P.A.M., 2016. Systematic pain assessment in horses. Vet. J. 209, 14–22. https://doi.org/10.1016/j.tvjl.2015.07.030

Dixon, P., 2000. Removal of equine dental overgrowths. Equine Vet. Educ. 12, 68–81. https://doi.org/10.2746/095777308X380346

Dixon, P.M., Treimane, W.H., Pickles, K., Kuhns, L., Hawe, C., McCann, J., McGorum, B., Railton, D.I., Brammer, S., 1999. Equine dental disease Part 1: A long-term study of 400 cases: disorders of incisor, canine and first premolar teeth. Equine Vet. J. 31, 369–377. https://doi.org/10.1111/j.2042-3306.1999.tb03835.x

Dixon, P.M., Tremaine, W.H., Pickles, K., Kuhns, L., Hawe, C., Mccann, J., Mcgorum, B.C., Railton, D.I., Brammer, S., 2000. Equine dental disease Part 3: A long-term study of 400 cases: Disorders of wear, traumatic damage and idiopathic fractures, tumours and miscellaneous disorders of the cheek teeth. Equine Vet. J. 32, 9–18. https://doi.org/10.2746/042516400777612099

Dixon, P.M., Dacre, I., 2005. A review of equine dental disorders. Vet. J. 169, 165–187. https://doi.org/10.1016/j.tvjl.2004.03.022

Dixon, P.M., Barakzais, S. Z., Collins, N.M., Yates, J., 2007. Equine idiopathic cheek teeth fractures. Part 3: A hospital-based survey of 68 referred horses. Equine veterinary journal. 39,149-153. https://doi.org/10.2746/042516407X182983

Dixon, P.M., 2017. The Evolution of Horses and the Evolution of Equine Dentistry. AAEP Proceedings. 63, 79-116. https://aaep.org/sites/default/files/2018-08/Proceedings%2063rd%20Annual%20Convention%202017_0.pdf (acessed 25 march 2019).

Dalla Costa, E., Minero, M., Lebelt, D., Stucke, D., Canali, E., Leach, M.C., 2014. Development of the Horse Grimace Scale (HGS) as a pain assessment tool in horses undergoing routine castration. PLoS One. 9,1–10. https://doi.org/10.1371/journal.pone.0092281

Du Toit, N., Buren, F.A., Dixon, P.M., 2009. Clinical dental examinations of 357 donkeys in the UK. Part 2: Epidemiological studies on the potential relationships between different dental disorders, and between dental disease and systemic disorders. Equine Vet. J.41, 395-400. http://dx.doi.org/10.2746/042516409X368903

Easley, K.J.,Dixon, P.M., Schumacher, J.S., 2011. Equine dentistry, third ed. Elsevier Saunders, Edinburgh, UK.

Filho, A.F., Borelli, V., Gilberto, J., Pereira, L., Fernandes, M., Castro, D.S., 2014. Prevalência do primeiro pré-molar (dente-de-lobo) em equinos puro sangue inglês. J Heal. Sci Inst. 32, 198–202.

Fleming, P., 2006. Exame diagnóstico de palpação da acupuntura em cavalos, in: Shoen, A.M., (Ed.), Acupuntura veterinária: da arte antiga à medicina moderna. Editora Roca, São Paulo. pp. 424- 432.

Galloway, S.S., Easley, K.J.,2008..Establishing a scientific basis for equine clinical dentistry Introduction. The Veterinary Journal. 178(3), 307-10. https://doi.org/10.1016/j.tvjl.2008.09.030

Gleerup, K.B., Forkman, B., Lindegaard, C., Andersen, P.H., 2015. An equine pain face. Vet. Anaesth. Analg. 42, 103–114. https://doi.org/10.1111/vaa.12212

Le Jeune,S.S., Jones, J.H., 2014. Prospective study on the correlation of positive acupuncture scans and lameness in 102 performance horses. American Journal of Traditional Chinese Veterinary Medicine. 9,33-41.

May, K.J., 2008. Interrelationship betweem equine acupuncture, chiropractic and dentistry. Proceedings of the 34th Annual International Congress On Veterinary Acupuncture. 207-216.

McCormick, W.H.,1997. Oriental channel diagnosis in foot lamness of the equine forelimb. Journal of Equine Veterinary Science. 17(6), 315-321. https://doi.org/10.1016/S0737-0806(97)80090-1

McCormick, W.H., 1998. The Origins of acupuncture channel imbalance in pain of the equine hindlimb. Journal of Equine Veterinary Science. 18(8), 528-534. https://doi.org/10.1016/S0737-0806(98)80074-9

Michelotto, P.V., Bastos, L.F.C., Sotomaior, C.S., Pimpão, C.T., 2014. Acupuncture Diagnosis in Equine Stifle Disease. Journal of Equine Veterinary Science. 34(8), 967–971.doi:10.1016/j.jevs.2014.05.001

Michelotto, P.V., Rego,D.X., Dombusch, P.T., 2007. Acupuncture palpation diagnosis in prepurchase evaluation of horses. Revista Acadêmica. 5,231-236. https://doi.org/10.7213/cienciaanimal.v5i3.10074

Moon, H.J., Lee, Y.K.,2011. The Relationship Between Dental Occlusion/Temporomandibular Joint Status and General Body Health: Part 1. The Journal of Alternative and Complementary Medicine.17(11), 995–1000. https://doi.org/10.1089/acm.2010.0739

Ozanam, F., Silva, C.E., Severino, R.S., 2005. Erupção do dente Lupino em cavalos PSI e campolina. Biosci. J. 30, 420–423. <http://www.seer.ufu.br/index.php/biosciencejournal/article/view/15204/14554>

Pagliosa, G.M., Alves, G.E.S., Faleiros, R.R., Saliba, E.O.S., Sampaio, I.B.M., Gomes, T.L.S., Gobesso, A.A.O., Fantini, P., 2006. Influence of excessive enamel points on nutrients digestibility in diets of horses. Arq. Bras. Med. Veterinária e Zootec. 58, 94–98. https://doi.org/10.1590/S0102-09352006000100014

Paiva Neto, A.O., Leite, C.T., Duarte, C.A.,.Dias, D.P.M; Döwich, G., Queiroz Neto, A., Lacerda Neto, J.C., 2018. Biomechanical analysis of the masticatory movement before and after adjusting dental occlusion in equine Arq. Bras. Med. Vet. Zootec. 70(1), 6-12 http://dx.doi.org/10.1590/1678-4162-9491

Pellegrini, D.Z., Muller, T.R., Fonteque, J.H., Souza, L.P., Souza, A.F., Joaquim, JG.F., 2018. Equine acupuncture methods and applications: A review. Equine vet. Educ. https://doi.org/10.1111/eve.12928

Pence P., 2002. EquineDentristry, a practicalguide. Lippincott Williams and Wilkins. Philadelphia.

Ramey,D.W., 2005. Acupuncture and traditional Chinese medicine in the horse. Part 2: A scientific overview. Equine Veterinary Education.17(2),106 – 112. https://doi.org/10.1111/j.2042-3292.2005.tb00347.

Schoen, A.M., 2000. Equine acupuncture: incorporation into lameness diagnosis and treatment. AAEP Proceedings. 59, 80-83.

Scognamillo-Szabo, M.V.R., Bechara, G.H., 2010. Acupuntura: histórico, bases teóricas e sua aplicacão em medicina veterinária. Ciência Rural. 40, 461-470. http://dx.doi.org/10.1590/S0103-84782010005000004.

Scrutchfield, W.,2006. Wolf teeth: How to safely and effectively extractand is it necessary. In: AAEP Focus on dentistry work book. 56-60.

Shmalberg, J., Xie, H., 2009. The clinical aplication of equine acupuncture. Journal of EquineVeterinary Science. 29(10),753-760. http://dx.doi.org/10.1016/j.jevs.2009.08.001

Speirs, V.C. Exame Clínico de equinos Artmed, Porto Alegre. 1999; 366p.

Stashak, T.S., Hill, C., 2006. Conformação e Movimento, In: Stashak, T.S., (Ed.), Claudicação em equinos segundo Adams. fifth ed. Editora Roca, SãoPaulo, pp. 55-77.

Xie, H., Preast, V., 2011. Acupuntura Veterinária XIE, MedVet, São Paulo.

Xie, H., Trevisanello, L, 2007. Equine transpositional acupoints, in: Xie, H., Preast, V., (eds). Xie's veterinary acupuncture. Ames: Blackwell Publishing, pp. 27-87.

CAPÍTULO 4 - CONSIDERAÇÕES FINAIS

Concluiu-se que diferentes alterações odontológicas resultam em dor ou desconforto em equinos, e que esta pode ser avaliada e quantificada com segurança usando a HGS tanto na avaliação direta, quanto por meio de avaliação fotográfica. Vale ressaltar que parâmetros bem determinados (demonstrando cada estrutura facial a ser observada) foram relevantes para a acurácia da avaliação da dor. Pode-se concluir que as alterações dentárias influenciaram negativamente no bem-estar geral dos equinos, demonstrado pela redução do escore de dor e pelo número de pontos de acupuntura reativos após o tratamento odontológico.

O exame de acupuntura baseado na sensibilidade dos pontos: ponto do dente, E-10, TA-17 e TA-16 mostrou-se eficaz no auxílio ao diagnóstico de alterações dentárias e principalmente auxiliar na determinação do sucesso do tratamento odontológico. Além disso, através do exame de acupuntura foi possível determinar que o tratamento odontológico é importante para reduzir a sensibilidade nos pontos de acupuntura relacionados à dor na coluna e nos membros posteriores. Dentre as alterações odontológicas avaliadas a presença de ulceração na mucosa oral foi a que apresentou maior influência tanto no escore de dor, como no número de pontos de acupuntura reativos e queixas em relação ao trabalho com embocadura. A integração da avaliação da dor utilizando a HGS e o exame através da palpação dos pontos de acupuntura pode ser útil na avaliação rotineira de equinos, além de oferecer a possibilidade de ampliar o entendimento das interconexões entre o sistema mastigatório e o restante do corpo.

REFERÊNCIAS

Alencar-Araripe MG, Castelo-Branco DSCM, Nunes-Pinheiro DCS. Alterações anatomopatológicas na cavidade oral equina. Acta Veterinaria Brasilica. 2013; 7(3): 184-92.

Alfaro A. Correlation of Acupuncture Point Sensitivity and Lesion Location in 259 Horses. American Journal of Traditional Chinese Veterinary Medicine. 2014; 9(1): 83-7.

Angeli AL, Joaquim JGF, Luna SPL. Acupuntura aplicada à medicina esportiva equina. Revista acadêmica de Curitiba. 2007; 5: 325-33.

Angeli AL, Luna SPL, Garotti JR. Aquapuntura no tratamento das síndromes equinas: há efeito sobre os valores hematológicos e de cortisol? ARS Veterinária. 2008; 24: 153-60.

Amaya JMC, Sánchez, JA, Hernandez, LGV. Caracterizacion y prevalencia de las enfermedades orales en el caballo criollo, departamento de Caldas,Colombia. Revista de Medicina Veterinária. 2012; 23: 39-50

Ardila MC, Montoya, L. Desórdenes bucales eqüinos. Revista Salude Animal. 2009; 31(3): 143-51.

Ashley FH, Waterman-Pearson AE, Whay HR. Behavioural assessment of pain in horses and donkeys: Application to clinical practice and future studies. Equine Veterinary Journal. 2005; 37: 565–75.

Bennett DG. An overview of bits and bitting. American association of equine practitioners Annual convention, focus meeting & annual resort symposium, equine dentistry - AAEP Focus Meeting. Indianapolis, IN, USA. 2006; disponível em: www.ivis.org/proceedings/aaepfocus/2006/bennett1.pdf.

Brigham EJ, Duncanson G. An equine dental post-mortem study: 50 cases. Equine Vet Educ. 2000a; 12: 59-82.

Brigham EJ, Duncanson GR. Case study of 100 horses presented to an equine dental technician in the UK. Equine Vet. Educ. 2010b; 12: 63–67.

Bussières G, Jacques C, Lainay O, Beauchamp G, Leblond A, Cadoré JL, Desmaizières LM, Cuvelliez SG, Troncy E. Development of a composite orthopaedic pain scale in horses. Res. Vet. Sci. 2008; 85: 294–306.

Cain M. editor. Acupuncture diagnosis and treatment of the equine. 4th ed. Lexington: Bioscan Inc. 2015; 115p.

Carmalt J, Rach D. Equine Dentistry – Moving into the 21st Century. Large Animal Veterinary Rounds. 2013; 3(5).

Chvala S, Nowotny N, Kotzab E, Cain M, DVM; van den Hovenet R. Use of the meridian test for the detection of equine herpesvirus type 1 infection in horses with decreased performance. Journal of American Veterinary Medical Association. 2004; 225(4): 554-59.

Dalla Costa E, Minero M, Lebelt D, Stucke D, Canali E, Leach MC. Development of the Horse Grimace Scale (HGS) as a pain assessment tool in horses undergoing routine castration. PLoS One. 2014; 9(3): 1-10.

de Grauw, JC, van Loon JPAM, Systematic pain assessment in horses. Vet. J. 2016; 209: 14–22.

Dixon PM. The Evolution of Horses and the Evolution of Equine Dentistry. AAEP Proceedings. 2017; 63: 79-83

Dixon PM, Barakzai S Z, Collins NM, Yates J. Equine idiopathic cheek teeth fractures. Part 3: A hospital-based survey of 68 referred horses. Equine veterinary journal. 2007; 39: 149-53.

Dixon PM, Dacre I. A review of equine dental disorders. Veteinary Journal. 2005; 169:165-87.

Dixon PM, Treimane WH, Pickles K, Kuhns L, Hawe C, McCann J, McGorum B, Railton DI, Brammer S. Equine dental disease Part 1: A long-term study of 400 cases: disorders of incisor, canine and first premolar teeth. Equine Vet. J. 1999; 31: 369–77.

Dixon PM, Tremaine WH, Pickles K, Kuhns L, Hawe C, Mccann J, Mcgorum BC, Railton DI, Brammer S. Equine dental disease Part 3: A long-term study of 400 cases: Disorders of wear, traumatic damage and idiopathic fractures, tumours and miscellaneous disorders of the cheek teeth. Equine Vet. J. 2000; 32: 9–18.

Easley KJ, Dixon PM, Schumacher JS, eds. Equine dentistry. 3rd ed. Edinburgh, UK: Elsevier Saunders, 2011; 424p.

Fleming P. Exame diagnóstico de palpação da acupuntura em cavalos. In: Shoen AM. Acupuntura veterinária: da arte antiga à medicina moderna. 2006; Editora Roca, São Paulo. p.424- 432.

Filho AF, Borelli V, Gilberto J, Pereira L, Fernandes M, Castro DS. Prevalência do primeiro pré-molar (dente-de-lobo) em equinos puro sangue inglês. J Heal. Sci Inst. 2014; 32: 198–202.

Galloway SS, Easley KJ. Establishing a scientific basis for equine clinical dentistry Introduction. The Veterinary Journal. 2008; 178(3): 307-10.

Guedes A. Pain Management in Horses. Veterinary Clinics of North America: Equine Practice. 2017; 33: 181–211.

Gleerup KB, Forkman B, Lindegaard C, Andersen PH. An equine pain face. Veterinary Anasthesia and analgesia. 2015; 42: 103-14.

Jagger DH, Robinson NG. História da acupuntura veterinária. In: Shoen AM. Acupuntura veterinária: da arte antiga à medicina moderna. 2006; Editora Roca, São Paulo. p.2-16.

Jiménez LMR, Díaz APU. Odontología en equinos: generalidades e importância en medicina veterinária. Revista de MedicinaVeterinária. 2011; 22: 69-83.

Johnson TJ, Porter CM. Common Disorders of Incisor Teeth and Treatment. In: Annual convention of the American association of equine practitioners. [AAEP], Proceedings. 2006a.

Johnson TJ, Porter CM. Dental overgrowths and acquired displacement of cheek teeth.In: Annual convention of the American association of equine practitioners. [AAEP], Proceedings. 2006b; 52.

Lima RAS, Shirota R, Barros GSC. Estudo do complexo do agronegócio cavalo. Piracicaba: ESALQ/USP, 2006. 250p.

MAPA – Ministério da Agricultura, Pecuária e Abastecimento. 2016. Revisão do Estudo do Complexo de Agronegócio do Cavalo. Brasília. 7.

May KJ. Interrelationship betweem equine acupuncture, chiropractic and dentistry. Proceedings of the 34th Annual International Congress On Veterinary Acupuncture. 2008; 22: 207-216.

McCormick WH. The Origins of acupuncture channel imbalance in pain of the equine hindlimb. Journal of Equine Veterinary Science. 1997; 18(8):528-34.

McCormick WH. Oriental channel diagnosis in foot lamness of the equine forelimb. Journal of Equine Veterinary Science. 1998; 17(6):315-21.

Michelotto Jr PV, Rego DX, Dornbusch, PT. Acupuncture palpation diagnosis in pre purchase evaluation of horse. Revista Acadêmica. 2007; 5(3):231-36.

Michelotto Jr PV, Bastos LFC, Sotomaior CS, Pimpão CT. Acupuncture Diagnosis in Equine Stifle Disease. Journal of Equine Veterinary Science. 2014; 34(8): 967–71.

Ozanam F, Silva CE, Severino RS. Erupção do dente Lupino em cavalos PSI e campolina. Biosci. J. 2005; 30: 420–23.

Pagliosa GM, Alves GES, Faleiros RR, Saliba EOS, Sampaio IBM, Gomes TLS, Gobesso AAO, Fantini P. Influência das pontas excessivas de esmalte dentário na digestibilidade e nutrientes de dietas de eqüinos. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2006; 58(1):94-98.

Pellegrini DZ, Muller TR, Fonteque JH, Souza LP, Souza AF, Joaquim JGF Equine acupuncture methods and applications: A review. Equine vet. Educ. 2018; 1:10.

Pence P. EquineDentristry, a practicalguide. Lippincott Williams and Wilkins. Philadelphia. 2002. 705p

Peters JWE, De Boer B, Broeze-Ten Voorde BM, Broeze J, Wiemer P, Sterk T, Spoormakers TJP. Survey of Common Dental Abnormalities in 483 Horses in the Netherlands. Focus on Dentistry. American Association of Equine Practitioners. 2006; 196–202

Price J, Catriona S, Welsh EM, Waran NK. Preliminary evaluation of a behaviour-based system for assessment of post-operative pain in horses following arthroscopic surgery. Vet. Anaesth. Analg. 2003; 30: 124–37.

Pritchett LC, Ulibarri C, Roberts MC, Schneider RK, Sellon DC. Identification of potential physiological and behavioral indicators of postoperative pain in horses after exploratory celiotomy for colic. Appl. Anim. Behav. Sci. 2003; 80: 31–43.

Rietmann TR, Stauffacher M, Bernasconi P, Auer JA, Weishaupt MA. The association between heart rate, heart rate variability, endocrine and behavioural pain measures in horses suffering from laminitis. J. Vet. Med. Ser. A Physiol. Pathol. Clin. Med. 2004; 51: 218–25.

Schwartz C. Quatro patas cinco direções. Ícone editora, São Paulo. 2008; 470p.

Schoen AM. Equine acupuncture: incorporation into lameness diagnosis and treatment. Proc. Am. Ass. Equine Practnrs. 2000; 59: 80-83

Schoen AM. Acupuntura veterinária: da arte antiga à medicina moderna. Ediora Roca, São Paulo. 2006; 624p.

Scognamillo-Szabo MVR, Bechara GH. Acupuntura: histórico, bases teóricas e sua aplicacão em medicina veterinária. Cien. Rural. 2010; 40: 461-70.

Scrutchfield W. Wolf teeth: How to safely and effectively extract
and is it necessary. In: AAEP Focus on dentistry work book. 2006; 56-60.

Shmalberg J, Xie H. The clinical aplication of equine acupuncture.Journal of Equine Veterinary Science. 2009; 29(10):753-60.

Speirs VC. Exame Clínico de equinos Artmed, Porto Alegre. 1999; 366p.

Stashak TS, Hill C. Conformação e Movimento. In: Stashak TS . Claudicação em equinos segundo Adams. 5° ed. Editora Roca, SãoPaulo. 2006; p.55-77.

Thomassian A. Enfermidades dos cavalos. 4ª Ed. São Paulo: Livraria Varela. 2005; 573p.

VanDierendonck, MC, van Loon JPAM. Monitoring acute equine visceral pain with the Equine Utrecht University Scale for Composite Pain Assessment (EQUUS-COMPASS) and the Equine Utrecht University Scale for Facial Assessment of Pain (EQUUS-FAP): A validation study. Vet. J. 2016; 216: 175–77.

Van Lonn JPAM, Van DierendonckMC. Monitoring equine head-related pain with the Equine Utrecht University scale for facial assessment of pain (EQUUS-FAP). The Veterinary Journal. 2017; 220:88–90

Velazquez RJL. Equine dentistry. Published in IVIS with the permission of the AAEP.2009.

Wathan J, Burrows AM, Waller BM, McComb K. EquiFACS: The equine facial action coding system. PLoS One. 2015; 10: 1–35.

Xie H, Preast V. Acupuntura Veterinária XIE. MedVet, São Paulo. 2011; 363p.

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Curitiba, 06 de Outubro de 2016.

PARECER DE PROTOCOLO DE PESQUISA

REGISTRO DO PROJETO: 01083 / 2016 1ª versão

TÍTULO DO PROJETO: Efeitos das afecções da cavidade oral sobre o estado clínico geral de equinos, avaliados através da pressão em acupontos diagnósticos

PESQUISADOR RESPONSÁVEL

Pedro Vicente Michelotto Junior

EQUIPE DE PESQUISA

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Authors may also wish to refer to the ethical guidelines published on the website of the International Society for Applied Ethology http://www.applied-ethology.org/ethicalguidelines.htm, or read the following article: Sherwin, C.M., Christiansen, S.B., Duncan, I.J., Erhard, H., Lay, D., Mench, J., O'Connor, C., and Petherick, C. (2003), 'Guidelines for the ethical use of animals in applied animal behaviour research', Applied Animal Behaviour Science, 81: 291-305.
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