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Sedative and cardiorespiratory effects of detomidine combined or not with diazepam in horses subjected to dental examination

Efeitos sedativos e cardiorrespiratórios da detomidina associada ou não ao diazepam em equinos submetidos ao exame odontológico

Efectos sedantes y cardiorrespiratorios de la detomidina asociada o no al diazepam en caballos sometidos a examen odontológico

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Abstract

A standing oral examination under sedation in horses is essential to facilitate the handling and ensure an excellent examination. This study aimed to determine if the use of diazepam associated with detomidine would increase the action of the α2 agonist (detomidine), mainly by tongue motion and chewing reduction without excessive destabilization. Twelve adult horses of different age, breed and gender underwent dental correction. The animals were randomly assigned to two treatments: detomidine (20 μg.kg^{-1}, IV) and diazepam (20 μg.kg^{-1}, IV) (DD; n=6) or detomidine (20 μg.kg^{-1}, IV) and 0.9% NaCl (DS; n=6). Tongue relaxation and exposure, heart rate, PR interval, respiratory rate, systolic blood pressure, rectal temperature, second-degree atrioventricular blocks, degree of ataxia and head movement were assessed at nine separated time points. Height of head above ground (HHAG) was assessed

at three separate times. Detomidine rescue (5µg.kg^{-1}, IV) was administered whenever a sedation increase was needed. All treatments provided adequate sedation and muscle relaxation. Differences between time points were observed for tongue relaxation (tongue VAS mm) and this was best observed at T7 for the DD treatment and T5 for the DS treatment and remained significantly lower than baseline throughout the times assessed. It was concluded that detomidine provided adequate sedation in horses undergoing dental examination, but there was no significant difference between treatments for any variable studied. The addition of diazepam did not improve the sedation or reduce the tongue movement.
Keywords: Dentistry; Alpha-2 adrenergic agonist; Sedation; Benzodiazepine.

Resumo

A sedação para o exame odontológico, em estação, nos equinos é essencial para facilitar o manejo e garantir um exame de excelência. O presente estudo objetivou avaliar se o diazepam associado à detomidina potencializaria a sedação do agonista α2 (detomidina), principalmente pela redução do movimento da língua e mastigação sem excessiva desestabilização. Foram examinados 12 equinos adultos de diferentes idades, raças e sexos submetidos a correção dos arcos dentários e distribuídos aleatoriamente entre dois tratamentos: detomidina (20 µg.kg^{-1}, IV) e diazepam (20 µg.kg^{-1}, IV) (DD; n=6) ou detomidina (20 µg.kg^{-1}, IV) e solução salina de NaCl 0,9% (DS; n=6). Foram avaliados o relaxamento e a exposição da língua, frequência cardíaca, intervalo PR, frequência respiratória, pressão arterial sistólica, temperatura retal, bloqueios atrioventriculares de segundo grau, grau de ataxia, movimento da cabeça, em nove momentos. E a distância da cabeça ao solo (HHAG) foram avaliadas em três momentos. Sempre que houve necessidade de aumentar a sedação, foi administrado um bolus de detomidina adicional (5µg.kg^{-1}, IV). Todos os tratamentos proporcionaram sedação e relaxamento muscular. Foram observadas diferenças entre os momentos para o relaxamento da língua (língua VAS mm) e isto foi melhor observado em T7 para o tratamento DD e T5 para o tratamento DS e manteve-se significativamente inferior à linha de base durante todo o tempo avaliado. O presente estudo sugere que a detomidina forneceu sedação adequada em cavalos submetidos ao exame odontológico mas não há necessidade de utilizar o diazepam da forma proposta porque não foi encontrada qualquer diferença entre tratamentos para qualquer variável estudada. A adição de diazepam não melhorou a sedação e nem diminuiu o movimento da língua.
Palavras-chave: Odontologia; Agonista alfa-2 adrenérgico; Sedação; Benzodiazepínico.

Resumen

La sedación para procedimientos de pie, como el examen oral y dental en caballos, es esencial para facilitar el manejo y garantizar un examen excelente. El presente estudio tuvo como objetivo determinar si el uso de diazepam asociado a detomidina potencializaría la sedación del agonista α2 (detomidina), principalmente reduciendo el movimiento de la lengua y la masticación sin una desestabilización excesiva. Se examinaron doce caballos adultos de diferente edad, raza y sexo sometidos a corrección de la arcada dental, los cuales se asignaron aleatoriamente a dos tratamientos: detomidina (20 µg.kg^{-1}, IV) y diazepam (20 µg.kg^{-1}, IV) (DD; n=6) o detomidina (20 µg.kg^{-1}, IV) y NaCl al 0,9% (DS; n=6). Se evaluaron las siguientes variables en nueve puntos temporales distintos: Relajación y exposición de la lengua, frecuencia cardíaca, intervalo PR, frecuencia respiratoria, presión arterial sistólica, temperatura rectal, bloqueos auriculoventriculares de segundo grado, grado de ataxia y movimiento de la cabeza del caballo. La altura del labio inferior o mandíbula sobre el suelo (HHAG) se evaluó en tres puntos temporales distintos. Siempre que hubo necesidad de aumentar la sedación se administró una dosis adicional de detomidina (5µg.kg^{-1}, IV). Todos los tratamientos proporcionaron sedación y relajación muscular adecuadas. Se observaron diferencias entre los puntos de tiempo para la relajación de la lengua (VAS de la lengua en mm) y esto se observó mejor en T7 para el tratamiento DD y T5 para el tratamiento DS y se mantuvo significativamente más bajo que la línea de base a lo largo de los tiempos evaluados. El presente estudio sugiere que la detomidina proporciona sedación adecuada en caballos sometidos a examen dental, pero no hay necesidad de administración de diazepam como fue propuesto, ya que no fue encontrada ninguna diferencia significativa entre los tratamientos para las variables estudiadas. La adición de diazepam no mejoró la sedación ni disminuyó el movimiento de la lengua
Palabras clave: Odontología; Agonista adrenérgico alfa-2; Sedación; Benzodiacepina.

1. Introduction

Standing field procedures in oral cavity of horses requires cautious use of sedatives to avoid ataxia and tongue and head movements. Maintenance of standing horses provides lower anesthetic risk to the patient compared to general anesthesia (De Bont, 2021; Gozalo-Marcilla & Ringer, 2021) once the disadvantages of the decubitus will not occur, including the impairment on the gas exchange, neuropathy or myopathy, and injuries risks during induction and recovery. Moreover, sedation tends to promote lower hemodynamic depression compared to inhalant anesthesia (Menzies & Easley, 2014; Vigani &

Garcia-Pereira, 2014).

Oral cavity access is easier on standing position since horses have a limited jaws opening angle, which are thinner at the maxilla, thus imposing greater difficulty in inspection and palpation clinical examination. In addition, the presence of tongue and its movement are disputing factors in this small area (Dixon & Dacre, 2005; Hopster et al., 2013).

The common sedatives used for standing procedures are acepromazine, α2 agonist, opioids, ketamine and lidocaine. Acepromazine causes only mild sedation and does not provide analgesia but is used frequently as part of a standard protocol. The use of opioids in horses is still a major debate but currently its use is recommended in premedication to increase sedation and provide analgesia. Ketamine can be an useful analgesic, mostly in inflammatory cases, and can also be used as rescue intervention in case of lightening level of α2 agonist sedation. Lidocaine provides visceral analgesia, sedation among, it also promotes gastrointestinal motility, but has lower therapeutic index (Love et al., 2011; Vigani & Garcia-Pereira, 2014; Gozalo-Marcilla & Ringer, 2021).

The α2 agonist drugs represent the main pharmacological group indicated for sedation and maintenance of equines in standing position. This group promotes analgesic, muscle relaxant, anxiolytic, sedatives and cardiovascular function effects which are dose-dependent (Valverde, 2010; Vigani & Garcia-Pereira, 2014; Rohrbach et al.,2009). In addition, this group has antagonists that can be used to reverse sedative effects, but sedation recurrence can be observed if high doses of alpha2 agonist drugs have been used (Hubbell et al., 2013).

Detomidine is the most widely used sedative in horses because it has longer sedation compared with xylazine and the ataxia appears less significant (Valverde, 2010; Vigani & Garcia-Pereira, 2014). The combination of drugs with different pharmacological actions allows reduction in individual doses, thereby decreasing their side effects. α2-agonists have a short period of action, it is common to combine them with other drugs to induce neuroleptanalgesic and prolonged sedative effects (Shini, 2000; Valverde, 2010; Vigani & Garcia-Pereira, 2014).

Diazepam is a benzodiazepine that has been used widely as a good muscle relaxant in horses. Decreased tongue movement and increased tongue exposure after benzodiazepine administration have been cited but not properly reported in literature, which demonstrates the need for further research (Burnett, 2005; Hopster et al., 2013; Hubbell et al., 2013). Development of safer and effective protocols are necessary to allow standing approach and provide better visualization of oral cavity and less tongue movement, according to dental surgeon demand (Burnett, 2005; Menzies and Easley, 2014). This study aimed to determine if the use of diazepam associated with detomidine would increase the action of an α2 agonist (detomidine), mainly by tongue motion and chewing reduction without excessive destabilization.

2. Methodology

This quantitative experimental clinical trial was approved by the Ethical Committee for Animal Use (CEUA-UFMG) of Universidade Federal de Minas Gerais (UFMG) under the protocol 325/2018. The experimental research was done in the Laboratory of Horses Teaching, Research and Extension (LEPET) at Veterinary Hospital of UFMG Veterinary School.

Animals were placed in stocks where they remained for 30 minutes in order to adapt to containment before the experiment began. After this period, the following baseline (BL) variables were measured: heart rate (HR, in beats per minute-method base-apex lead) and cardiac rhythm assessment by digital electrocardiogram (Mediana®, model M20, USA); Respiratory Rate (RR, in movement per minute) by observing the movement of the ribs; Systolic Blood Pressure (SBP, in mmHg) by vascular Doppler (Parks Medical®, model 811-B, USA) with the probe positioned over the coccygeal artery; rectal temperature (RT, in °C) obtained through a digital clinical thermometer (Geratherm Medical, rapid GT model, Germany). All clinical variables were always measured by the same evaluator throughout the experimental period. During the procedure, the

duration of each stage of oral evaluation, the duration of the entire procedure and the number of rescues performed were also recorded.

After registered baseline variables, a 14-gauge catheter (Insyte Autoguard®, BD, Brazil) was aseptically inserted into one of the jugular veins for drug administration. An occlusion device (PRN adaptor®, BD, Brazil) was used to permit intermittent administrations.

Animals were randomly allocated in two groups (n=6/treatment), DD: detomidine (Detomidin®, Syntec, Brazil) 20 µg.kg^{-1} followed by diazepam (Ciazepam®, USP, Brazil) 20 µg.kg^{-1} two minutes later or DS: detomidine 20 µg.kg^{-1} followed by saline solution (Fisiológico, JP, Brazil) 0.004 mL.Kg^{-1} after the same time. All drug treatments were administered through the IV catheter. Sedation and muscle relaxation were assessed through the degree of ataxia (Table 1), head movement with tongue relaxation (Table 2) and Height of Head Above Ground (HHAG), with the latter evaluated by measuring tape. Tongue exposure was measured by graduated ruler. Animals showing tongue relaxation and head movement score ≤ 3 received an additional dose of detomidine (5µg.kg^{-1}, IV). The number of detomidine rescue required was recorded.

Table 1 - Evaluation of the degree of ataxia in horses.

Degree of Ataxia	Score
Muscular coordination, animal keeps its balance	1
Postural stability, with slight lateral movements	2
More intense movements, tendency to inclinations to one side of the stock	3
Supports the body in one side of the stock, pelvic limbs are crossed and presents sudden and frequent push ups on carpal articulation.	4

The table includes scoring system used to evaluate the degree of ataxia in horses within purpose of evaluate sedation and muscle relaxation of animals that received detomidine 20 µg.kg^{-1} and diazepam 20 µg.kg^{-1} (DD) or detomidine 20 µg. kg^{-1} and 0.9% NaCl saline 0.004 ml. kg^{-1}(DS) protocols. Modified from Roscoe, 2007. Source: Prepared by the authors.

Table 2 - Evaluation of the degree of tongue relaxation and head movement in horses.

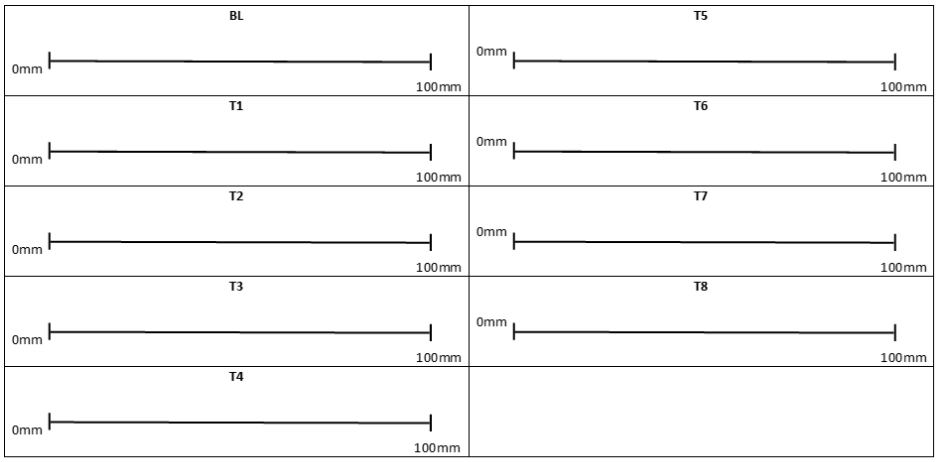
Score for degree of tongue relaxation and head movement in horses	Score
Not possible to manipulate and continuous head movement	1
Collects the tongue after stimuli or moves intensively/ Chewing and/or head movement continuous after stimuli.	2
Moderate movement on all tongue after stimuli/ Chewing and/or head movement moderate sometimes.	3
Little tongue movements after stimuli and chewing or head movement are occasional and discreet.	4
No tongue and head movement	5

The table includes scoring system used to evaluate the degree of tongue relaxation and head movement in horses within purpose of evaluate sedation and muscle relaxation of animals that received detomidine 20 µg.kg^{-1} and diazepam 20 µg.kg^{-1} (DD) or detomidine 20 µg.kg^{-1} and 0.9% NaCl saline 0.004 ml.kg^{1} (DS) protocols. Source: Modified from Roscoe, 2007. Source: Prepared by the authors.

Clinical variables were evaluated at the following experimental moments: BL (baseline variables); T1 (5 minutes after diazepam/ saline administration, when dental examination started with oral inspection without mouth gag and palpation); T2 (after the position of Hausmann mouth gag, when a thorough inspection and also cleaning of the oral cavity were done); T3 (after the end of the oral inspection, when speculum, light source and odontology mirror were used); T4 (after occlusal adjustment using a motorized diamond drill on left superior hemiarch); T5 (after occlusal adjustment of right superior

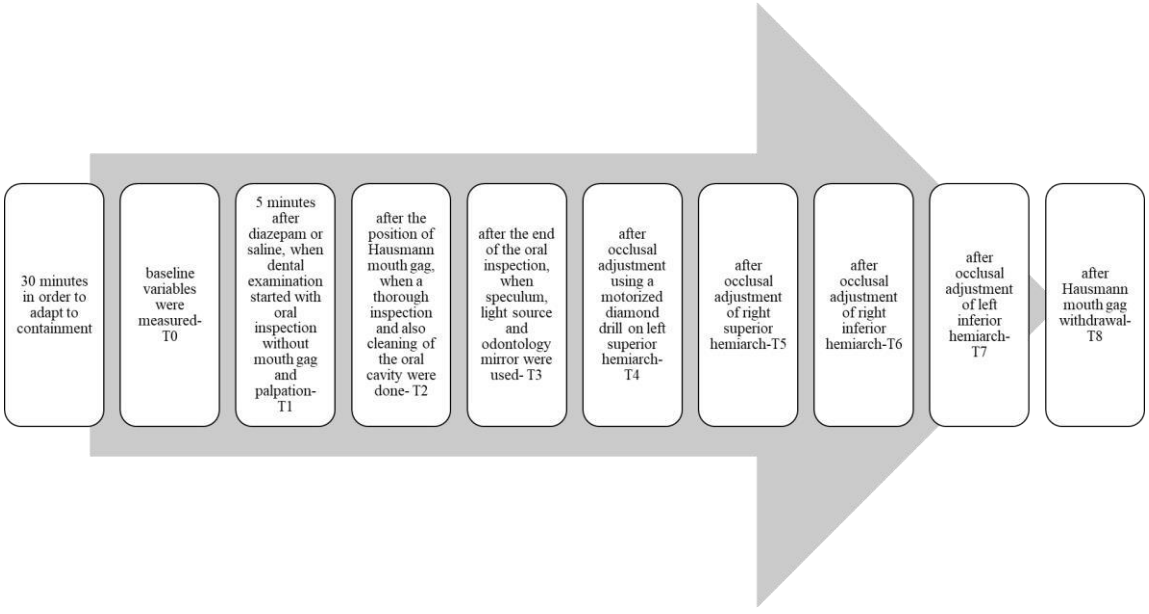
hemiarch); T6 (after occlusal adjustment of right inferior hemiarch) T7 (after occlusal adjustment of left inferior hemiarch); and T8 (after Hausmann mouth gag withdrawal). HHAG(%) was evaluated only at the moments BL, T1 and T8, since the animal's head was lifted after T1 for the dental procedure. Degree of ataxia, muscle relaxation of the tongue, head movement, tongue exposure (cm) and visual analog scale of tongue relaxation, with 0 being no relaxation and 100 the maximum relaxation, were evaluated (tongue VAS, mm) (Figure 1). Each variable was measured in all experimental moments by the same evaluator unaware of the treatments (Figure 2).

Figure 1 - Visual analog scale of tongue rela -xation (VAS, mm).



The figure includes the opinion of the surgeon about tongue relaxation in all experimental moments of animals that received detomidine 20 µg.kg^{-1} and diazepam 20 µg.kg^{-1} (DD) or detomidine 20 µg. kg^{-1} and 0.9% NaCl saline 0.004 ml. kg^{-1}(DS) protocols with 0 being no relaxation and 100 the maximum relaxation. Source: Prepared by the authors.

Figure 2 - Timeline of animal's experiment.



Timeline of animal's experiment. Source: Prepared by the authors.

Data were presented as mean and standard-deviation or median and total range. Normal distribution was evaluated by the Kolmogorov-Smirnov test and graphical methods. Time and protocol effects for the clinical variables HR, RR, SBP, PR interval, TºC, chin-floor distance and tongue VAS were evaluated by ANOVA after adjusting of a mixed model. The model

included fixed effects of protocol, time and protocol x time interaction The animal effect was considered random to account for repeated measures over time as in a repeated measure design (Bate and Clark, 2014). Symmetric, first-order, and unstructured component covariance structures were evaluated to model the dependence of repeated measurements. The chosen structure was based on the Akaike Information Criterion (AIC). Means comparisons were performed by the T-test. The Mann-Whitney test was used to analyze the protocol effect within each assessment time for second-degree atrioventricular block, score of ataxia, score of tongue relaxation and head movement and tongue exposure. For these variables, the effect of the evaluation time was analyzed using the Friedman test within each protocol. Comparisons between each pair of moments were performed using the Bonferroni-corrected Conover test. For tests, the null hypothesis was rejected when $P<0.05$. Statistical analyzes were performed using the softwares Statistical Analysis System (SAS 9.4) and R 9.0.4 (R Core Team, 2020).

3. Results and Discussion

The present study evaluated twelve adult horses from several breeds (seven males and five females) undergoing dental arch correction. Animals were between 2 and 19 years old and weighing 286±56kg (mean ± standard deviation) (Table 3).

Sedation quality and muscle relaxation were not different between DD and DS. At T7 tongue was more relaxed with the DD protocol while the DS protocol showed better results at T5, although it remained lower than the baseline during all evaluated times. There was no difference between protocols for procedure length and the number of detomidine rescue with predominance at T2 and T5 in both treatments. Total procedure time for DS and DD was 73.33 ± 13.40 and 66.83 ± 8.16 minutes, respectively (Table 4).

Table 3 - Age and weight values.

Treatment	Animal	1	2	3	4	5	6	Mean ± Standard deviation
DS	Age (years)	19	7	4	1.5	7	14	8.75 ± 6.54
	Weight (Kg)	235	368	315	230	413	240	300 ± 77.9
DD	Age (years)	17	3	1.5	4.5	2	2	5 ± 1.19
	Weight (Kg)	285	280	250	304	240	280	273.16 ± 23.75

The table includes values of age and weight of animals that received detomidine 20 µg.kg^{-1} and diazepam 20 µg.kg^{-1} (DD) or detomidine 20 µg. kg^{-1} and 0.9% NaCl saline 0.004 ml. kg^{-1}(DS) protocols. Source: Prepared by the authors.

Table 4 - Number of detomidine rescue received during the procedure.

PARAMETER	GROUP	T1	T2	T3	T4	T5	T6	T7	T8	T0TAL
DETOMIDINE RESCUE	DS	-	2	5	6	5	7	1	-	26
	DD	-	-	7	6	3	5	-	-	21
TIME LENGTH (min)	DS	5 (±0)	13 (±6)	9 (±4.8)	11.5 (±5.8)	10 (±4)	9 (±4.2)	10 (±4.2)	5.5 (± 3.2)	73.3 (±13.4)
	DD	5 (±0)	15 (±2.4)	7 (±3.3)	11 (±3.8)	8.5 (±1.5)	6 (±2.5)	6 (±2.3)	5.5 (±1)	66.8 (±8.16)

The table includes the first administration of detomidine rescue (5µg.kg^{-1}, IV), stages length and total time length of the procedure in animals that received detomidine 20 µg.kg^{-1} and diazepam 20 µg.kg^{-1} (DD) and detomidine 20 µg.kg^{-1} and 0.9% NaCl saline 0.004 ml.kg^{-1}(DS) (Mean ± Standard deviation). Source: Prepared by the authors.

There were no differences on hemodynamic variables evaluated (HR, RR, SBP, and PR interval) between the protocols. Compared to baseline, differences were observed in several time points. HR in DD and DS had a significant decrease with lower values measured after the end of oral inspection (T3). RR was lower at the final time (T8) in both groups (Table 5). SBP showed higher values after upper left hemiarch inspection (T4). PR interval remained at physiological values throughout the procedure, with similar values observed at 6 moments: after placing the mouth opener (T2), end of inspection (T3), upper left hemiarch (T4), upper right hemiarch (T5), lower left hemiarch (T6) and final moment (T8). with values ranged between 36.5 and 37.8 in both protocols There were no differences for rectal temperature over time with values ranged between 36.5 and 37.8°C in both protocols (Table 5).

Table 5 - Values of Heart Rate (HR), Respiratory Rate (RR), Systolic Blood Pressure (SBP), PR Interval, Tongue VAS and Rectal Temperature in horses (n = 6) before (baseline) and after administration of one of the 2 protocols: detomidine 20 µg.kg^{-1} and diazepam 20 µg.kg^{-1} (DD) or detomidine 20 µg.kg^{-1} and 0.9% NaCl saline 0.004 ml.kg^{-1}(DS).

PARAMETER	GROUP	BL	T1	T2	T3	T4	T5	T6	T7	T8
HR (beat/min)	DS	37 ±6^{a}	24 ±4^{c}	26 ±4^{b}	23 ±3^{c}	27 ±3^{b}	26 ±4^{b}	26 ±3^{c}	28 ±3^{b}	26 ±3^{b}
	DD	39 ±4 ^{a}	32 ±4 ^{c}	33 ±4 ^{b}	31 ±4 ^{c}	33 ±4^{b}	33 ±4 ^{b}	32 ±4 ^{c}	33 ±3 ^{b}	32 ±3 ^{b}
RR (breath/min)	DS	24 ±4^{a}	15 ±2^{b}	15 ±1^{bc}	14 ±1^{bc}	15 ±1^{b}	14 ±2^{bc}	13 ±1^{c}	13 ±2^{c}	11 ±1^{d}
	DD	24 ±3^{a}	12 ±1^{b}	12 ±1^{bc}	14 ±5^{bc}	13 ±1^{bc}	13 ±1^{bc}	11 ±1^{c}	11 ±2^{c}	11 ±2^{d}
SBP (mmHg)	DS	124 ±18^{c}	147 ±21^{bc}	147 ±20^{bc}	153 ±23^{a}	179 ±26^{a}	164 ±22^{a}	166 ±18^{a}	160 ±12^{a}	133 ±12^{bc}
	DD	150 ±12^{c}	173 ±22^{bc}	173 ±17^{bc}	175 ±27^{a}	185 ±15^{a}	185 ±16^{a}	185 ±14^{a}	179 ±13^{a}	159 ±11^{bc}
Interval PR (s)	DS	0.30 ±0.01^{b}	0.30 ±0.01^{b}	0.32 ±0.01^{a}	0.32 ±0.01^{a}	0.33 ±0.01^{a}	0.32 ±0.01^{a}	0.31 ±0.01^{a}	0.30 ±0.01^{b}	0.31 ±0.01^{a}
	DD	0.29 ±0.01^{b}	0.32 ±0.01^{b}	0.32 ±0.01^{a}	0.32 ±0.01^{a}	0.31 ±0.01^{a}	0.34 ±0.02^{a}	0.32 ±0.01^{a}	0.33 ±0.01^{b}	0.32 ±0.01^{a}
Tongue VAS (mm)	DS	85.8 ±6.6^{a}	26.8 ±12.0^{b}	31.0 ±11.9^{b}	22.5 ±5.9^{b}	26.7 ±8.3^{b}	22.0 ±9.7^{b}	26.5 ±7.9^{b}	24.3 ±7.9^{b}	26.7 ±8.8^{b}
	DD	87.2 ±4.4^{a}	25.5 ±8.8^{b}	24.7 ±9.0^{b}	25.8 ±7.7^{b}	26.3 ±7.8^{b}	27.2 ±5.7^{b}	31.2 ±7.3^{b}	24.0 ±4.2^{b}	24.5 ±4.4^{b}
Rectal T°	DS	37 ±0.3	37.1±0.3	36.9±0.3	37±0.3	36.9±0.3	36.9±0.3	36.6±0.3	36.8±0.3	36.5±0.3
	DD	37.6±0.4	37.8±0.4	37.6±0.4	37.6±0.4	37.6±0.4	37.4±0.4	37.4±0.4	37.3±0.4	37.3±0.4

Values presented as Mean ± Standard deviation
* Distinct lowercase letters on lines represent time difference by T-test ($p < 0.05$)
T1: 5 minutes after treatment administration, when dental examination started with oral inspection without mouth gag and palpation; T2: after position of Hausmann mouth gag, when a thorough inspection and also cleaning of oral cavity were done; T3: after the end of oral inspection, when speculum, light source and odontology mirror were used; T4, T5, T6 and T7: after occlusal adjustment using motorized diamond drill on left and right superior and right and left inferior hemiarch, respectively; T8: after Hausmann mouth gag withdrawal.
Source: Prepared by the authors.

For second degree atrioventricular block, tongue exposure, degree of ataxia, tongue muscle relaxation with or not head movement and percentage distance HHAG, differences were observed between the evaluation periods and the baseline. In both protocols the behavior of those variables were similar (Table. 6). For the Q-S Distance (%) a reduction on the distance in 44.46 % for the DS protocol at 5 minutes after administration (T1) and 49.01% at the final moment (T8) was noticed.

Moreover, a reduction of 40.83% for DD at 5 minutes after administration and 50.74% at the end of the experiment (T8) was also observed (Table 6). Seven animals (58.3%) had second-degree atrioventricular block at some point of the experiment. For tongue exposure, the highest values were found at the inspection without mouth opening (T1) for DS and inspection with mouth opening (T2) for DD (Table 6). The highest degree of ataxia was observed at 5 minutes after administration (T1) for both groups. For degree of tongue muscle relaxation and head movement (MRT/HM) the best relaxation was observed at 5 minutes after administration (T1) for both protocols. The MRT/HM remained significantly lower than baseline during all evaluated times (Table 6). There was no difference between protocols for total procedure length and number of detomidine rescue.

Table 6 - Second degree atrioventricular block (2 ° AVB), tongue exposure, degree of tongue muscle relaxation and head movement (MRT / HM), degree of ataxia, height of head above ground (HHAG-%).

PARAMETER	GROUP	T0	T1	T2	T3	T4	T5	T6	T7	T8
2° AVB (%)	DS	0(0) [a]	0(0-17.8)[b]	0(0-9.3)[b]	0(0-14.2)[b]	0(0-14.2)[b]	0(0-14.8)[b]	0(0-14.8)[b]	0(0-19.2)[b]	0(0-19.2)[b]
	DD	0(0)[a]	0(0-19)[b]	0(0-35)[b]	0(0-22.7)[b]	0(0-15.3)[b]	0(0-38.8)[b]	0(0-22.7)[b]	0(0-18.1)[b]	0(0-22.7)[b]
TONGUE EXPOSURE (cm)	DS	0 (0)[a]	15 (14-16)[b]	13.5 (11-16)[b]	12 (11-15)[b]	12 (10-15)[b]	12 (10-14)[b]	12 (11-15)[b]	11 (10-15)[b]	13 (10-14)[b]
	DD	0 (0)[a]	12.5 (11-14)[b]	13.5 (9-15)[b]	11.25 (9-15)[b]	13.5 (10-16)[b]	11.5 (9-16)[b]	12 (9-14.5)[b]	10.5 (9-15)[b]	11 (8-14)[b]
MRT/HM	DS	1(1)[a]	5(2-5)[b]	3(3-4)[b]	2.5(2-3)[b]	3(2-4)[b]	3(2-4)[b]	3(2-4)[b]	3(2-4)[b]	3(1-4)[b]
	DD	1(1)[a]	4.5(3-5)[b]	3(2-4)[b]	3(2-4)[b]	4(3-4)[b]	3.5(2-4)[b]	4(3-4)[b]	3(3-4)[b]	3(3-4)[b]
DEGREE OF ATAXIA	DS	1(1)[a]	4(2-4)[b]	4(2-4)[b]	4(2-4)[b]	4(2-4)[b]	2(2-3)[b]	3.5(2-4)[b]	2.5(2-4)[b]	2.5(2-4)[b]
	DD	1(1)[a]	3.5(2-4)[b]	3.5(2-4)[b]	3.5(3-5)[b]	3.5(2-5)[b]	4(2-4)[b]	4(3-4)[b]	3.5(3-4)[b]	3(3-4)[b]
HHAG (%)	DS	100[a]	44.46[b]	-	-	-	-	-	-	49.01[b]
	DD	100[a]	40.83[b]	-	-	-	-	-	-	50.74[b]

Distinct lowercase letters in lines represent time difference using the Friedman Test ($p < 0.05$).
T1:5 minutes after treatment administration, when dental examination started with oral inspection without mouth gag and palpation; T2: after position of Hausmann mouth gag, when a thorough inspection and also cleaning of oral cavity were done; T3: after the end of oral inspection, when speculum, light source and odontology mirror were used; T4, T5, T6 and T7: after occlusal adjustment using motorized diamond drill on left and right superior and right and left inferior hemiarch, respectively; T8: after Hausmann mouth gag withdrawal.
Source: Prepared by the authors.

This study intended to associate diazepam with detomidine due to its synergistic effect with detomidine, resulting in better muscle relaxation. An α2 agonist (detomidine) primarily acts by reducing tongue movement and chewing without excessive destabilization. Nevertheless, this effect was not observed. Explanations for this may be due to individual variation or the short effect of diazepam since it was administered only as bolus (2 minutes after detomidine), although the half-life elimination of diazepam in horses is 6.9- 13.2 hrs with peak of action in few minutes (Muir et al.,1982; Shini, 2000; Schenk et al., 2021).

In order to maintain sedation for a longer period, rescue bolus of detomidine was chosen rather than increase initially dose because the procedure might require longer sedation time.

The animals were sedated to perform clinical procedures such as dental examination, oral inspection with mouth gag and occlusion adjustment. Physical restraint and mouth manipulations represent potential stress-inducing factors for animals. In this study, three animals from DD treatment, which were the most reactives, did not have adequate detomidine sedation with decreased sedative effect, which may have interfered in the results (Burnett, 2005; Fernandes et al., 2016; Müller, et al., 2017).

Animals presented some degree of ataxia and head lowering 5 minutes after drug application. Those effects have

already been described in horses after α2 agonists administration (Burnett, 2005; Roscoe 2007; Hopster et al., 2013; Ringer et al., 2013). Both proposed protocols have a fast latency period, allowing them to be used in practice (Burnett, 2005). Excessive ataxia is unwanted in stand-up procedures in horses, thus a lower dose of diazepam was chosen in order not to increase the ataxia promoted by detomidine because detomidine promotes important dose-dependent muscle relaxation (Muir et al., 1982; Burnett, 2005; Fernandes et al., 2016; Hopster et al., 2013; Müller, et al., 2017; Ringer, et al., 2013). In the present study with 20 $\mu g.kg^{-1}$ administration, relaxation did not cause adverse effects. Furthermore, relaxation and side effects were not increased with diazepam combination.

Detomidine use for sedation may cause bradycardia, as observed in all treatments, due to the predominance of central effects, presynaptic receptor activation on Sympathetic Nervous System (England et al.,1992; Valverde, 2010). Detomidine will also increase vagal tonus with decreased myocardial contractility and predisposition to cardiac arrhythmias, such as increased PR interval and 1º AV block (Buhl et al., 2007; Valverde, 2010). According to Buhl et al. (2007), detomidine at a dose of $10\mu g.kg^{-1}$ is already capable of cause significant effect on cardiac function such as valve regurgitation. Occurrence of 1º and 2º degree AV blocks may occur when animal has excessive bradycardia. AV blocks in horses may have no clinical meaning being normal in resting horses (England et al., 1992; Menzies-Gow, 2001; Valverde, 2010). Seven horses experienced this arrhythmia at some moment in both groups due to detomidine administration as no treatments effects were observed.

SBP increased in both protocols, indicating transitory hypertension. This can be explained by the additional dose administered at times T2 to T7 when hypertension remained sustained. Many studies using α2agonists in horses have shown episode of hypertension (Yamashita et al., 2000; Valverde, 2010). The sedative and physiologic effects of detomidine were correlated in a dose-dependent manner, in a way that when high doses (above 40 $\mu g.kg^{-1}$) are used it will result in a prolonged increase of peripheral vascular resistance and hypertension (Valverde, 2010; Yamashita et al., 2000).

The respiratory rate (RR) decreased in both groups, although without clinically significant importance (Nyman et al., 2009). The alteration is explained due to the central depressing effect caused by the use of α2 agonists.

There are only a few studies describing drugs effect on horse tongue movement. The use of diazepam (10 $\mu g .kg^{-1}$, IV) was mentioned in a procedure for tongue relaxation in horses, however this was not a controlled clinical study (Burnett, 2005; Stoll, 2007). These studies reported improved sedation quality, decreased chewing and better mouth gag tolerance in sedated horses (Hopster et al., 2013; Müller et al., 2017).

The main limitations of this study were breed and sex variation between the experimental groups. However, in a clinical study it is sometimes not possible to control all concurrent variables. Nonetheless, probably the dose used was not sufficient to achieve and maintain an effective plasma concentration of the drugs which may have been responsible for the short-term effect on the tongue.

4. Conclusion

Administration of detomidine $20\mu g.kg^{-1}$ plus diazepam $20\mu g.kg^{-1}$ or detomidine $20\mu g.kg^{-1}$ promotes adequate sedation and allows well-performed dental examination procedures. Due to the lack of differences between protocols for tongue relaxation, HHAG %, and detomidine rescue administration it is inferred that there is no need to use diazepam at the dose or methodology that we proposed in this study. Both sedation protocols led to minimal cardiovascular changes without increasing the risk of cardiorespiratory complications. Considering the limitations, more studies are required with a greater number of animals.

Further studies using other doses are needed in this species and we suggest additional studies to evaluate the pharmacological association of diazepam and detomidine in a constant rate infusion.

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