

# Opioid-free sedation for atlantoaxial cerebrospinal fluid collection in adult horses

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## Abstract

**Background:** Atlantoaxial (AA) cerebrospinal fluid (CSF) collection in standing horses utilizes the controlled narcotic morphine, thereby limiting feasibility in field practice settings.

**Objectives:** To compare AA CSF collection time and reaction scores in horses sedated with morphine-containing and opioid-free sedation protocols: detomidine + morphine (DM), detomidine + xylazine (DX), detomidine + detomidine (DD), detomidine alone (D0); To develop a novel method for assessing sedation in standing horses using open-source motion-tracking software.

**Animals:** Six healthy adult horses.

**Methods:** Randomized crossover. Atlantoaxial CSF collections were performed weekly for 4 weeks. Horses received sedation protocols in random order. Procedure time and procedure reaction scores were compared between groups using Friedman test. Associations between procedure reaction scores and motion tracking variables (total distance and farthest excursion traveled by horse's head) were examined using scatter diagrams and linear regression.

**Results:** Procedure times were lower in horses sedated with DX (median: 36 seconds; range: 28 – 188), compared to D0 (121; 35 – 196;  $P = 0.04$ ). Procedure reaction scores were lower in horses sedated with DX (median 1.0, range 1.0 to 2.0), or DM (1.8; 1.3 – 3.0) compared to D0 (3.0; 2.3 – 3.0;  $P = .03$ ). Reactions to dura mater puncture were recorded in 3 of 6 horses in D0 and DD groups, and 0 of 6 horses in DX and DM groups. Positive associations were observed between reaction score vs total distance or farthest excursion distance from baseline.

**Conclusions and Clinical Importance:** Both opioid-free and morphine-containing sedation protocols are acceptable for AA CSF collection. Motion-tracking software represents a novel method for assessing sedation in standing horses.

## KEYWORDS

cerebrospinal fluid, CSF, equine neurology, equine sedation, neurology diagnostics

**Abbreviations:** AA, atlantoaxial; CSF, cerebrospinal fluid; D0, detomidine alone; DD, detomidine + detomidine; DEA, Drug Enforcement Administration; DM, detomidine + morphine; DX, detomidine + xylazine; HHAG, height of the head above the ground; LS, lumbosacral.

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## 1 | INTRODUCTION

Cerebrospinal fluid (CSF) is a critical sample in the diagnostic work up of horses with neurologic disease. The traditional method of CSF collection from the lumbosacral (LS) space in standing horses is often avoided by equine practitioners because of technical difficulty and unpredictable, sometimes violent, horse reactions when the spinal needle punctures the lumbar spinal cord dura. More recently, a method describing ultrasound-guided CSF collection from the atlantoaxial (AA) space in standing horses<sup>1</sup> offers advantages over LS CSF collection including direct visualization of the anatomic target and less blood contamination of the sample.<sup>2</sup> This method, however, recommends the administration of morphine for its central nervous system analgesic effects and its purported ability to prevent negative reaction to puncture of the dura mater.<sup>1</sup> Morphine, a mu agonist opioid, is classified by the Drug Enforcement Administration (DEA) as a Schedule II controlled narcotic, making it legally and logistically difficult for veterinarians to use in the field practice setting. Identification of opioid-free sedation protocols that provide an acceptable plane of sedation for AA CSF collection in standing horses would enable veterinarians to more readily perform this procedure in practice.

The primary objective of this study was to compare (a) time to AA CSF collection and (b) procedure reaction scores in response to 1 of 4 sedation protocols containing detomidine + morphine (DM), detomidine + xylazine (DX), detomidine + detomidine (DD), or detomidine alone (D0) in healthy adult horses. We hypothesized that opioid-free sedation protocols (DX, DD, and D0) would provide comparable sedation quality, procedural time, and CSF collection success when compared to the morphine-containing protocol (DM). A secondary objective was to develop a novel method to measure sedation depth and quality in standing healthy horses using open-source motion tracking software. We hypothesized that motion tracking outcomes such as the total distance traveled by the horse's head and farthest excursion from baseline position during CSF collection would correlate with procedure reaction scores assigned by study personnel.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals

Six adult university-owned horses were prospectively enrolled in this study. Horse ages ranged from 3 to 15 years (mean 7.8 years; SD 4.1 years) and included 3 mares and 3 geldings. Weights ranged from 417 to 572 kg (mean 504.9 kg; SD 54.5 kg). Breeds represented included Thoroughbred (5) and Oldenburg (1). All experimental procedures were approved by the University of Florida Institutional Animal Care and Use Committee; protocol #201910880. All horses were evaluated with physical examinations and deemed healthy before the start of the study. Horses were group housed in outdoor paddocks throughout the study and brought into individual box stalls on CSF collection days. Horses were allowed ad libitum access to water and coastal Bermuda hay for the duration of the study.

### 2.2 | Study design and sedation protocols

In this randomized crossover clinical trial, standing AA CSF collections were performed on all horses at weekly intervals for 4 weeks. In addition to the standard morphine-containing protocol (DM), study sedation protocols (DX, DD, and D0) were selected using non-controlled sedatives at fixed doses commonly administered in equine practice. Each horse received all 4 sedation protocols, in random order, over the course of the study. The order in which treatments were administered was determined using an online random number generator (<http://www.randomizer.org/>). The person performing all CSF collection (G. Cock) was blinded to all sedation protocols for the duration of the study.

For each CSF collection, a 2-part sedation administration model was used wherein horses are sedated with an initial dose of detomidine (Dormosedan, Zoetis, Parsippany, New Jersey) before aseptic skin preparation and then administered an additional sedation 3-5 minutes before centesis.<sup>1</sup>

Treatment groups were as follows:

DM: detomidine 5 mg IV + morphine sulfate 30 mg IV

DX: detomidine 5 mg IV + xylazine 150 mg IV

DD: detomidine 5 mg IV + detomidine 2 mg IV

D0: detomidine 5 mg IV alone

### 2.3 | Cerebrospinal fluid collection

Horses were restrained in stocks for all CSF collections. The proximalateral neck directly over the AA joint was clipped. Each horse was then administered detomidine (5 mg IV) and the clipped area was aseptically prepared. Lidocaine hydrochloride (6 mL total; 2% solution) was administered subcutaneously and intramuscularly at the intended injection site to provide local analgesia. A second sedative dose was then administered according to protocol (morphine [30 mg IV], xylazine [150 mg IV], detomidine [2 mg IV], or nothing) 3-5 minutes before centesis. Atlantoaxial CSF collection was performed under ultrasound guidance as previously described<sup>1</sup> using a 3.5-in. 18-gauge spinal needles (Mila International, Florence, Kentucky). Horses' heads were rested, but not restrained, on a head stand throughout the procedure. All CSF collections were video captured using a Nikon D5600 digital single lens reflex camera. Procedure success was defined as CSF flowing into the syringe, after which the spinal needle was removed and 1% diclofenac sodium cream (Surpass, Boehringer Ingelheim, Duluth, Georgia) was applied to the centesis site. Horses were administered flunixin meglumine (Banamine, Merck Animal Health, Madison, New Jersey; 500 mg IV) immediately after the procedure. Horses were supported with their heads at wither level or above until fully recovered from sedation and returned to their respective paddocks at the end of the day. At 24-hour post-procedure, horses were monitored with physical examination and were administered flunixin meglumine (500 mg IV). Centesis sites were evaluated for swelling, heat, or pain upon palpation.

## 2.4 | Motion-tracking software and data acquisition

During all CSF collections, open-source motion tracking software was utilized to track and quantify horse head motion as follows: All procedures were video recorded using a MacBook Pro webcam operating on Catalina 10.15.5. The distance from the webcam to the horse's head (97 cm), as well as the webcam height from the floor (132 cm), was measured and remained constant throughout the study. Before CSF collection, a 3-4-cm-diameter water-soluble purple paint dot was applied to the center of horse's forehead. The color purple was chosen because of its stark contrast with the colors of the horses and surrounding environment. An open-source software algorithm, "Ball Tracker," available through Python3 (version 3.8.2; <https://www.python.org/>)<sup>3,4</sup> and OpenCV (version 4.3.0; <https://opencv.org/>)<sup>5,6</sup> was modified to identify the purple paint dot and track it as a focal region of interest, termed a "centroid." During CSF collection, the Ball Tracker algorithm traced the path of the horse's head through continuous plotting of the centroid's x,y coordinates as a function of time. Details of the modified code used for this study are provided in Data S1 (*Modified Ball Tracker source code for centroid tracking*).

## 2.5 | Data collection

### 2.5.1 | CSF collection

After completion of the study, video recordings of all 24 CSF collections were reviewed. Procedure time, defined as the total time from initial needle insertion to acquisition of CSF, was recorded. All CSF collections were performed with only 1 needle insertion, that is, a second attempt was not required at any timepoint. Successful acquisition of CSF (yes/no) and reactions to the needle puncturing the dura mater (yes/no; defined as sudden head movement when needle entered dura) were also recorded. Three blinded reviewers reviewed all videos and assigned each CSF collection procedure a "procedure reaction score" of 1-4 based on the horse's response to the procedure using a scale adapted from Chidlow et al.<sup>2</sup> Scores were defined as: 1 = no reaction, head remains still; 2 = small movement of head or neck; 3 = head jerk; 4 = severe adverse reaction, CSF collection not possible. For data analysis, reviewer scores were averaged to produce a mean procedure reaction score for each CSF collection.

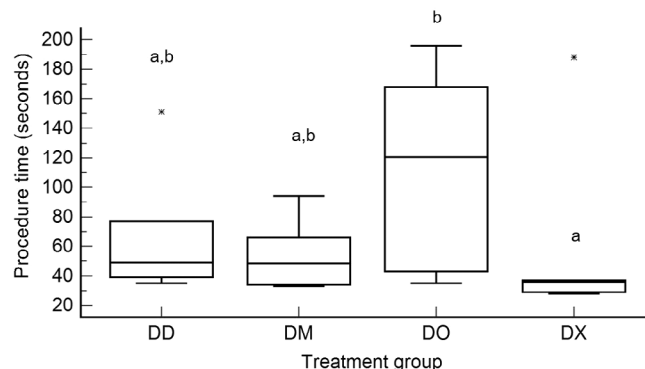
### 2.5.2 | Motion tracking

For each CSF collection, continuous x,y coordinate data generated by the Ball Tracker algorithm were used to calculate the total distance traveled by the centroid (in pixels) as well as the farthest excursion distance from baseline (in pixels). Graphical plots of the centroid path and total distance traveled as functions of time were generated using open-source software at Matplotlib (version 3.2.1; <https://matplotlib.org/>)<sup>7</sup> and NumPy (version 1.19.0; <https://numpy.org/>)<sup>8-10</sup>

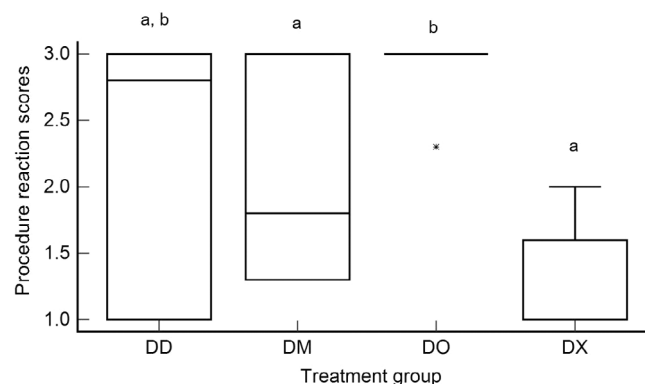
## 2.6 | Data analysis

### 2.6.1 | CSF collections

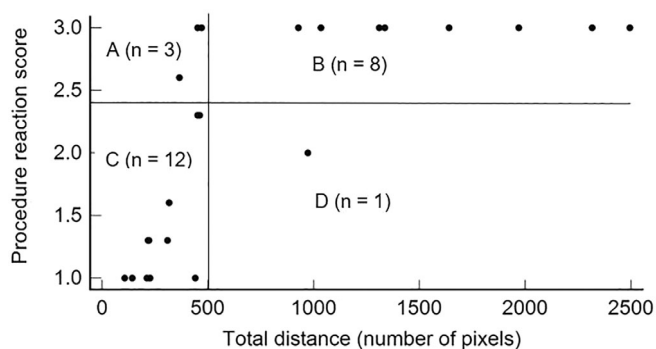
Normality of the data was assessed using histogram examination and the Shapiro-Wilk test. The variables for procedure time and procedure reaction scores were not independent and were not normally distributed. The null hypotheses that procedure times and procedure



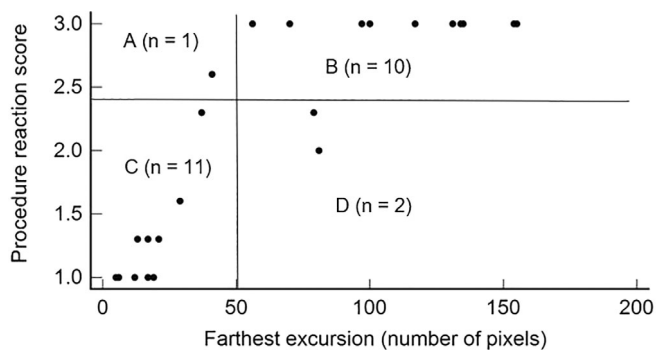
**FIGURE 1** Procedure time (time from needle introduction to acquisition of CSF) compared across treatment groups of studied horses. DD = detomidine + detomidine; DM = detomidine + morphine; DO = detomidine alone; DX = detomidine + xylazine. Horizontal line inside box indicates median value. Top and bottom of the box indicate first and third quartiles. Vertical lines on the top and the bottom of the box indicate minimum and maximum values. Asterisks show possible outliers (151 and 188 seconds). Treatment groups with different superscripts (a,b) indicate distributions for procedure times were statistically different ( $P < .05$ )



**FIGURE 2** Procedure reaction scores compared across treatment groups of studied horses. DD = detomidine + detomidine; DM = detomidine + morphine; DO = detomidine alone; DX = detomidine + xylazine. Horizontal line inside box indicates median value. Top and bottom of the box indicate first and third quartiles. Vertical lines on the top and the bottom of the box indicate minimum and maximum values. Asterisk shows a possible outlier (score = 2.3). Treatment groups with different superscripts (a, b) have statistically different median procedure reaction scores ( $P < .05$ )



**FIGURE 3** Scatter diagram showing an association between procedure reaction score and total distance traveled (number of pixels) in 24 horses, that is, number of paired observations (data points) of sedation scores (y-axis) and total distance (x-axis) in 24 study horses. Horizontal reference line indicates the median score (2.4). The vertical reference line indicates the median total distance (500.0). Quadrant B + C vs quadrant A + D = 8 + 12 vs 3 + 1 (or  $0.33 + 0.50 = 0.83$  vs  $0.13 + 0.04 = 0.17$ ). In quadrant C, 2 data points are 2 paired data, where the sedation score = 1.0 and total distance = 142.4 or 143.1; 2 additional data points are 2 paired data, where the sedation score = 1.3 and total distance = 218.5 or 220.6

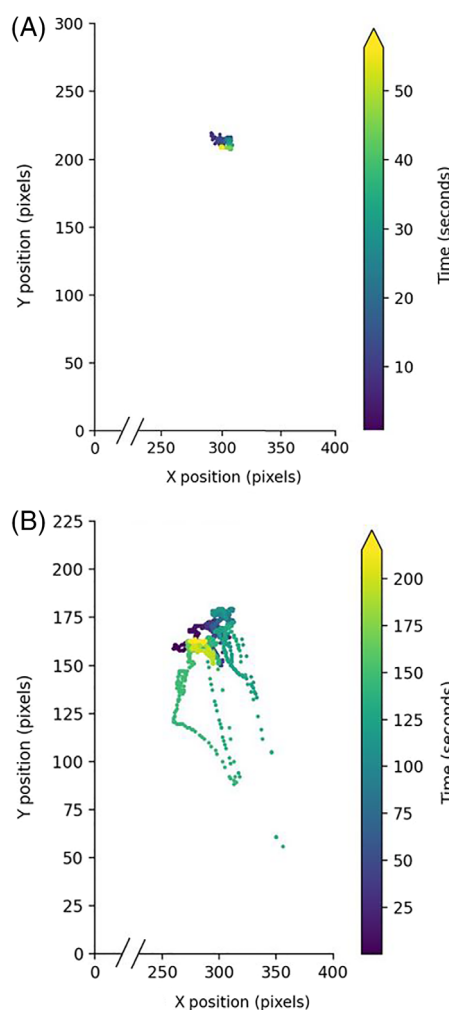


**FIGURE 4** Scatter diagram showing an association between procedure reaction score and farthest excursion distance from baseline (number of pixels) in 24 horses, that is, number of paired observations (data points) of sedation scores (y-axis) and farthest excursion (x-axis) in 24 study horses. Horizontal reference line indicates the median score (2.4). The vertical reference line indicates the median total distance (50.0). Quadrant B + C vs quadrant A + D = 10 + 11 vs 1 + 2 (or  $0.42 + 0.46 = 0.88$  vs  $0.04 + 0.08 = 0.12$ ). In quadrant C, 2 data points are 2 paired data, where the sedation score = 1.0 and farthest excursion = 6.0 or 6.1

reaction scores were not different between treatment groups were tested using the non-parametric Friedman test.

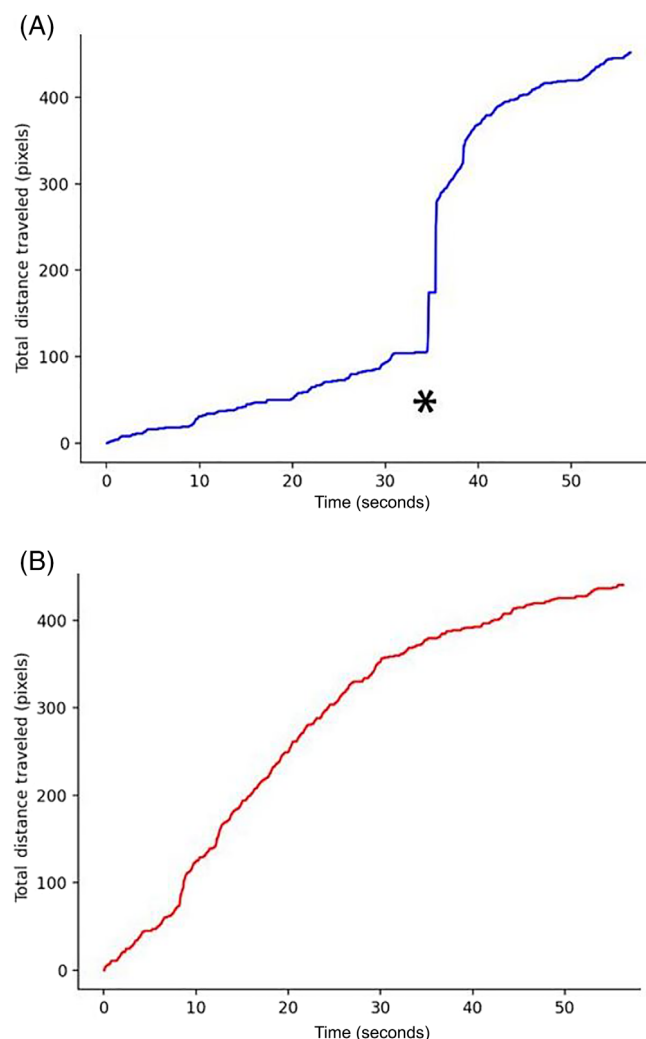
### 2.6.2 | Motion tracking

The correlations between procedure reaction score and total distance traveled or farthest excursion distance from baseline were examined by constructing scatter diagrams.<sup>11</sup> Scatter diagrams were constructed using 24 paired observations (data points) of procedure reaction scores



**FIGURE 5** Examples of motion paths traveled (in pixels) by 2 study horses' heads during AA CSF collection, captured using motion tracking software and displayed as x,y coordinates over time. (A) This horse was sedated with detomidine + xylazine (DX) and displayed minimal movement during CSF collection. Procedure time was 37 seconds and procedure reaction score was 1. (B) This horse was sedated with detomidine alone (DO), and displayed multiple head movements throughout the procedure. Procedure time was 196 seconds and procedure reaction score was 3

(y-axis) and total distance traveled or farthest excursion distance from baseline (x-axis). Two reference lines were drawn to estimate proportions of observations in positive quadrants (B and C) and negative quadrants (A and D) and to reflect the strength of association between procedure reaction score and total distance traveled or farthest excursion distance. Reference lines were based on the median distribution of procedure reaction score (Y) and total distance traveled or farthest excursion distance (X). In addition, the association between procedure reaction score and total distance traveled or farthest excursion distance from baseline were examined using linear regression analysis. For all analyses, values of  $P < .05$  were considered significant. Analyses were performed using commercially available statistical software (MedCalc Software Ltd., Ostend, Belgium).



**FIGURE 6** Examples of total distance traveled (in pixels) by 2 study horses' heads during AA CSF collection, captured using motion tracking software and displayed as a function of time. (A) This horse was sedated with detomidine + detomidine (DD). The sharp increase in distance traveled noted at approximately 34 seconds (asterisk) correlates to a head jerk in response to dural puncture. Procedure time was 43 seconds and procedure reaction score was 3. (B) This horse was sedated with detomidine + xylazine (DX) and displayed minimal head movement throughout the procedure. Procedure time was 37 seconds and procedure reaction score was 1

### 3 | RESULTS

#### 3.1 | CSF collection

All 6 horses remained healthy throughout the study. Slight swelling at the centesis site was occasionally noted in the 24-hour period post-CSF collection. All swelling was self-limiting and no additional intervention was required. Cerebrospinal fluid was successfully collected in all horses at all timepoints. Median procedure time across all groups was 43 seconds (minimum: 28 seconds; maximum: 196 seconds). Median procedure times at each timepoint (weeks 1, 2, 3, 4) were 99, 52, 34, and 36 seconds, respectively. Procedure times by

treatment group were as follows: DD (median: 49 seconds; range: 35 – 151 seconds), DM (49; 33 – 94), D0 (121; 35 – 196), and DX (36; 28 – 188). Procedure times were significantly lower in horses sedated with DX compared to D0 ( $P = 0.04$ ; Figure 1). Median procedure reaction score across all groups was 2.3 (minimum: 1.0; maximum: 3.0). Cerebrospinal fluid was successfully obtained at every procedure, thus, no procedure reaction scores of 4 were assigned during the study. Median procedure reaction scores at each timepoint (weeks 1, 2, 3, 4) were 3.0, 1.65, 1.95, and 1.95, respectively. Procedure reaction scores by treatment group were as follows: DD (median score: 2.8; minimum: 1.0, maximum: 3.0), DM (1.8; 1.3, 3.0), D0 (3.0; 2.3, 3.0), and DX (1.0; 1.0, 2.0). Procedure reaction scores were significantly lower in horses sedated with DX or DM compared to D0 ( $P = 0.03$ ; Figure 2). Reactions to dura mater puncture were noted in 3 of 6 horses in the D0 and DD treatment groups, and 0 of 6 horses in the DX and DM treatment groups.

#### 3.2 | Motion tracking

The Ball Tracker motion-tracking algorithm performed successfully throughout all CSF collections. Median total distance traveled by the horse's head (as determined by centroid path) during CSF collection was 451 pixels (minimum: 107 pixels; maximum: 2494 pixels). Median farthest excursion distance from baseline during CSF collection was 49 pixels (minimum: 5 pixels; maximum: 155 pixels). Evaluation of the scatter diagrams revealed positive associations between procedure reaction score and total distance traveled (Figure 3) as well as farthest excursion distance from baseline (Figure 4) in study horses. Using linear regression, the estimated correlations ( $r$ ) between procedure reaction score and total distance traveled or farthest excursion distance from baseline were .71 and .87, respectively ( $P < .001$ ). Plots displaying the centroid's path as a function of time (Figure 5) as well as the centroid's total distance traveled (Figure 6) were generated for each CSF collection procedure.

### 4 | DISCUSSION

In this study, we have identified non-narcotic sedation protocols suitable for standing AA CSF collection in adult horses, thereby enabling veterinarians to perform this diagnostic procedure in their practices without the risks and regulations associated with the use of controlled medications. In our study horses, sedation with a combination of detomidine and xylazine provided similar procedural times and sedation quality when compared to morphine-containing protocols for performing AA CSF collection. Furthermore, sedation with a combination of detomidine and xylazine was associated with 100% procedural success and no dura mater puncture reactions in any study horse. Detomidine and xylazine are alpha-2 adrenoreceptor agonists with similar mechanisms of action, differing primarily in time to onset and duration of action.<sup>12,13</sup> Both produce reliable sedation and analgesia with minimal side effects when used in healthy horses and are widely used in equine practice in both field and hospital settings.



Morphine is classified as a schedule II controlled substance, making it legally and logistically difficult for veterinarians to carry on their trucks for use in a field setting. A mu agonist opioid, morphine is a potent central nervous system analgesic, and has been reported to reduce horses' reactions to dura mater puncture during AA CSF collection.<sup>1</sup> In response to the current nationwide opioid crisis involving both manufacturing shortage and an increasing opioid abuse epidemic, the FDA and American College of Veterinary Anesthesia and Analgesia have published recommendations urging veterinarians to seek opioid-free alternatives in clinical practice.<sup>14,15</sup> Butorphanol, an opioid agonist-antagonist frequently utilized in equine practice, was not selected for use in the present study nor in previous AA CSF reports<sup>1,2</sup> because of its tendency to cause excitement and increased head movement and locomotion when administered intravenously to horses.<sup>16,17</sup> Romifidine, another alpha-2 agonist, has been used for AA CSF collection,<sup>2</sup> but was not chosen for this study because of its inconsistent use in equine practice. Rather than administering treatments on a mg/kg basis, fixed doses commonly administered in equine practice were selected to better emulate a field practice setting.

In this study, administration of a single dose of 5 mg detomidine IV did not provide an acceptable depth of sedation for standing AA CSF collection, resulting in significantly longer procedure times and higher procedure reaction scores when compared to other sedation protocols evaluated. Both sedation protocols containing only detomidine (D0 and DD) were associated with dura mater puncture reactions in 50% of the study horses, while no dural puncture reactions were noted in horses sedated with detomidine in combination with xylazine or morphine (DX and DM). These findings are similar to those reported in a recent study comparing CSF collection methods in standing horses, in which 1 out of 3 horses sedated with a single 10 µg/kg IV dose of detomidine required additional sedation to facilitate successful CSF collection.<sup>2</sup> In contrast to our findings, a recent study utilizing detomidine and morphine for repeated AA CSF collection reported multiple horse reactions to both needle insertion into cervical musculature and dural puncture. The authors of that study noted that the reactions were more commonly noted in young horses and those with refractory behavior.<sup>18</sup>

Median procedure times and reaction scores were highest during week 1 and relatively similar during weeks 2, 3, and 4. This trend is likely explained by the fact that during week 1, the majority of horses received either D0 (2/6) or DD (3/6), with only 1 horse receiving DM and no horses receiving DX. In this study, horses receiving D0 had longer procedure time and higher reaction scores and could explain the higher median values noted during week 1 compared to subsequent weeks. Alternatively, this finding could be explained by an increase in procedure proficiency on the part of the CSF collector after the first week resulting in faster CSF collections and less horse reaction throughout the course of the study.

Mild swelling and sensitivity at the centesis site was noted in some of the horses after CSF collection. In all cases, the swelling was self-limiting and did not require additional intervention. Recently, anecdotal reports of horses displaying severe pain and "head-ache" like signs after AA CSF collection have been posted on veterinary listservs and online discussion groups. These signs have been

presumptively attributed to local inflammation, CSF leakage, or a combination of both, into the epidural and deep cervical fascial spaces. Severe headaches and neck pain are frequently observed in human patients with CSF leakage from the subarachnoid space and are hypothesized to result from pain-sensitive meningeal structures, CSF hypovolemia, and intracranial hypotension.<sup>19</sup> In this study, no neck pain or headache-like signs were noted at any horses after CSF collections. To prevent local inflammation and pain, all horses received 2 doses of flunixin meglumine, a non-steroidal anti-inflammatory. To prevent CSF hypovolemia and leakage, respectively, CSF collection volumes were limited to less than 10 mL per procedure, and horses recovered from sedation with their heads supported at or above the level of the withers. The rationale for head elevation was to prevent excessively low head carriage typical of sedated horses from creating increased intracranial and CSF pressures and potentially increasing the risk of CSF leakage after centesis.

In this study, we also developed a novel method to measure sedation depth and quality in standing healthy horses using open-source motion tracking software. Sedation protocols vary greatly in clinical practice, and objective methods for assessing sedation depth and quality are critical for studies seeking to identify optimal protocols for potentially painful procedures. Published methods of sedation assessment in horses often rely on the height of the head above the ground (HHAG) and observation of behaviors such as ataxia, or observation of behaviors such as ataxia; and chewing and head movement.<sup>20-23</sup> More recently, a facial scale has been described for evaluating sedation in horses.<sup>24</sup> While well-suited for assessing sedation depth in standing, undisturbed horses, these methods are not easily applied to sedated horses undergoing a potentially painful stimulus. Scoring systems have been described for assessing horse responses to various tactile, auditory, and visual stimuli<sup>2,22,25,26</sup>; however, reviewer-assigned scoring systems are inherently prone to subjectivity and scorer bias. Alternatively, automated analysis of animal behavior is widely utilized in biomedical research, and open-source availability of software code enables modification to perform the specific needs of a particular study.<sup>27</sup> In the equine veterinary realm, automated motion tracking has been used extensively for kinematic gait analysis and lameness diagnosis,<sup>28-31</sup> but to the authors' knowledge has not yet been evaluated for assessing sedation depth and quality in horses.

In this study, we modified an open-source motion-tracking algorithm, *Ball Tracker*, to trace and measure the path traveled by a single point (centroid) on the horse's forehead. Our rationale for this method was the assumption that horses undergoing AA CSF collection with inadequate plane of sedation would be more likely to move their heads excessively (translating to a longer total distance traveled) and would have reactions of greater severity (translating to a greater excursion from baseline distance) when compared to well-sedated horses undergoing the same procedure. To assess the utility of this novel method, we compared total distance traveled and farthest excursion from baseline to a standardized procedure reaction score by scatter diagram analysis and calculation of correlation coefficients, both of which demonstrated positive association. Both motion tracking outcomes (total distance traveled and farthest excursion distance from

baseline) are quantitative, continuous variables, making them less prone to subjectivity and reviewer bias when compared to scoring systems. Additionally, the motion path tracings provide unique information regarding each horse's individual behavior during CSF collection. For example, a visual comparison of motion path tracings enables one to identify and characterize unique types of head motion, for example, minimal movement throughout procedure (Figure 5A), small back-and-forth movements suggestive of head sway or ataxia, single large head jerks in response to painful stimulus, or continuous head movements suggestive of overall inadequate plane of sedation (Figure 5B). Similarly, graphs displaying total distance traveled as a function of time enable one to easily differentiate a horse reacting to a specific step in the procedure (eg, dural puncture; Figure 6A) from a horse demonstrating negligible head motion throughout the procedure (Figure 6B).

This study had several limitations. The small sample size used in this study might have precluded identification of subtle differences between treatment groups. Researcher bias in reaction score assignments and CSF collection procedures was low or negligible, respectively. We mitigated this potential source of bias by using standardized definitions in the procedure reaction scoring system. In addition, all 3 scorers and the person performing CSF collections were blinded to the treatment groups for the duration of the study. An additional limitation was the estimation of the association between procedure reaction scores and total distance traveled or farthest excursion distance from baseline using correlation coefficients, as these 2 variables were not normally distributed. However, the evaluation of the scatter diagram revealed a positive association between procedure reaction scores and total distance traveled or farthest excursion distance in study horses.

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#### CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

#### OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

#### INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the University of Florida IACUC, Protocol #201910880.

#### HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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