

STANDARD ARTICLE

Comparison of 2 collection methods for cerebrospinal fluid analysis from standing, sedate adult horses

Hayley Chidlow¹  | Steve Giguère¹ | Melinda Camus² | Bridgette Wells² | Elizabeth Howerth² | Roy Berghaus³ | Erin McConachie Beasley¹

¹Department of Large Animal Medicine and Surgery, University of Georgia, College of Veterinary Medicine, Georgia

²Department of Pathology, University of Georgia, College of Veterinary Medicine Athens, Georgia

³Department of Population Health, University of Georgia, College of Veterinary Medicine Athens, Georgia

Correspondence

Hayley Chidlow, Newmarket Equine Hospital, Cambridge Road, Newmarket, CB8 0FG, United Kingdom.

Email: h.b.chidlow@gmail.com

Funding information

University of Georgia New Faculty Research Funding—Veterinary Medical Experiment Station, Grant/Award Number: n/a

Abstract

Background: Cerebrospinal fluid (CSF) analysis is an important component of the evaluation of horses with neurologic disease. Lumbosacral (LS) centesis is routine, but CSF is also collected from the space between the first and second cervical vertebrae (C1-C2).

Objectives: To compare collection times, CSF cytology results, and equine protozoal myelitis (EPM) titers of CSF collected from the C1-C2 and LS sites.

Animals: Fifteen university-owned adult horses with no evidence of neurologic disease, and 9 horses with signs of neurologic disease: 3 university-owned and 6 client-owned.

Methods: Prospective study. Cerebrospinal fluid collection from the LS space and C1-C2 space of each horse was performed in randomized order. Continuous data were analyzed using mixed-effects linear models and count data using mixed-effects negative binomial regression. Statistical significance was set at $P < .05$.

Results: Cerebrospinal fluid collected from the C1-C2 site had a significantly lower mean protein concentration (49 [95% CI: 43-55.8] mg/dL C1-C2 versus 52.1 [95% CI: 45.7-59.3] mg/dL LS; $P = .03$) and red blood cell count (6 [95% CI: 2-16] cells/ μ L versus 33 [95% CI: 13-81] cells/ μ L; $P = .02$). Collection time, total nucleated cell count, EPM titers, and serum:CSF EPM titer ratios were not significantly different between collection sites.

Conclusions and Clinical Importance: Cerebrospinal fluid from the C1-C2 space provides an acceptable alternative to LS CSF collection with decreased likelihood of clinically important blood contamination of samples.

KEYWORDS

centesis, cervical, equine, lumbosacral, neurologic diagnostics

1 | INTRODUCTION

Abbreviations: AO, atlanto-occipital; CSF, cerebrospinal fluid; EPM, equine protozoal myeloencephalitis; LS, lumbosacral; RBC, red blood cell; WBC, white blood cell.

Complete neurologic system evaluation requires examination of cerebrospinal fluid (CSF). However, CSF collection in horses is often

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2020 The Authors. *Journal of Veterinary Internal Medicine* published by Wiley Periodicals, Inc. on behalf of the American College of Veterinary Internal Medicine.

limited by technical difficulty and perceived risk of injury to personnel and the horse. The most commonly used technique for CSF collection in horses is standing lumbosacral (LS) centesis; however, this can present a number of challenges. In overweight or muscular horses, it can be difficult to palpate landmarks, and although ultrasound-guided LS centesis can assist with needle placement,¹ it is not uncommon to obtain CSF with iatrogenic blood contamination. Blood contamination can limit the diagnostic usefulness of the sample, artifactually increasing the protein concentration and nucleated cell count in the sample,² or giving false-positive results when testing for equine protozoal myeloencephalitis (EPM) using Western blot analysis.³ Although LS centesis is the technique of choice in horses with suspected spinal cord lesions or multifocal disease, if intracranial disease is suspected it may be preferable to collect CSF from a location closer to the foramen magnum.^{4,5} Finally, penetration of the dura mater with the needle has been associated with violent reactions in some horses.⁴ These reactions occur rarely but can result in injury to the veterinarian, horse handler, or to the horse itself.

Until recently, the only alternative technique was atlanto-occipital (AO) thecal puncture, which typically requires general anesthesia. This technique is simpler than LS centesis but does present a greater risk of iatrogenic trauma, particularly in horses with increased intracranial pressure.⁴⁻⁶ Recovery from general anesthesia presents a greater risk for horses that are weak or uncoordinated due to neurologic disease.⁴ Recently, a percutaneous, ultrasound-guided technique has been described to obtain CSF from the C1-C2 space in standing horses.⁷ However the description of the technical and safety aspects of the technique were reported in a small number of primarily normal horses. The C1-C2 technique has not been directly compared to LS centesis, in terms of fluid sample characteristics and ease of sample collection. Cerebrospinal fluid collected from the AO and LS spaces differs slightly in horses.⁸ However, most laboratories report a single reference interval that is applied to fluid collected from either site. In dogs, distinct reference intervals are used for CSF obtained from the AO space versus the LS space due to significant differences found in the nucleated cell count and protein concentration.⁹ Comparisons between CSF obtained through the C1-C2 approach and LS site, to the authors' knowledge, have not been reported. The objectives of this study are (1) to compare horse response and technical aspects of CSF collection between the 2 standing approaches on both normal and neurologically abnormal horses, (2) to compare the CSF characteristics from each site, and (3) to evaluate the centesis sites postmortem in a subset of horses.

2 | MATERIALS AND METHODS

2.1 | Animals

University- and client-owned horses were prospectively enrolled in this study. All experimental procedures were approved by the University of Georgia Institutional Animal Care and Use Committee for university-owned horses and the Clinical Research Committee for client-owned horses. The study population consisted of 24 adult

horses, aged 2-30 years (mean 14.9 years), consisting of 8 mares and 16 geldings. Breeds represented were Thoroughbred (n = 8), Quarter Horse (n = 4), Arabian (n = 3), Warmblood (n = 2), Welsh (n = 2), Appaloosa (n = 1), Percheron (n = 1), Morgan (n = 1), Paint (n = 1), and Saddlebred (n = 1). Six horses were client-owned and presented to the University of Georgia Veterinary Teaching Hospital for a complaint of neurologic disease. Eighteen horses had been donated to the University of Georgia for conditions associated with a poor quality of life, including orthopedic disease (n = 11), neurologic disease (n = 3), non-healing wounds (n = 1), dental disease (n = 1), pituitary pars intermedia dysfunction (n = 1), and behavioral issues (n = 1). All horses underwent a complete physical examination and neurologic examination before enrollment, and individuals were classified as normal (non-neurologic; 15 horses) or with signs of neurologic disease (9 horses). Donation horses were allowed 24 hours to acclimate to surroundings before undergoing CSF collection. Sixteen of 18 university-owned horses were euthanized 48 hours after the procedure. The horses were kept in individual box stalls with access to ad libitum water and coastal Bermuda hay for the duration of the study period.

2.2 | Cerebrospinal fluid collection

Horses were restrained in stocks for CSF collection. The procedures were performed using standing sedation with romifidine (80 µg/kg, IV; Sedivet, Boehringer Ingelheim Vetmedica, Duluth, Georgia) or detomidine hydrochloride (10 µg/kg, IV; Dormosedan, Zoetis, Parsippany, New Jersey). Cervical and LS CSF collections were performed consecutively, and an online random number generator (<https://www.randomizer.org/>) was used to determine the order of sampling. Lidocaine hydrochloride (100 mg; 2%; VetOne, Boise, Idaho) was infiltrated SC and into the musculature at both C1-C2 and LS sites before the final aseptic preparation of the skin to provide local anesthesia. Cervical and LS CSF collection was performed as previously described.^{4,7,8} An 8-in. 18 gauge spinal needle (Mila International, Florence, Kentucky) was used for LS centesis, and a 3.5-in. 18 gauge spinal needle (Mila International) was used for cervical centesis. A total of 5 mL of CSF were collected in 1 mL aliquots from each site. After collection of CSF, horses were administered flunixin meglumine (1.1 mg/kg IV; Banamine, Merck Animal Health, Madison, New Jersey). Whole blood (10 mL) was collected into a serum tube by venipuncture of the jugular vein. The blood was allowed to clot at room temperature, centrifuged at 3000 rpm for 15 minutes, and the serum separated and frozen at -80°C until analysis.

2.3 | Data collection

Findings of physical and neurologic examinations were recorded for each horse. A neuroanatomic diagnosis was recorded for horses with neurologic disease. For clinical cases of neurologic disease, in addition to CSF analysis and EPM titers, the results of ancillary diagnostic testing including cervical radiographs were available if performed. The total time from initial needle insertion to complete sample acquisition

was recorded. Gross blood contamination of any of the samples was noted. Horse response to each procedure was recorded based on a scale of 1-4 (1 = no reaction, 2 = tail swishing, small movement of head or neck, 3 = head jerk, attempted to kick or did kick, walked or jumped forward, 4 = severe adverse reaction; attempted to jump out or did jump out of stocks, fell down in the stocks).

2.4 | Cerebrospinal fluid analysis

The third aliquot of CSF collected from each site was submitted to the clinical pathology laboratory for analysis within 30 minutes of collection. A nucleated white blood cell (WBC) count and a red blood cell (RBC) count were performed manually on each sample using a standard Neubauer hemocytometer. Total protein concentration was quantitated via the biuret method (Cobas 6000 c 501; Roche Diagnostics, Basel, Switzerland). A clinical pathology resident (B.W.) and a board-certified clinical pathologist (M.C.) performed cytologic evaluation on cytospin preparations in batches and were blinded to the collection site and all case details. Grossly blood contaminated samples were excluded from the analysis.

2.5 | Equine protozoal myeloencephalitis CSF titers

Cerebrospinal fluid (fourth aliquot) and serum from each horse was stored at -80°C and shipped to Equine Diagnostic Solutions Laboratory (Lexington, Kentucky) for batched analysis within 6 months of collection. An enzyme linked immunosorbent assay to detect antibodies to *Sarcocystis neurona* (surface antigens 2, 4, and 3) was performed. The laboratory technicians were blinded to collection site and all case details.

2.6 | Post-procedure monitoring

Horses were monitored with physical examinations and abbreviated neurologic examination (including cranial nerve examination, gait analysis on level ground, and cervical range of motion) twice daily for 48 hours following the procedure. Centesis sites were monitored for heat, swelling, or pain upon palpation.

2.7 | Necropsy

Sixteen of 18 horses donated to the University of Georgia were euthanized after the 48-hour monitoring period. Eleven horses underwent necropsy to evaluate for gross or histologic evidence of trauma induced by CSF collection. The cervical and LS regions were skinned to examine for changes in the soft tissues and muscle in the region of the tap. The cervical cord from the first 3 vertebrae was removed, and the canal and cord were examined for hemorrhage or other pathology.

The distal cord and cauda equina were removed from 4 inches proximal to the LS joint caudally. The canal and epidural tissues were examined for hemorrhage or other tissue injury. After fixation in 10% buffered formalin, the dura was opened to examine for subdural and arachnoid/subarachnoid hemorrhage and the cord was serially sectioned to assess parenchymal pathology.

2.8 | Statistical design

Normality of the data was assessed based on the examination of histograms and normal Q-Q plots of the residuals. Homogeneity of variance was assessed by plotting residuals against predicted values. Data were transformed to the natural logarithm when necessary. The effects of site of sampling and the order of sample collection on protein concentration, RBC count, EPM titer in CSF, and serum/CSF titer ratios were assessed using linear mixed-effects models with horse included as a random effect to account for repeated measurements, and sampling site and order of sample collection modeled as fixed nominal effects. Counts of WBC were fitted to a mixed-effects negative binomial model. The percentages of neutrophils, lymphocytes, and large mononuclear cells were analyzed using fractional probit regression with robust standard errors clustered on horse to account for the correlated structure of the data. For all analyses, model fit was assessed using Akaike's information criterion values. Time from the start of the procedure to sample collection was compared between sampling sites using the Wilcoxon signed rank test. For all analyses, values of $P < .05$ were considered statistically significant. Analyses were performed using commercially available statistical software (Stata version 15.1, StataCorp LLC, College Station, Texas).

3 | RESULTS

All horses had vital signs within normal limits on physical examination. Nine of 24 horses had abnormalities on neurologic examination and were assigned to the neurologic group; all 9 had spinal ataxia localized to C1-T2 (7/9 = grade 2/5, 1/9 = grade 3/5, 1/9 = grade 4/5). One horse (grade 2/5 ataxia) also had unilateral, right-sided cranial nerve VII and VIII dysfunction.

3.1 | Reaction to CSF collection

Sixteen of 24 horses had no reaction to CSF collection from the C1-C2 space (8/9 with neurologic disease and 8/15 normal). One horse with neurologic disease and 6 normal horses demonstrated minor head movement during C1-C2 centesis (score 2/4). One normal horse demonstrated a moderate reaction (score 3/4; head jerk). Head movement appeared unrelated to dura mater puncture in all horses.

Eleven of 24 horses had no reaction to CSF collection from the LS space (7/9 with neurologic disease and 4/15 normal). Seven normal horses had a mild reaction (score 2/4; tail swish). Two horses with

neurologic disease and 3 normal horses had a moderate reaction (score 3/4; kicked out) and 1 normal horse had a severe reaction (score 4/4; attempted to jump out of stocks). In all cases, the reaction was observed during presumptive dura mater puncture (based on the timing of the reaction in relation to CSF collection).

3.2 | Level of sedation

For initial sedation, 20 horses received romifidine (80 µg/kg, IV). An adequate level of sedation was achieved in 11 of 20 horses, with no requirement for additional sedation. Seven of 20 horses were well sedated for CSF collection from the first site but required additional sedation with xylazine (0.3 mg/kg, IV) for CSF collection from the second site. Two horses with reactive temperaments were not adequately sedated with the initial dose of romifidine and received a second dose of romifidine (40 µg/kg, IV) and 2 doses of xylazine (total dose 0.4 mg/kg, IV) to achieve a sufficient level of sedation to allow CSF to be collected from both sites. One horse with grade 4/5 ataxia was sedated initially with a lower dose of romifidine (40 µg/kg) but adequate sedation was not achieved; therefore, detomidine (10 µg/kg, IV) was administered.

Three horses were sedated with detomidine (10 µg/kg, IV) due to the lack of availability of romifidine at the time of the procedures; adequate sedation was achieved in 2 of 3 horses, and the third horse required an additional dose of detomidine (6 µg/kg) to maintain adequate sedation for CSF collection from the second site.

3.3 | Comparison of CSF collected from C1-C2 and the LS space

Cerebrospinal fluid from normal horses ($n = 15$) and horses with neurologic disease ($n = 9$) from both sites were analyzed together. Cerebrospinal fluid analytes are summarized by collection site in Table 1. Compared to CSF samples collected from the C1-C2 site, samples collected from the LS site had a higher mean protein concentration and higher mean RBC count. One horse had visibly blood contaminated CSF collected from the LS space and was excluded from analysis. Cerebrospinal fluid from the C1-C2 site in the same horse was not visibly blood contaminated. This horse was recorded as having 70% neutrophils and 18% lymphocytes at the LS site compared to 0% neutrophils and 80% lymphocytes at the C1-C2 site.

None of the horses with neurologic disease in this study had an EPM serum:CSF titer ratio consistent with a clinical diagnosis of EPM, and there was no significant difference in EPM titer between the C1-C2 and LS sites.

The C1-C2 site was sampled first in 15 horses and second in 9 horses. There was no significant main effect of sample collection order and no significant interaction between anatomic site and sample collection order for any of the analytes evaluated. There was no significant difference between the median time required to collect samples from the C1-C2 and LS sites (4.0 versus 5.5 minutes, respectively;

TABLE 1 Estimated marginal means (95% confidence intervals) for CSF analytes collected on the same day from cervical (C1-C2) and lumbosacral (LS) anatomic sites in 24 adult horses, adjusted for the order of sample collection

Analyte	C1-C2	LS	P ^a
Protein (mg/dL) ^b	49.0 (43.0, 55.8)	52.1 (45.7, 59.3)	.03
WBC count (cells/µL)	2 (1, 3)	3 (2, 4)	.17
RBC count (cells/µL) ^b	6 (2, 16)	33 (13, 81)	.02
Neutrophils %	2 (0, 3)	6 (2, 10)	.07
Lymphocytes %	88 (83, 93)	82 (76, 88)	.12
Large mononuclear cells %	10 (6, 14)	10 (6, 13)	.79
EPM titer ^b	3 (2, 5)	3 (2, 5)	.41
Serum:CSF EPM titer ratio ^b	91 (66, 124)	87 (63, 118)	.44

Note. Cerebrospinal fluid was not collected from the C1-C2 site in 1 horse, and the LS sample from 1 horse was excluded due to visible blood contamination.

Abbreviations: CSF, cerebrospinal fluid; EPM, equine protozoal myelitis; RBC, red blood cell; WBC, white blood cell.

^aContrast of anatomic site marginal means.

^bGeometric means back transformed from the logarithmic scale.

$P = .15$). Cerebrospinal fluid collection from the C1-C2 site was unsuccessful in 1 horse that failed to respond appropriately to sedation.

3.4 | Post-procedure monitoring

All horses had normal vital signs after CSF collection. No neurologic deficits were noted in previously neurologically normal horses, and there was no change in neurologic examination findings in horses with neurologic disease. Three horses had mild swelling and sensitivity to palpation at the site of C1-C2 CSF collection. One horse had mild swelling at the site but no sensitivity on palpation. Sensitivity to palpation resolved by completion of the 48-hour monitoring period.

3.5 | Necropsy

Eleven horses (8 normal, 3 with neurologic disease) underwent necropsy to evaluate for gross or histologic evidence of trauma induced by CSF collection. Eight horses had mild to moderate hemorrhage in the musculature and fascia of the neck corresponding to the C1-C2 centesis site. Four of these horses had mild hemorrhage and edema of the cervical epidural soft tissues. Seven horses had evidence of cervical subarachnoid hemorrhage microscopically; subarachnoid hemorrhage was visible grossly in 2 of these horses. Of the 7 horses with cervical subarachnoid hemorrhage, 2 had no hemorrhage of cervical musculature or epidural soft tissues; 2 had hemorrhage of cervical musculature without hemorrhage of epidural soft tissues; and 3 had hemorrhage of both cervical musculature and epidural soft tissues. One horse that had subarachnoid, epidural, and musculature

hemorrhage also had an evidence of parenchymal damage possibly associated with C1-C2 CSF collection, with unilateral focal hemorrhage in the dorsal horn consistent with needle puncture.

One horse had LS dorsal soft tissue hemorrhage. Seven horses had mild to moderate hemorrhage in the epidural fat or epineurium of the cauda equina nerve roots outside the dura mater. Subarachnoid hemorrhage was not observed in the LS region.

In the 3 horses with neurologic disease that underwent postmortem examination, diagnoses of C2-C3 articular facet dysplasia with degenerative intervertebral discs ($n = 1$), cholesterol granuloma within the lateral ventricle ($n = 1$), and C7-T1 intervertebral disc degeneration ($n = 1$) were made.

4 | DISCUSSION

Based on the findings in this study, CSF can be successfully collected from both the C1-C2 and LS sites in standing, sedated horses. Horse responses vary widely, but reaction to needle placement at the C1-C2 site was more predictable and well tolerated than reactions to needle advancement in the LS space.

When the horse with marked blood contamination of CSF from the LS space was excluded from analysis, the only statistically significant differences were decreased RBC count and protein concentration in CSF from the C1-C2 space compared to CSF from the LS space. Decreased RBC contamination of CSF from the C1-C2 space is clinically relevant, as blood contamination of CSF can falsely increase protein concentration in CSF, affect cytologic interpretation due to artifactual increases in nucleated cells, and can result in false-positive results when using Western blot testing for EPM.^{2,3} Although the mean RBC count for CSF from both sites was below the cutoff for blood contamination for Western blot analysis (>50 RBC/ μ L),¹⁰ CSF collected from the LS space had >50 RBC/ μ L in 12 horses (50%) versus only 3 horses (13%) of samples collected from the C1-C2 space. Protein concentration for both sites was within previously described reference intervals⁴ (49.0 mg/dL C1-C2 versus 52.1 mg/dL LS). Therefore, the difference in protein concentration is unlikely to be clinically relevant.

Normal horses appeared to be more reactive to CSF collection, in particular to penetration of the dura mater during LS centesis, with 4 of 15 (27%) normal horses having no reaction to dura penetration during LS centesis, compared to 7 of 9 (78%) horses with neurologic disease. Likewise, although reaction to C1-C2 centesis did not appear directly related to dura penetration, 8 of 9 (89%) horses with neurologic disease had no reaction to the procedure compared to 8 of 15 (53%) normal horses. There was no obvious difference in the degree of sedation achieved between the normal and neurological horse groups. Further study may be warranted to investigate whether there is a significant difference in reaction to CSF collection between normal horses and horses with neurologic disease, and the clinical relevance of this observation.

In a previous description of C1-C2 CSF collection,⁷ morphine sulfate was used to decrease the anticipated reaction to dura penetration. In this study, sedation with an alpha-2 agonist alone was chosen

to avoid the use of controlled drugs. Lidocaine hydrochloride, infiltrated in the subcutis and muscle of planned needle puncture location, was used to reduce reaction to needle penetration through the skin and cervical musculature. In all horses that demonstrated head movement during C1-C2 centesis, this occurred during needle passage through the cervical musculature. Although it was perceived that there was minimal reaction to dura penetration, the addition of an opioid such as morphine sulfate may be beneficial in reducing head movement during all stages of the procedure. Additional lidocaine infiltration in the SC tissues and cervical musculature could also theoretically reduce adverse reactions to the procedure. Horse temperament influenced the ease of sample collection using cervical centesis. Highly strung, head-shy horses or those with a poor response to sedation were more reactive to all stages of the procedure, including preparation of the site and needle penetration through the skin and cervical musculature. This correlated to increased likelihood of head movement during the procedure, which can result in changes in needle orientation and a requirement for needle redirection.

Cerebrospinal fluid collection from the C1-C2 site was unsuccessful in 1 horse, for which an adequate plane of sedation was not achieved. The horse was adequately sedated for LS centesis, which was performed first. The horse demonstrated repeated head movements during initial attempts to collect CSF from the C1-C2 space on the left side despite additional sedation. Following this, artifact from the needle tract prevented accurate ultrasonographic identification of the dura and spinal cord. Therefore, collection from the right side of the neck was attempted. When the horse continued to be refractory to sedation, attempts were discontinued.

Time of collection was used as an objective measure to compare the 2 techniques in terms of ease of collection. There was no significant difference in median time of collection between techniques (4.0 minutes C1-C2 versus 5.5 minutes LS; $P = .15$), suggesting that cervical centesis is no more technically challenging than LS centesis. However, the time of collection and the number of needle redirections for C1-C2 centesis improved as the study progressed and as operator experience increased. Median time of collection for C1-C2 centesis in the final 12 horses sampled was 1.5 minutes. This was in contrast to LS centesis, for which the median time of collection did not improve (5.5 minutes for final 12 horses). In overweight or heavily muscled horses, anatomic landmarks of the LS space can be difficult to palpate. In horses with neurologic disease and ataxia, it can be difficult to optimally position the hindquarters squarely, particularly after sedation, increasing the difficulty of LS centesis. In comparison, an adequate plane of sedation allows correct positioning of the horse's head for C1-C2 centesis regardless of the degree of ataxia.

Four horses developed mild post-procedure swelling or soreness at the C1-C2 centesis site. All 4 horses were enrolled early in the study, before more experience being gained in the technique of cervical centesis. Two of these horses underwent necropsy; 1 horse had evidence of subarachnoid hemorrhage microscopically, and 1 had hemorrhage of the cervical musculature, epidural soft tissues, and subarachnoid space. The other 2 horses were available for long-term follow-up and had no further adverse effects related to cervical

centesis. As operator experience improved, no further post-procedure reactions at the C1-C2 centesis site were noted.

Microscopic hemorrhage in the subarachnoid region was observed in 7 of 11 horses at the C1-C2 site, 2 of which also had gross evidence of subarachnoid hemorrhage. One horse with microscopic subarachnoid hemorrhage had mild swelling and sensitivity at the centesis site that had resolved by 48 hours post-procedure, and another with microscopic subarachnoid hemorrhage had mild swelling at the centesis site but no sensitivity. The remaining 5 horses with subarachnoid hemorrhage had no evidence of sensitivity and swelling at the centesis site and no alterations in neurologic status during the 48-hour post-procedure monitoring period. Subarachnoid hemorrhage was not observed at the LS centesis site in any horses. In human medicine, iatrogenic spinal subarachnoid hemorrhage does occur after lumbar puncture but is normally associated with anticoagulant treatment or preexisting coagulopathies or with technical difficulties during the procedure.¹¹ Clinical signs of subarachnoid hemorrhage in humans include pain at the level of the hemorrhage, and neurologic dysfunction.¹² The development of subarachnoid hematoma and subarachnoid air has been described in a horse undergoing myelography.¹³ The horse developed neurologic deficits after surgery that resolved within 7 days. Although subarachnoid hemorrhage in the cases described here was subclinical, it should be considered a risk factor for the procedure, and horses should be closely monitored for local pain or neurologic dysfunction following C1-C2 centesis. Of the 11 horses submitted for necropsy, 7 horses were enrolled early in the study and 4 were enrolled in the later stages. Five of the 7 horses with subarachnoid hemorrhage were enrolled early in the study, compared to 2 of the 4 enrolled later in the study. The authors hypothesize that operator inexperience contributed to the increased incidence of subarachnoid hemorrhage following C1-C2 centesis. Unfortunately, a limitation of this study is the small number of horses that underwent necropsy, and the fact that the majority that underwent necropsy were enrolled earlier in the study. This precludes statistical comparison of complications between horses enrolled early in the study versus later. However, it is likely that the risk of complications is higher when C1-C2 is performed by an inexperienced operator.

The finding at necropsy of spinal cord parenchymal hemorrhage secondary to cervical centesis in 1 horse illustrates that this technique is not without risk. There was no indication during the procedure that spinal cord penetration had occurred, either ultrasonographically or in horse reaction. The horse in question was the 10th enrolled in the study, by which time operator experience had improved as evidenced by the time of CSF collection and the number of needle redirections required. However, the horse had an anxious temperament and appeared refractory to sedation. No reaction was observed specifically during penetration of the dura mater, but the horse exhibited repeated jerking head movements during sterile preparation of the skin for the procedure and during CSF collection. No neurologic deficits were observed during or after CSF collection, and there was no pain or swelling observed at the centesis site during the 48-hour monitoring period. In the authors' opinion, spinal cord penetration occurred in this case as a consequence of the horse's refractory behavior; therefore, it should be considered that some horses may not be appropriate candidates for this procedure due to temperament.

Limitations of this study include the small number of horses with neurologic disease and the fact that none of the neurologic horses enrolled had abnormalities in their CSF. This precluded assessment of whether C1-C2 CSF collection may provide more useful information than LS CSF collection in cases of intracranial neurologic disease. Evaluation of C1-C2 CSF collection in horses with meningitis and encephalitis is warranted. Equine protozoal myeloencephalitis serum:CSF titers were also not consistent with clinical EPM in any case. Further study is required in horses with EPM serum:CSF titer ratios <100 to confirm that the site of collection does not have an effect on EPM titers in CSF. A larger sample size of horses enrolled in the study might have identified more subtle differences in CSF collected from the 2 sites.

This study demonstrates that the degree of difficulty is similar between C1-C2 and LS centesis. In horses with neurologic disease, particularly those with hind limb ataxia, C1-C2 centesis might be technically less challenging than LS centesis and might also involve less risk to the veterinarian performing the procedure due to positioning at the front of the horse versus close proximity to the hind limbs. Increased experience with the technique resulted in fewer needle redirections during the procedure. Spinal cord penetration and subarachnoid hematoma are risks of the procedure and temperament of the horse should be considered before choosing this technique. Cerebrospinal fluid collection from the C1-C2 space may provide an acceptable alternative to LS CSF collection with decreased likelihood of clinically important blood contamination of samples.

ACKNOWLEDGMENTS

All work was performed at University of Georgia College of Veterinary Medicine (UGA CVM), Athens, Georgia. This study was supported by funding from the UGA CVM Veterinary Medical Experiment Station. The authors thank Dr. Jennifer Morrow at Equine Diagnostic Solutions for facilitating batched sample analysis. Originally presented as an abstract at the 2018 ACVIM Forum, Seattle, Washington.

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

This study was approved by the University of Georgia IACUC for university-owned horses and the Clinical Research Committee for client-owned horses.

HUMAN ETHICS APPROVAL DECLARATION

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

ORCID

Hayley Chidlow  <https://orcid.org/0000-0001-5152-1613>

REFERENCES

1. Aleman M, Borchers A, Kass PH, Puchalski SM. Ultrasound-assisted collection of cerebrospinal fluid from the lumbosacral space in equids. *J Am Vet Med Assoc.* 2007;230:378-384.
2. Sweeney CR, Russell GE. Differences in total protein concentration, nucleated cell count, and red blood cell count among sequential samples of cerebrospinal fluid from horses. *J Am Vet Med Assoc.* 2000; 217:54-57.
3. Miller MM, Sweeney CR, Russell GE, Sheetz RM, Morrow JK. Effects of blood contamination of cerebrospinal fluid on western blot analysis for detection of antibodies against *Sarcocystis neurona* and on albumin quotient and immunoglobulin G index in horses. *J Am Vet Med Assoc.* 1999;215:67-71.
4. Johnson PJ, Constantinescu GM. Collection of cerebrospinal fluid in horses. *Equine Vet Educ.* 2000;12:7-12.
5. Schwarz B, Piercy RJ. Cerebrospinal fluid collection and its analysis in equine neurological disease. *Equine Vet Educ.* 2006;18: 243-248.
6. Green EM, Constantinescu GM, Kroll RA. Equine cerebrospinal fluid: physiologic principles and collection techniques. *Compend Contin Educ Vet.* 1992;14:229-237.
7. Pease A, Behan A, Bohart G. Ultrasound-guided cervical centesis to obtain cerebrospinal fluid in the standing horse. *Vet Radiol Ultrasound.* 2012;53:92-95.
8. Mayhew IG, Whitlock RH, Tasker JB. Equine cerebrospinal fluid: reference values of normal horses. *Am J Vet Res.* 1977;38:1271-1274.
9. Bailey CS, Higgins RJ. Comparison of total white blood cell count and total protein content of lumbar and cisternal cerebrospinal fluid of healthy dogs. *Am J Vet Res.* 1985;46:1162-1165.
10. Furr M, MacKay R, Granstrom D, Schott H. Clinical diagnosis of equine protozoal myeloencephalitis (EPM). *J Vet Intern Med.* 2002;16:618-621.
11. Domenicucci M, Ramieri A, Paolini S, et al. Spinal subarachnoid hematomas: our experience and literature review. *Acta Neurochir.* 2005; 147(7):741-750.
12. Kreppel D, Antoniadis G, Seeling W. Spinal hematoma: a literature survey with meta-analysis of 613 patients. *Neurosurg Rev.* 2003;26:1-49.
13. Prange T, Derksen FJ, Stick JA, Garcia-Pereira FL, Carr EA. Cervical vertebral canal endoscopy in the horse: intra- and post-operative observations. *Equine Vet J.* 2011;43:404-411.

How to cite this article: Chidlow H, Giguère S, Camus M, et al. Comparison of 2 collection methods for cerebrospinal fluid analysis from standing, sedate adult horses. *J Vet Intern Med.* 2020;34:972–978. <https://doi.org/10.1111/jvim.15702>