Year: 2016

Ultrasonographic examination of the spinal cord and collection of cerebrospinal fluid from the atlanto-occipital space in cattle

Braun, Ueli ; Attiger, Jeannette

Abstract: Ultrasonography is useful for the visualization of the spinal cord and associated structures and facilitates the safe collection of cerebrospinal fluid from the atlanto-occipital space in cattle. This technique is less stressful than the blind puncture technique because it does not require strong ventroflexion of the head. Furthermore, painful puncture of the spinal cord can largely be avoided when ultrasound guidance is used.

DOI: https://doi.org/10.1016/j.cvfa.2015.09.012

Originally published at:
Braun, Ueli; Attiger, Jeannette (2016). Ultrasonographic examination of the spinal cord and collection of cerebrospinal fluid from the atlanto-occipital space in cattle. The Veterinary clinics of North America. Food animal practice, 32(1):109-118.
DOI: https://doi.org/10.1016/j.cvfa.2015.09.012

Ultrasonographic examination of the spinal cord and collection of cerebrospinal fluid from the atlanto-occipital space in cattle

Ueli Braun, Prof Dr med vet, Dr med vet h c, Dipl. ECBHM, Jeannette Attiger, Med Vet

Ueli Braun, Prof Dr Med Vet, Professor for Internal Medicine of Cattle, Department of Farm Animals, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland

E-mail address: ubraun@vetclinics.uzh.ch

Telephone number: 0041-44-6358241

Fax number: 0041-44-6358904

Summary

Ultrasonography is useful for the visualization of the spinal cord and associated structures and facilitates the safe collection of cerebrospinal fluid from the atlanto-occipital space in cattle. This technique is less stressful than the blind puncture technique because it does not require strong ventroflexion of the head. Furthermore, painful puncture of the spinal cord can largely be avoided when ultrasound guidance is used. Ultrasonographic examination of the spinal cord between the 5th and 6th lumbar vertebrae or from the lumbosacral foramen is feasible in calves and has been used to diagnose diplomyelia.

KEYWORDS

• Cattle • Ultrasonography • Spinal cord • Atlanto-occipital space • Cerebrospinal fluid

Introduction

The examination of cerebrospinal fluid (CSF) plays a major role in the diagnosis of central nervous system diseases in cattle. There are two sites from which CSF can be collected in cattle: the first is the atlanto-occipital (AO) space and the second is the lumbosacral foramen (LSF).[1,2] The exact site of needle insertion at both locations is determined by skeletal landmarks but puncture is carried out blindly without visualization of the subarachnoid space.[1-4] For collection from the AO space, the head is ventroflexed at a 90° angle and the needle is inserted at the intersection between the dorsal midline and an imaginary line connecting the cranial edges of the wings of the atlas[2,4] or slightly cranial to that intersection.[1] A spinal needle is introduced into the subarachnoid space parallel to the longitudinal axis of the flexed head.[1,4] The depth to which the needle is inserted is not exactly predictable, and the needle is advanced slowly and carefully and monitored for free flow of CSF by removing the stylet at regular intervals.[2] Puncture of the spinal cord must be avoided because it can lead to nerve damage or even death of the patient.[2,5] Strong ventroflexion of the head required for this technique often provokes avoidance movements in the animal and may impair respiration. Furthermore, blind aspiration of CSF from the AO space frequently results in contamination of the sample with blood,[6,7] which can impair the diagnosis.[8-12] Finally, the spinal cord may be punctured during blind aspiration despite the precautions outlined above and results in pain evidenced by violent twitching. Based on experiences in the collection of CSF under ultrasonographic guidance in the horse,[13-15] the spinal canal of cattle was examined ultrasonographically and the feasibility of ultrasound-guided collection of CSF investigated.[16,17] Another study described the ultrasonographic findings of diplomyelia of the lumbar spine in a calf,[18] and the ultrasonographic examination of the spinal cord in healthy calves was presented.[19] The purpose of this article is to describe the ultrasonographic findings of the spinal cord and its surrounding structures and the ultrasound-guided collection of CSF from the AO space in cattle.

Anatomy of the atlanto-occipital space

The AO space is bordered by the occiput cranially and by the atlas caudally and is covered by the skin, the nuchal ligament, various muscles, and the AO membrane.[2,20] Ventral to this membrane is the cranial-most section of the vertebral canal, which contains the spinal cord surrounded by three meninges. The outermost meninx is the dura mater, which is separated from the vertebral periosteum by the epidural space.[21] The middle meninx is the dura arachnoidea, which is enveloped by the dura mater and consists of three layers. The outermost layer is made up of fibrocytes and collagen fibers and is separated from the dura mater by a so-called neurothelium. Avascular bundles of collagen fibers covered by neurothelium, referred to as arachnoid trabeculae, connect the outer layer with the inner layer of the dura arachnoidea, which also consists of collagen fibers and fibrocytes.[21,22] These trabeculae are in the middle layer and form a spider web-like network surrounded by CSF. The middle layer of the dura arachnoidea is referred to as the subarachnoid space[22] and contains the arteries that supply the central nervous system.[21] The innermost layer of the dura arachnoidea follows the superficial surface of the brain and spinal cord, whereas the outermost layer, together with the dura mater, forms a straight sac, which envelops the spinal cord. The innermost meninx, the pia mater, adheres to the surface of the brain and spinal cord and closely follows their contours. Cerebrospinal fluid-filled spaces referred to as subarachnoid cisternae are formed in the regions where the dura arachnoidea and pia mater separate over depressions in the brain or spinal cord. The cerebellomedullary cistern, also called the cisterna magna, is formed between the caudal aspect of the cerebellum and the medulla oblongata and in most domestic animals is of clinical importance for the collection of CSF from the AO space.[21,22] However, in cattle, the cerebellomedullary cistern cannot be accessed because of the caudal elongation of the occipital bone, and therefore the caudal extension of the cistern is punctured for collection of

CSF.[23] The pia mater consists of loose connective tissue including blood vessels and nerves. It is tightly associated with the surface of the brain and spinal cord and is adjacent to the superficial glial cells of the central nervous system. The pia mater forms two narrow fibrous strips on either side of the spinal cord, called denticulate ligaments, with extensions that attach to the dura mater and provide stability to the spinal cord within the dural sac.[21,22]

The spinal cord is a cylindrical structure characterized by a dorsal median sulcus, two dorso-lateral sulci and a deep ventral median fissure. The dorsal afferent nerve roots enter the spinal cord at the dorso-lateral sulci, and the efferent nerve roots exit the spinal cord ventrolaterally on both sides. The dorsal and ventral nerve roots unite in the subarachnoid space to form the spinal nerves, which exit the spinal canal through the intervertebral foramina. In the center of the spinal cord is the central canal, which is continuous with the ventricular system of the brain.[22]

Ultrasonographic examination of the spinal cord from the AO space

The ultrasonographic findings of the spinal cord and the collection of CSF under ultrasonographic guidance from the AO space in 73 cows immediately after euthanasia and in 14 live cattle of various age with central nervous disease were described.[16,17]

Preparation of cattle for the ultrasonographic examination

For ultrasonographic examination and collection of CSF, cattle are placed in lateral recumbency. Cows are sedated with 0.07 to 0.10 mg/kg xylazine intravenously, followed by 0.05 mg/kg xylazine intramuscularly depending on the level of sedation. The cow is then placed on a tilt table and all four legs and the head are secured with straps. A 15 cm x 10 cm area over the AO space is clipped and cleaned with ethanol. The head is fixed to the table with a halter in mild ventroflexion (about 30°) to improve the ultrasonographic visibility of the spinal cord. Rarely, moderate ventroflexion of about 45° is required for successful imaging of the spinal cord and CSF collection but strong ventroflexion of 90°, which is needed for blind CSF aspiration, is never required.

Technique of ultrasonographic examination

A 5.0- to 7.5-MHz linear or convex transducer is used and after the application of conductive gel, the spinal cord and its surrounding structures are imaged in longitudinal and cross section.

Ultrasonographic findings of the AO space

Ultrasonograms of the AO space show, from dorsal to ventral, the skin, the nuchal ligament, various muscles including the rectus capitis minor und major muscles, the AO membrane, and the vertebral canal, which is bordered by the hyperechoic dura mater. In longitudinal section, the muscles appear as echoic structures with longitudinal striations, and the nuchal ligament is hypoechoic. The spinal cord is seen as a hypoechoic band, some areas of which have a heterogeneous internal structure (**Fig. 1**). It is surrounded dorsally (toward the skin) as well as ventrally (away from the skin) by the subarachnoid space and is anechoic to hypoechoic and sometimes has a heterogeneous internal structure. Blood vessels often seen dorsolateral and adjacent to the dural sac can be interpreted as a venous sinus based on findings in the horse.[13] In cross section, the spinal cord is circular and surrounded by the subarachnoid space (**Fig. 2**). The hyperechoic denticulate ligaments are often seen on both sides of the spinal cord between the pia mater and dura mater. The central canal is frequently seen as a hyperechoic spot in the middle of the spinal cord. The pia mater appears as an echoic line adjacent to the spinal cord. The dura mater and arachnoid membrane are also seen as a hyperechoic line but cannot be differentiated.

Measurements in the AO space in 73 euthanized cows

The ultrasonographically visible structures were measured to generate reference intervals for the cows with central nervous system disorders.[16,17] Optimal sagittal and transverse ultrasonograms were frozen and various variables measured using the electronic cursors. The measurements made in the longitudinal and transverse planes are very similar (**Table 1**). In the longitudinal section, the distance between the skin and arachnoidea ranges from 30 to 52 mm (mean ± sd = 38.6 ± 4 mm) and the height of the subarachnoid spaces dorsal and ventral to the spinal cord ranges from 5 to 12 mm (8.9 ± 1.6 mm) and from 4 to 11 mm (median = 8.4 mm), respectively. The height of the spinal cord varies from 6 to 13 mm (9.9 ± 1.2 mm) and the height of the entire dural sac from 20 to 34 mm (26.9 ± 3 mm). The spinal cord can be seen in the sagittal plane over a distance of 19 to 72 mm (43.1 ± 10.3 mm).

Ultrasound-guided collection of CSF from the atlanto-occipital space

Preparation of cattle and CSF collection technique

After ultrasonography, the clipped area over the AO space is cleaned with iodine soap and disinfected and the skin at the site of puncture is anesthetized using 5 ml of 2% lidocaine. The so-called freehand technique[24] with a spinal needle (0.90 x 90 mm, Terumo® Spinal needle, Terumo Medical Corporation, USA) is used to puncture the arachnoidea under ultrasonographic guidance (**Figs. 3, 4**). Positioning the needle so that it is aligned perfectly with the sagittal orientation of the sound waves can pose a problem initially, but this technique becomes easier with practice and the accidental puncture of blood vessels can be avoided. The needle is introduced in the median plane in a caudoventral direction. As described for CSF collection in the horse, the angle between the needle and the dura mater is critical.[13,15] When the angle is too small, the needle does not perforate the dura mater but pushes it ventrally. This complication has occurred regardless of the angle of the needle and is referred to as tenting in human medicine.[25] The tenting phenomenon increases the risk of accidental puncture of the spinal cord and must be avoided at all cost. After perforation of the arachnoidea and observation of the tip of the needle in the subarachnoid space, the stylet is removed and 3 to 5 ml of CSF is aspirated using a syringe. If the attempt is unsuccessful, the stylet is re-inserted and the needle withdrawn partly or completely and the puncture repeated at a slightly different angle. A new needle is used after accidental puncture of a blood vessel or aspiration of blood. When done correctly and without spinal cord puncture, this technique does not elicit pain or avoidance behavior in cows.

Examination of the cerebrospinal fluid

Ultrasound-guided collection of CSF reduces the incidence of contamination of the CSF with blood, which is common when the blind puncture technique is used.[6,7] Therefore, most CSF samples are clear and colorless but it must be remembered that blood contamination is not always recognized macroscopically.[7,9,10] In CSF samples collected under ultrasound guidance at our clinic, the red blood cell count ranged from 0 to 820 erythrocytes/µl CSF (median = 2.5 erythrocytes/µl CSF) (**Fig. 5**). A minimum erythrocyte count of about 2,000 to 3,000 cells/µl is required to render a CSF sample grossly discolored or turbid,[8,9,26] which explains why practically all of our samples appeared uncontaminated. It also means that CSF collected using the described technique is well suited for diagnostic purposes in cattle with central nervous system disease. It should also be noted that it is possible to collect a clean CSF sample in a second attempt after a blood vessel has been punctured and hemorrhagic CSF aspirated initially. This is a major advantage over the blind puncture technique, which usually does not allow for the collection of a blood-free CSF sample at the same collection site once a hemorrhagic sample or frank blood has been aspirated.

Ultrasonographic examination of the spinal cord from the lumbosacral area in the calf

In calves, the spinal cord also can be examined ultrasonographically between the 5th and 6th lumbar vertebrae or from the lumbosacral foramen[18,19] but a detailed description of this technique in adult cows was not available at the time of this writing. There are anecdotal reports that lateral ultrasonograms of the spinal cord can be obtained at the lumbosacral foramen in adult cows. A 7.5-MHz linear transducer is best suited for the examination in calves. The calf is placed in lateral recumbency and positioned such that the lumbar vertebrae are slightly arched dorsally. Similar to the technique described for the AO space, the spinal cord and the surrounding structures are examined in the sagittal and transverse planes. The ultrasonographic appearance of the spinal cord is analogous to that at the AO space except that two spinal nerves are seen on transverse images. This technique allows for the diagnosis of spinal cord malformations, for instance diplomyelia, which is duplication of the spinal cord including the central canal.[27]

Conclusions

The spinal cord and its surrounding structures can readily be identified using ultrasonography. Also, it is possible to collect cerebrospinal fluid without blood contamination. In addition, ultrasound guidance eliminates the need for marked ventroflexion of the head, which in turn minimizes defensive reactions that commonly occur when the blind technique is used. Ultrasound-guided collection of CSF is convenient and safe and therefore the method of choice for collection of CSF in cattle.

References

1. Vandevelde M, Jaggy A, Lang J. Spezielle Untersuchungsmethoden. Untersuchung des Liquor cerebrospinalis (LCS); Entnahmetechnik. In: Vandevelde M, Jaggy A, Lang J, editors. Veterinärmedizinische Neurologie. Ein Leitfaden für Studium und Praxis. Berlin, Parey Buchverlag. 2001, pp. 63-9.
2. De Lahunta A, Glass E. Cerebrospinal fluid and hydrocephalus. In: De Lahunta A, Glass EN, editors. Veterinary Neuroanatomy and Clinical Neurology. St Louis, Saunders Elsevier. 2009, pp. 54-76.
3. Di Terlizzi R, Platt SR. The function, composition and analysis of cerebrospinal fluid in companion animals: Part II – Analysis. Vet J 2009; 180 (1): 15-32.
4. Kumar V, Kumar N. Diagnostic value of cerebrospinal fluid evaluation in veterinary practice: An overview. J Adv Vet Res 2012; 2 (3): 213-7.
5. Luján Feliu-Pascual A, Garosi L, Dennis R, Platt S. Iatrogenic brainstem injury during cerebellomedullary cistern puncture. Vet Radiol Ultrasound 2008; 49 (5): 467-71.
6. Averill DR. Examination of the cerebrospinal fluid. In: Kirk RW, editor. Current Veterinary Therapy: V. Small Animal Practice. Philadelphia, WB Saunders Company. 1974, pp. 645-8.
7. Kornhuber ME, Kornhuber J, Kornhuber AW, Hartmann GM. Positive correlation between contamination by blood and amino acid levels in cerebrospinal fluid of the rat. Neurosci Lett 1986; 69 (2): 212-5.
8. Ylitalo P, Heikkinen ER, Myllylä VV. Evaluation of sucessive collections of cisternal cerebrospinal fluid in rats, rabbits, and cats. Exp Neurol 1976; 50 (2): 330-6.
9. 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 (1): 67-71.

10. 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 (1): 54-7.
11. Finno CJ, Packham AE, Wilson WD, Gardner IA, Conrad PA, Pusterla N. Effects of blood contamination of cerebrospinal fluid on results of indirect fluorescent antibody tests for detection of antibodies against Sarcocystis neurona and Neospora hughesi. J Vet Diagn Invest 2007; 19 (3): 286-9.
12. Doyle C, Solano-Gallego L. Cytologic interpretation of canine cerebrospinal fluid samples with low total nucleated cell concentration, with and without blood contamination. Vet Clin Pathol 2009; 38 (3): 392-6.
13. Audigié F, Tapprest J, Didierlaurent D, Denoix JM. Ultrasound-guided atlanto-occipital puncture for myelography in the horse. Vet Radiol Ultrasound 2004; 45 (4): 340-4.
14. Pease A, Behan A, Bohart G. Ultrasound-guided cervical centesis to obtain cerebrospinal fluid in the standing horse. Vet Radiol Ultrasound 2012; 53 (1): 92-5.
15. Depecker M, Bizon-Mercier C, Couroucé-Malblanc A. Ultrasound-guided atlanto-occipital puncture for cerebrospinal-fluid analysis on the standing horse. Vet Rec 2014; 174 (2): 45.
16. Attiger J. Liquorentnahme aus dem Spatium atlanto-occipitale unter Ultraschallkontrolle beim Rind. 2014, Master-Thesis, University of Zurich.
17. Braun U, Attiger J, Brammertz C. Ultrasonographic examination of the spinal cord and collection of cerebrospinal fluid from the atlanto-occipital space in cattle. BMC Vet Res 2015;11:227.
18. Testoni S, Franz S, Dalla Pria A, Cipone M, Gentile A. Sonographie zur Untersuchung des Wirbelkanals bei Kälbern mit neurologischen Symptomen. KTP 2014; 22: 125-130.

19. Gentile A, Testoni S, Franz S, Dalla Pria A. Spinal cord: Ultrasonographic windows in calves. Proceedings XXVII World Buiatrics Congress, Lisbon, 2012, 154.
20. Popesko P. Rind, junge Färse. Medianschnitt durch den Kopf. In: Popesko P, editor. Atlas der topographischen Anatomie der Haustiere. Stuttgart, Enke Verlag. 2011, p 30.
21. Stoffel MH. Meningen. In: Stoffel MH, editor. Funktionelle Neuroanatomie für die Tiermedizin. Stuttgart: Enke Verlag. 2011, pp. 100-5.
22. König HE, Liebich HG, Červeny C. Nervensystem (Systema nervosum). In: König HE, Liebich HG, editors. Anatomie der Haussäugetiere: Lehrbuch und Farbatlas für Studium und Praxis. Stuttgart, Schattauer. 2005, pp. 485-556.
23. Berg R, Müller K. Hals und Brustwand. In: Budras KD, Buda S, editors. Atlas der Anatomie des Rindes. Supplement: Klinisch-funktionelle Anatomie. Hannover, Schlütersche Verlagsgesellschaft. 2007, pp. 14-5.
24. Tucker RL. Ultrasound-guided biopsy. In: Rantanen RW, McKinnon AO, editors. Equine Diagnostic Ultrasonography. Baltimore, Williams & Wilkins. 1998, pp. 649-53.
25. Orrison WW, Eldevik OP, Sackett JF. Lateral C1-2 puncture for cervical myelography. Part III: Historical, anatomic, and technical considerations. Radiology 1983; 146 (2): 401-8.
26. Patten BM. How much blood makes the cerebrospinal fluid bloody? J Am Med Assoc 1968; 206 (2): 378.
27. Testoni S, Grandis A, Diana A, Dalla Pria A, Cipone M, Bevilacqua D, Gentile A. Imaging diagnosis – ultrasonographic diagnosis of diplomyelia in a calf. Vet Radiol Ultrasound 2010; 51 (6): 667-9.

Table 1

Ultrasonographic measurements of the vertebral canal at the atlanto-occipital space in 73 euthanized cattle (mm, mean ± standard deviation, median, range) (reproduced from Braun et al.[17])

Variable	Section: Longitudinal	Section: Transverse
Distance between skin and arachnoidea	38.6 ± 4 (30 – 52) n = 68	39.5 ± 4.2 (32 – 52) n = 73
Depth of the subarachnoid space dorsal to the spinal cord	8.9 ± 1.6 (5 – 12) n = 67	9.2 ± 1.6 (6 – 13) n = 73
Diameter of spinal cord	9.9 ± 1.2 (6 – 13) n = 67	10.1 (8 – 15) n = 72[2]
Depth of the subarachnoid space ventral to the spinal cord	8.4 (4 – 11) n = 68	8.8 ± 1.8 (5 – 14) n = 73
Diameter of entire dural sac	26.9 ± 3 (20 – 34) n = 68	28.2 ± 3.5 (21 – 40) n = 73
Length of visible spinal cord	43.1 ± 10.3 (19 – 72) n = 67	-

Legend to Figures

Fig. 1 Longitudinal ultrasonogram and schematic representation of the vertebral canal at the level of the atlanto-occipital space obtained immediately after euthanasia in a 3.5-year-old Swiss Braunvieh cow. Left is cranial and right is caudal. 1 Nuchal ligament, major and minor rectus capitis muscles, 2 Atlanto-occipital membrane, 3 Subarachnoid space dorsal to the spinal cord, 4 Spinal cord, 5 Central canal, 6 Subarachnoid space ventral to the spinal cord, A Distance between skin and arachnoidea, B Depth of the subarachnoid space dorsal to the spinal cord, C Diameter of the spinal cord, D Depth of the subarachnoid space ventral to the spinal cord.

Fig. 2 Transverse ultrasonogram and schematic representation of the vertebral canal at the level of the atlanto-occipital space obtained immediately after euthanasia in a 3.5-year-old Swiss Braunvieh cow. 1 Nuchal ligament, major and minor rectus capitis muscles, 2 Atlanto-occipital membrane, 3 Subarachnoid space, 4 Spinal cord, 5 Denticulate ligaments, 6 Venous sinus within the epidural space, 7 Epidural space, A Distance between skin and arachnoidea, B Depth of the subarachnoid space dorsal to the spinal cord, C Diameter of the spinal cord, D Depth of the subarachnoid space ventral to the spinal cord.

Fig. 3. Collection of cerebrospinal fluid in a sedated cow in lateral recumbency. The head and legs are tied to the operating table. The fluid is collected from the atlanto-occipital space using a spinal needle and ultrasonographic guidance provided by a 5-MHz convex transducer.

Fig. 4. Schematic diagram of puncture of the subarachnoid space for collection of cerebrospinal fluid. The diagram is based on MRI images of the head of a 10-year-old Simmental cow. 1 Occiput, 2 Atlas, 3 Subarachnoid space, 4 Spinal cord.

Fig. 5. Frequency distribution of different levels of blood contamination of cerebrospinal fluid collected under ultrasound guidance in 73 cows.