Cerebrospinal fluid collection and interpretation

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Abstract

Cerebrospinal fluid analysis is an important ancillary diagnostic test indicated in cases where the results of a physical and neurological exam indicate central nervous system disease. Analysis of cerebrospinal fluid is most valuable in diagnosing inflammatory or traumatic conditions and, in some cases, may demonstrate the etiologic agent. Cerebrospinal fluid collection is a field-friendly procedure and serum preservation is used to stabilize cells in the fluid for shipping to a reference laboratory. This manuscript details the cerebrospinal fluid collection procedure and interpretation of protein concentration, total nucleated cell counts and other analytes.

Keywords: Sheep, goats, camelids, cerebrospinal fluid

Cerebrospinal fluid (CSF) is obtained from 2 sites: the atlanto-occipital (AO) space and the lumbosacral (LS) space. The AO site is preferred for suspected intracranial disease due to proximity of the lesion, but requires heavy sedation or full anesthesia due to the risk of damage to the brainstem with animal movement. For this reason, the LS site is most commonly used, which can generally be done with the animal standing or in sternal recumbency, awake or with

light sedation. It is best for spinal disease, but due to caudal flow of CSF produced in the brain, it can be useful for more cranial disease.

For AO taps, the patient is positioned under heavy sedation or anesthesia in lateral recumbency with the neck flexed to open the AO space. The space is palpable just caudal to the occipital bone. For LS taps, the patient is restrained standing or in sternal recumbency with no or light sedation. For patients in sternal recumbency, it is helpful to flex the hips and fully extend the hindlimbs cranially, which opens up the LS space dorsally. The wide LS space is palpable just caudal to a line drawn across the ileal wings.

For both locations, surgically clip and prepare the site aseptically. Block the skin with 0.5mL of 2% lidocaine to provide anesthesia of the skin at the needle entrance point. A potential drawback of this block is that it may increase peripheral blood contamination of the needle and the subsequent fluid sample. Using a 20-18 ga, 3.5" (9cm) spinal needle or, in smaller sheep and goats, a 20 gauge hypodermic needle, pierce the skin on midline perpendicular to the spine and with the bevel facing cranially. At first, simply pierce the skin to allow for any reaction from the patient and to avoid inadvertent deep puncture. Once the animal is still, confirm the needle's perpendicular placement to the spine in the saggital and transverse planes. Then, advance the needle slowly through the interarcuate ligament, which will make a palpable 'pop'. Continue to advance slowly into the subarachnoid space, which may also make a 'pop' and/or will result in a twitch reaction from the patient. This may include tail twitching or attempting to push off with the hindlimbs. If using a spinal needle, pull the stylet and collect any fluid coming from the needle hub. If no fluid spontaneously exits the needle, take the stylet and swipe it across the back of your glove. If you are in the subarachnoid space partially or fully, the stylet will leave a wet streak on your glove. Attempt to aspirate fluid. If none is obtained, replace the stylet and advance

slightly more. Repeat the stylet removal and swipe as above until fluid is obtained. Place some the fluid into a red top or other additive-free tube and the remainder in an EDTA tube. If the volume obtained is small, I prioritize the EDTA tube for cytology and protein if the sample is of sufficient volume to fill the tube to the recommended volume. The additive-free tube may be used for culture and also protein.

CSF must be analyzed by a reference laboratory for an accurate total protein determination. Protein in CSF is in mg/dL, whereas an in-clinic refractometer lacks that sensitivity and is in g/dL. Further, for a cell count and differential, CSF needs to undergo a cytospin and be rapidly analyzed by a reference laboratory. In situations where a reference lab is not immediately available and samples must be shipped, there is a technique for cell preservation. First, 0.2mL or more of CSF should be placed in a plastic tube for protein determination. Then, collect blood from the patient and allow it to clot in a non-additive tube for 30 minutes. At that time, centrifuge the sample and harvest the serum. Then, take 0.25mL CSF and place that into an EDTA tube and add 0.03mL of serum. Label this tube as serum-spiked so the laboratory knows not to report a total protein on this tube. Finally, ship both samples overnight on cold packs for analysis.

Depending on the reference lab, a variety of values may be provided in the final report. An example of chemistry analyte reference ranges and interpretation follows.

- Analyte reference ranges
    - Protein – <40mg/dL
    - Pandy – negative
    - Glucose – 60-80% of blood
    - Sodium – 134-144mEq/L

- Creatine kinase (CK) – 2-48IU/L
- Xanthochromia – negative

Total protein is a measure of the protein in the CSF with elevations indicating inflammation or other immune stimulation. Pandy's test or Pandy's reaction indicates elevated protein levels, mostly indicating globulins, indicative of immune stimulation. Glucose levels should be 60-80% of the blood glucose and lower levels suggest bacterial consumption of glucose. Sodium concentration elevations are indicative of salt toxicity. An isoenzyme of CK is found in the brain and elevations have been found in humans with traumatic brain injuries. Its interpretation and utility in veterinary medicine is not totally elucidated. Xanthochromia indicates a yellow discoloring to the CSF attributed to bilirubin and, among other things, may indicate hemorrhage into the CSF.

Laboratories will also provide a red blood cell count (as an indicator of peripheral blood contamination of the sample) and a white blood cell count and differential. Red blood cells (RBCs) in the CSF may indicate hemorrhage as part of the disease process (trauma) or peripheral blood contamination during aspiration. The presence of platelets in freshly analyzed samples can help differentiate these as the lifespan of platelets in the CSF is about 6 hours. If RBCs are present and platelets are present, it is likely contamination; however, if platelets are absent, then it may indicate hemorrhage as part of the disease.

White blood cell counts in excess of the reference range is referred to as pleocytosis and is further described by the predominant cell type.

- WBC reference range and common differential count interpretations for small ruminants
    - Reference range <3 cells/uL
    - Neutrophilic pleocytosis - bacterial infection, such as meningitis

- Eosinophilic pleocytosis - parasitic infection, such as meningeal worm
- Mononuclear pleocytosis or lymphocytic pleocytosis - listeriosis, viral infections or some degenerative conditions

A study from Switzerland has been published looking at CSF results in sheep and goats with various neurological conditions. I have summarized some of their findings in Table 1.[1] I would caution, however, that their results, particularly for cerebrospinal nematodiasis, are not consistent with what others and I see here in the United States, where we see significant eosinophilic pleocytosis.

It is tempting when working in a rural environment to be reticent to offer apparently costly diagnostics or those which are associated with a relatively high laboratory analysis cost, such as CSF analysis. Performing the CSF collection itself is associated with very low material cost - needle, syringe, 2 tubes, gloves and scrub material. It can be valuable to perform CSF collection even when laboratory submission is not in the budget. In some cases, the changes to CSF may be significant enough to cause visual changes that can be interpreted in light of the clinical presentation of the patient to provide a presumptive diagnosis. A foamy appearance to CSF indicates a total protein level of >200mg/dL (reference range <40mg/dL), while a cloudy appearance to the CSF indicates a total white blood cell count of >40 cells/uL (reference range <3 cells/uL). For diseases like septic meningitis, these visual changes may be sufficient to indicate a guarded to grave prognosis to allow for informed decisions regarding the pursuit of treatment and associated costs.

References

1. Schöb LC, Gerspach C, Stirn M, Hofman-Lehmann R, Riond B. Findings related to cerebrospinal fluid and central nervous system disorders in small ruminants - a retrospective study on sheep and goats. *Animals* 2024; 14(1):46.

Table 1. CSF results for sheep and goats with various neurological conditions. Adapted from: Schöb LC, et al, 2024.

Diagnosis	TP (mean)	TNCC (range)	Differential
Listeriosis Sheep	171 mg/dL	10 - 9941 cells/uL	Monos > lymphs > neuts
Listeriosis Goats	940 mg/dL	9 - 925 cells/uL	Lymphs>monos>neuts
Encephalitis Sheep	575 mg/dL	4 - 62 cells/uL	Monos > lymphs > neuts > eos
Meningoencephalitis Goats	333 mg/dL	20 - 13,170 cells/uL	Neuts>>lymphs>monos
Nematodiasis Sheep	640 mg/dL	68 - 83 cells/uL	Lymphs>>eos>monos
Nematodiasis			