

Satellite Article

Cerebrospinal fluid collection and its analysis in equine neurological disease

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Keywords: horse; neurological disease; CSF; atlanto-occipital; lumbosacral; cytology

Neurological diseases are commonly encountered in equine practice; however, establishing a definitive diagnosis *ante mortem* is at times difficult. Following neurological examination, additional ancillary diagnostic techniques are often indicated although one such technique, the collection and analysis of cerebrospinal fluid (CSF), is sometimes omitted due to practical constraints, perceived technical difficulty or safety concerns. Furthermore, because results obtained following CSF collection are rarely specific for any particular condition (Hayes 1987), some clinicians may feel that time or money is better spent elsewhere. However, as seen in the case report by Bentz *et al.* (2006) in this issue, CSF analysis may enable not only a diagnosis to be reached, but also provide a means with which to monitor response to treatment: with cases like these, the clinician is rewarded for the extra effort.

This article summarises normal anatomy and physiology of CSF, how it changes during disease and the techniques employed for CSF collection in adult horses and foals.

CSF anatomy, physiology and response to disease

CSF surrounds the brain and spinal cord (De Lahunta 1983). It is produced by the choroid plexi of the lateral, third and fourth ventricles as well as directly from the ependymal lining of the ventricular system, the pia arachnoid and meningeal blood vessels and central canal of the spinal cord (**Fig 1**) (De Lahunta 1983). The CSF production rate is not known in horses, but in man is equivalent to 3–5 times the total CSF volume per day (Lorenzo *et al.* 1970; De Lahunta 1983), a rate largely dependent on the plasma oncotic pressure (Mayhew and Beal 1980). Regional pressure changes caused by vertebral motion and pulsation of blood vessels force the CSF caudally from the ventricles into the subarachnoid space and along the spinal

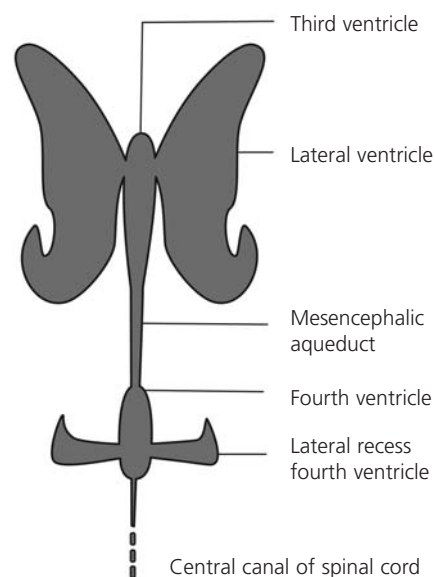


Fig 1: Diagram illustrating the ventricular system of the brain and its extension to the spinal canal. CSF is produced in the choroid plexi of the lateral, third and fourth ventricles, as well as by the ependymal lining of the ventricles and spinal canal. CSF gains access to the subarachnoid space (from where it is collected) via apertures at the lateral recesses of the fourth ventricle.

canal (De Lahunta 1983; Smith and George 2002). Arachnoid villi in the venous sinuses, acting as one-way valves, collect CSF because of its higher hydrostatic pressure than venous blood (Mayhew and Beal 1980; De Lahunta 1983), thereby returning the fluid to the circulation.

Functions of CSF

CSF serves as an intracerebral transport medium for neuroendocrine hormones and transmitters (Mayhew and Beal 1980). Other functions include protection of the brain and spinal cord, regulation of intracranial pressure and maintenance of ionic and acid-base balance (Mayhew and

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Fig 2: Atlanto-occipital CSF collection. a) Diagrammatic representation redrawn from Mayhew (1975) with permission. The cranial borders of the atlas are shown with red circles and the external occipital protuberance on the dorsal midline is marked with a red cross. b) With the horse anaesthetised in lateral recumbency and the head flexed 90°, the needle is inserted as described, directed towards the horse's lower lip. White tape marks the cranial border of the atlas. The stylet is removed frequently to check for CSF flow. c) Upon entering the subarachnoid space, CSF is collected by an assistant.

Beal 1980). The pH of CSF is determined by the pCO₂ and the cerebrospinal strong ion difference, in particular, relative changes in the ratio of sodium to chloride; buffering ability is not as tight as that seen in the plasma particularly in neonates where for example hypercapnia may contribute to the central nervous system dysfunction often encountered in sick foals (Geiser *et al.* 1996).

CSF collection

CSF may be collected from either the atlanto-occipital (AO) or lumbosacral (LS) sites and several factors influence the clinician's choice. For example, a LS tap is more likely to provide information about diseases caudal to the *foramen magnum* because of the caudal CSF flow (Mayhew 1975). In addition, AO collection requires general anaesthesia in adults. Larger volumes of CSF are readily obtained from the AO site in comparison with the LS site, although large volumes are rarely required (except when performing myelography), but removing a large volume can lead to brain herniation and

death in animals with increased intracranial pressure (Lorenz and Kornegay 2004). As a result, AO taps are usually contraindicated when the clinician considers that intracranial pressure may be raised (e.g. following trauma, or as a result of possible space-occupying lesions).

Complications of AO and LS puncture are extremely rare provided the procedure is performed aseptically, with care and under appropriate restraint. Since specific analytical equipment is usually required, the service of a specialist laboratory or a local hospital is needed for complete evaluation, which should be performed as soon as possible after sample collection (Mayhew and Beal 1980; Sweeney and Russell 2000). If immediate analysis is not possible, the sample can be divided with one part fixed by adding an equal volume of 50% ethanol (Hayes 1987) although, extrapolating from experiments on canine CSF, the addition of an equal volume of hetastarch or adding fetal calf serum (to make a 20% solution) may be preferable (Fry *et al.* 2006). The remaining (unaltered) sample should also be submitted and the dilution factor should be accounted for when interpreting results.

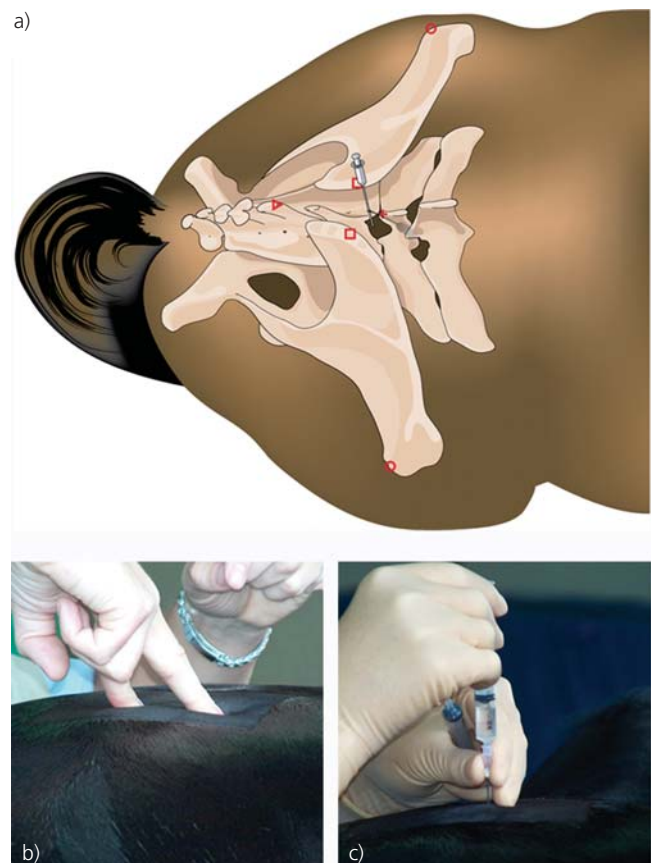


Fig 3: Lumbosacral CSF collection. a) Diagrammatic representation redrawn from Mayhew (1975) with permission. The palpable landmarks are the caudal border of each tuber coxae (red circles), the cranial edge of each tuber sacrale (red squares), the 2nd sacral vertebral dorsal spinous process (red diamond) and in some horses, the caudal edge of the spinous process of L6. b) Palpating the cranial edge of each tuber sacrale. c) gentle aspiration with a 5 ml syringe following correct spinal needle placement.

Atlanto-occipital puncture (Fig 2)

A CSF aspirate can be obtained by AO puncture in lateral recumbency during general anaesthesia in adults or occasionally under sedation and local anaesthesia in foals. The technique is simple, but carries potentially a greater risk of iatrogenic trauma than the LS site. Ultrasound guidance is reported (Audigie *et al.* 2004), but rarely necessary.

The head is flexed at the AO joint approximately 90° (ensuring that the airway remains patent during flexion) and the neck-poll region clipped and prepared for an aseptic procedure. The puncture site is in the midline along an imaginary line drawn between the cranial borders of the atlas wings: it is useful to mark the line with tape outside the prepared area.

Wearing sterile gloves, a spinal needle, (for an adult 450 kg horse: 18 or 20 gauge 9 cm needle with stylet; for a foal: 20 gauge 3.8 cm needle, with or without stylet) is inserted and slowly advanced in the direction of the horse's lower lip. The usual depth where the subarachnoid space is entered is approximately 4–6 cm in a 450 kg horse, 1.5–2.5 cm in a foal. Every few millimetres the stylet is removed to check whether the subarachnoid space has been entered (CSF should flow). If not, the stylet is replaced and the needle advanced further. Occasionally, rotating the needle is helpful. When the needle enters the subarachnoid space, CSF will drip rapidly from the needle as soon as the stylet is removed. The first few millilitres of CSF are usually discarded and then 2–3 ml are collected. Note: if CSF spurts from the needle this indicates increased pressure and the needle should be withdrawn immediately. The sterile stylet is replaced for needle withdrawal.

Lumbosacral puncture (Fig 3)

Obtaining a LS CSF sample in a standing horse is a generally safe procedure, but should ideally be performed in stocks. Moderate, but not heavy sedation with an α_2 agonist and butorphanol is recommended even though this reduces CSF pressure (Moore and Trims 1992). With the horse standing squarely, the landmarks are palpated. These include (1) a line drawn over the rump between the caudal borders of the *tuber coxae*; (2) approximately 1 cm cranial to the midline depression between the cranial aspect of each dorsal prominence of the paired *tuber sacrale*. Note: often this site is close to, or at the highest point, of the rump of a standing horse. Landmarks are more difficult to detect in overweight animals or those with previous pelvic injury. LS CSF collection is also possible in the laterally recumbent horse and, indeed, this is usually more practical in foals.

The site is clipped and prepared for an aseptic procedure and sterile gloves worn. Approximately 5 ml of local anaesthetic is infiltrated subcutaneously and a pilot hole at the correct insertion site can be made with a sterile 12 gauge needle. Then, standing to the side of the horse, the clinician inserts a spinal needle (15.2 cm 18 gauge for a 450 kg horse; occasionally an 20.3 cm needle is required for heavy draught breeds). The needle is advanced perpendicularly - it is helpful to

have an assistant check this. At the correct site, needle passage is unimpeded; however, the needle should be repositioned if any difficulty or grating sensation is encountered.

In a 450 kg horse, the needle usually needs to be inserted to a depth of 12.7–14 cm. In most cases a slight change of resistance with penetration of the dorsal *dura mater* is sensed. Frequently at this movement the horse may flinch, twitch the tail or occasionally exhibit more violent movements. If in doubt, it is helpful to check to see if CSF is obtained. The stylet is removed and a sterile 5 ml syringe is connected. Very gently, the plunger is withdrawn and, if the needle is positioned correctly, CSF should begin to flow. CSF flow can be increased by having an assistant occlude both jugular veins (Queckenstedt's phenomenon). The stylet is inserted prior to needle withdrawal. Note: occasionally several attempts are required or it is necessary to rotate the needle or to replace the stylet and advance the needle slightly deeper. If the needle hits bone, or is inserted maximally, it should be withdrawn and angled cranially or caudally.

Iatrogenic blood contamination is common at the LS site. Occasionally whole blood is obtained when the epidural vein is punctured, but usually contamination is slight and may be recognised as a red stream within a clear sample probably as a result of meningeal or spinal cord vessel damage. Gentle and steady CSF aspiration reduces iatrogenic blood contamination and discarding the first few millilitres of sample can make it easier to interpret results as iatrogenic haemorrhage usually quickly stops. Occasionally a completely new needle is required or the procedure needs repeating at a later time.

CSF assessment (Table 1)

Gross appearance

Normal CSF is clear and colourless (Mayhew and Beal 1980; De Lahunta 1983; Smith and George 2002). Bacteria, fungi or epidural fat may occasionally cause it to appear turbid in the absence of pleocytosis (Hayes 1987; Mayhew 1989; Lawrence 2005). Usually though, slight turbidity is appreciated with as few as 200 WBC/ μ l or 400 RBC/ μ l (Hayes 1987). CSF may clot if it contains increased fibrinogen (occasionally seen in suppurative meningitis or if the sample is significantly blood contaminated (De Lahunta 1983; Lorenz and Kornegay 2004).

More than 600 RBC/ μ l gives CSF a red discolouration. If the haemorrhage is recent or iatrogenic, the CSF usually becomes colourless with centrifugation (Mayhew and Beal 1980; De Lahunta 1983; Hayes 1987); however, if it persists, earlier haemorrhage should be suspected. Minor haemorrhage will not significantly influence leucocyte numbers or protein concentrations (De Lahunta 1983) and formulae used to predict the proportions of nucleated cells or protein concentration following blood contamination can be inaccurate and are probably unnecessary (Sweeney and Russell 2000). However blood contamination can generate false positive results when CSF is analysed by Western blot for *Sarcocystis neurona* antibodies when testing for equine protozoal myeloencephalitis (Miller *et al.* 1999).

TABLE 1: A guide to typical cerebrospinal fluid results according to certain conditions. Note that exceptions apply

Condition	Gross appearance	Total protein	Nucleated cells	Cytology/comments
Cervical vertebral malformation/stenosis	Clear	↔	↔	Usually normal; occasionally mild increase in nucleated cells and protein
Traumatic injury	Clear to mildly xanthochromic; red tinge	↔ ↑	↔ ↑	Erythrophagia, protein and later increased nucleated cells
Bacterial meningitis	Turbid, may clot; may be xanthochromic	↑	↑ ↑	Mononuclear pleocytosis with neutrophilic predominance; glucose may be decreased
Viral encephalitides	Clear to xanthochromic	↑	↑	Mononuclear pleocytosis with lymphocytic predominance; eastern and Venezuelan encephalitis may be associated with neutrophilic pleocytosis
Equine herpes virus 1 myeloencephalopathy	Often markedly xanthochromic	↑ ↑	↔ ↑	High total protein often without an increase in nucleated cells
Protozoal, verminous infection, abscess or neoplasia	Normal to mildly xanthochromic	↔ ↑	↔ ↑	Mild mononuclear pleocytosis with neutrophilic predominance or normal.
Fungal infection	Clear to xanthochromic	↑	↑ ↑	Neutrophilic pleocytosis

Key: ↔ - no change; ↑ - increased; ↑ ↑ - markedly increased.

Xanthochromia (a slight yellow tinge), that persists after centrifugation is usually due to previous RBC lysis, and appears within 2–4 h following subarachnoid haemorrhage (De Lahunta 1983; Smith and George 2002). Mild xanthochromia is also seen in neonates - a reflection of their higher CSF protein concentration (Furr and Bender 1994) or in pathological conditions associated with elevated CSF protein concentration, such as herpes (EHV1) myeloencephalitis, or during hyperbilirubinaemia (De Lahunta 1983; Lorenz and Kornegay 2004).

Protein concentration

As an ultrafiltrate, CSF contains much less protein than blood (approximately 1/100 of the plasma total protein; generally less than 0.8 g/l). Normally protein reaches the CSF by pinocytosis, (it is excluded by the blood-CSF barrier), although in neonatal foals, a more permeable blood-CSF barrier is believed to account for their higher CSF protein concentration, reflecting both increased IgG and albumin (Rossdale *et al.* 1982; Andrews *et al.* 1994). Ponies are also reported to have higher CSF protein concentrations than horses (Mayhew *et al.* 1977).

A urinary dipstick test is not sufficiently sensitive to determine CSF protein concentration (Scott 2004) and if possible, a quantitative measurement of total protein is preferred. However, in practice, and as used by the authors in this issue (Bentz *et al.* 2006) the Pandy test can provide a reasonable assessment of CSF protein concentration. The test relies on 10% carbolic acid solution causing precipitation of proteins, the degree being specified as 1+ to 4+. Normal CSF shows a negative result (Kraft *et al.* 1995). A foamy sample is also indicative of elevated protein concentrations (generally over 2 g/l) (Mayhew and Beal

1980; De Lahunta 1983).

Increased CSF protein concentration has low specificity and is seen in any condition that increases blood-CSF barrier permeability (eg. CNS infection, haemorrhage, EHV1 myeloencephalitis and tissue degeneration). It is also seen with conditions that result in intrathecal antibody production. So-called albuminocytological dissociation (elevated protein concentration without pleocytosis) occurs in cases of noninflammatory degeneration of brain parenchyma with tissue necrosis, vascular lesions, neoplasia and viral nonsuppurative encephalitis (De Lahunta 1983).

Protein fractions (mainly globulins and albumin) can be separated by electrophoresis. Increased albumin concentration or an increased albumin quotient (CSF albumin concentration/serum albumin concentration x 100) suggests the possibility of disruption of the blood-CSF barrier. An increased IgG-index - a calculated quotient, (ratio of IgG in CSF and IgG in serum/ratio of albumin concentration in CSF and albumin concentration in serum) suggests intrathecal synthesis of IgG (Andrews *et al.* 1994) though not in foals (Cook *et al.* 2002).

Cell count

A differential nucleated cell count is generated most commonly following a Cytospin preparation. With this system, a cytocentrifuge sediments all the cells from 0.5–1.0 ml of CSF to enable microscopic evaluation. DiffQuick or occasionally other stains are used.

Normal CSF contains far fewer cells than the peripheral blood, with no erythrocytes and only small numbers of mononuclear cells (<5 WBC/ μ l) (De Lahunta 1983); small lymphocytes and occasional monocytes (approximately 30%), responsible for immune surveillance (Beech 1983) are normally

detected. The cells gain access to CSF by migration across the blood-brain and blood-CSF barriers, only following activation (Kleine and Benes 2006).

Any other leucocytes are considered abnormal. A neutrophilic CSF pleocytosis is the most common abnormality seen with bacterial meningitis however neutrophilic predominance may occur early in viral meningitis and monocytes may form the majority of cells in late bacterial infections (Mayhew and Beal 1980). A predominantly monocytic or lymphocytic pleocytosis is more characteristic of viral encephalitis (Mayhew and Beal 1980). Note that the vasculitis associated with equine herpes virus myeloencephalitis is often not associated with pleocytosis.

Protozoal and parasitic infections and abscesses usually result in either normal CSF or occasionally a low number of neutrophils or mildly elevated mononuclear cell count. Eosinophils are occasionally encountered with fungal or parasitic disease or spinal neoplasia (Mayhew and Beal 1980). Brain or spinal lymphoma or haemangiosarcoma may produce cells with characteristic neoplastic features. Spinal cord compression as a result of cervical vertebral malformation or stenosis, usually results in normal CSF cytology or very minor alterations.

Pressure

The pressure of CSF can be measured but is not specific (De Lahunta 1983; Hayes 1987; Lorenz and Kornegay 2004) and is influenced by the technique, anaesthesia and systemic blood pressure (De Lahunta 1983). It is rarely measured in the clinical setting.

CSF glucose

Glucose concentrations in CSF are reported to be approximately 60–80% of plasma glucose and correlates with the plasma concentrations during the previous 1–3 h (Mayhew and Beal 1980). Concentrations are somewhat higher in foals, but decrease rapidly with age (Furr and Bender 1994). Decreased glucose concentrations (hypoglycorrachia) may be seen with acute meningitis, where it is utilised by bacteria or fungi and endogenous WBC (Mayhew and Beal 1980).

Other parameters

Both CSF and plasma have the same osmolality: approximately 289 mOsm/l, but CSF contains less potassium, bicarbonate and calcium, but more chloride, sodium and magnesium than plasma (Rossdale *et al.* 1982; De Lahunta 1983). Enzyme activities are occasionally measured, although evidence suggests that creatine kinase activity may rise as a result of contamination with epidural fat or *dura* and the test is probably best avoided (Jackson *et al.* 1996).

Gram stain and culture of CSF

If bacterial infection is suspected from clinical signs or after detecting turbid CSF, low CSF glucose concentration or

neutrophilic pleocytosis, a sample should be cultured. Prior to receiving antibacterial sensitivity results, a Gram stain can aid in early choice of antibiotic therapy (De Lahunta 1983; Lorenz and Kornegay 2004).

Conclusions

Performed properly, CSF collection is a safe procedure that gives valuable additional information about central nervous system disease. When used in conjunction with the history, physical and neurological examination and other diagnostic tests, it can aid in establishing diagnosis and prognosis as well as choice of therapy and response to treatment. Occasionally, as seen in the case report in this issue (Bentz *et al.* 2006) CSF collection becomes the essential component of the diagnostic workup.

Acknowledgement

The authors are grateful for the help of Mr Jack Sisterson, Royal Veterinary College for generating the redrawn digitised images of CSF collection.

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