Analysis of cerebrospinal fluid from 20 calves after storage for 24 hours

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Samples of CSF collected from 20 normal healthy calves were analysed either immediately or after having been stored for 24 hours at 4°C in the presence of 11 per cent autologous serum. There were no significant differences between the total and differential cells counts of the fresh and stored samples, but there was a positive linear correlation between them. There were some morphological changes to the nuclei of the mononuclear cells in the stored samples.

The analysis of cerebrospinal fluid (CSF) has an important role in the diagnosis of neurological diseases, especially for detecting inflammatory changes. Its total cell count and protein concentration can provide useful information for differentiating between inflammatory, neoplastic and degenerative processes, and the differential cell count and cellular morphology can further define the pathological process (Lorenz and Kornegay 2004). In cattle, CSF can be collected either from the cerebellomedullary cistern or the lumbosacral site, depending on the site of the lesion in the central nervous system (CNS). Clinically, CSF is often collected from the lumbosacral space rather than the atlanto-occipital space because of the risks inherent in the latter technique (Brewer 1987, Mayhew 1989, Jean and others 1997, Scott 2004). Because CSF is highly perishable, it must be processed within an hour of collection. One way to overcome this technical limit is to add ethanol or formalin to preserve the cells for a total cell count; a cytoconcentrate can be prepared with a sedimentation device directly in the field (Raskin and Freeman 2001), but this expedient is hardly applicable to bovine practice. The addition of autologous or fetal serum to canine and feline CSF has been shown to preserve the cells and stabilize the sample for up to 24 hours (Bienzle and others 2000, Fry and others 2006), but to the authors' knowledge no similar studies have been conducted with CSF from cattle. To evaluate the stabilising effect of autologous blood serum added to CSF the total and differential cell counts and cell morphology of CSF samples from 20 healthy calves were analysed within an hour of collection and after being stored for 24 hours at 4°C.

MATERIALS AND METHODS

The study was performed in accordance with animal welfare regulations. Twenty healthy male Holstein calves, two to three months old, housed in box stalls, were given a complete physical and neurological examination, and shown to have no clinical signs of disease. A blood sample (5 ml)

was collected from a jugular vein of each calf into a serum vacuum tube, and the serum was separated by centrifugation for 10 minutes at 1500 *g*.

COLLECTION OF CSF

A sample of CSF was collected from the lumbosacral site of each calf, while it was standing, as described by Mayhew (1989). The calf was sedated with 0·05 mg/kg xylazine (Rompun; Bayer HealthCare) administered intravenously. The collection site (5 cm x 10 cm) was clipped, surgically prepared and locally anaesthetised with 2·5 ml of 2 per cent lidocaine (Lidocaine 2 per cent; Fort Dodge Veterinaria). Disposable, 20 G 0·90 mm x 90 mm spinal needles (Terumo) were used to collect 5 ml CSF by gentle syringe aspiration into a sterile tube. The sample was divided into two equal parts, one of which was analysed immediately.

Autologous serum was added to the other to a final concentration of 11 per cent and it was stored for 24 hours at 4°C.

ANALYSIS OF CSF

The fresh samples were analysed within an hour of collection. To assess the total nucleated cell count, 100 µl CSF were added to 100 µl Turks stain and placed on a Nageotte haemacytometer, and the number of cells in an eight by 10 rectangle was counted. Two slides, one for a differential cell count and one for a morphological evaluation, were prepared by the cytocentrifugation (Cytospin2; Shandon) of 250 µl CSF for six minutes at 150 *g*, and stained with May-Grünwald Giemsa. Only samples with at least 10 cells per slide were used for the differential cell count. The percentages of neutrophils, eosinophils and mononuclear cells (subdivided into lymphocytes and monocytes) were calculated (Raskin and Freeman 2001). The total protein concentration of the samples was measured by the spectrophotometer method (Sentinel Diagnostics) using a pyrogallol red assay. The total and differential cell counts of the stored CSF samples were analysed in the same way 24 hours later.

STATISTICAL ANALYSIS

The data were analysed by using the freeware statistical software package R2 1.0 (R Development Core Team 2007). The Shapiro-Wilk normality test was used to determine whether the data followed a normal distribution, and statistical significance was examined by the Wilcoxon sum-rank test. Pearson's product-moment correlation coefficient (r) was used to test the strength of the linear correlations between the total and differential cell counts before and after storage. Statistical significance was set at $P \leq 0{\cdot}05$ (Petrie and Watson 2006).

RESULTS

Table 1 shows the mean (sd) protein concentration and total and differential cell counts of the samples. Five of the 20 samples were excluded from the differential cell count because they contained less than 10 cells per smear. There were no significant differences between the total and differential cell counts of the fresh and stored samples. Mononuclear cells were the most numerous in both the fresh and the stored samples; neutrophils were detected in nine of the 15 samples, in both aliquots. In the stored samples there were increases in mononuclear cells and decreases in neutrophils, but the differences were not statistically significant. The morphology of the cells was preserved in all the stored samples, except for some small mononuclear cells having cleaved nuclei and an increase in the number of vacuolated mononuclear cells (Fig1). Table 2 shows the coefficients of linear correlation between the total and differential cell counts in the fresh and stored samples of CSF.

TABLE 1: Mean (sd) total protein in fresh CSF and mean (sd) total and differential cell counts in fresh and stored samples of CSF

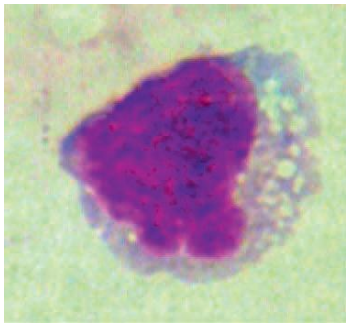
Measurement	Fresh CSF	Stored CSF	Mean difference	P
Total protein (mg/dl)	32·95 (7·65)			
Total cells (mm^3)	6·89 (7·12)	7·20 (4·81)	–0·33	NS
Neutrophils (%)	5·88 (9·22)	3·22 (5·51)	2·20	NS
Mononuclear cells (%)	88·77 (23·28)	96·55 (5·79)	–8·18	NS
Monocytes (%)	62·41 (17·15)	65·47 (13·42)	–4·00	NS
Lymphocytes (%)	29·83 (15·45)	32·06 (14·49)	–1·44	NS

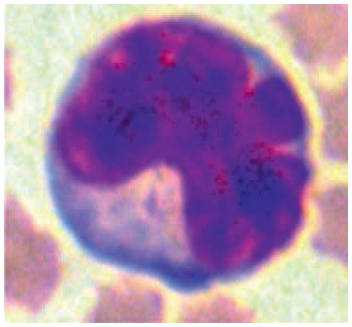
NS Not significant

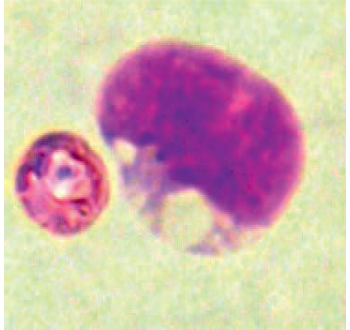
TABLE 2: Correlation coefficients (r) between the total and differential cell counts in fresh and stored samples of CSF

Measurement	Correlation Coefficient (r)	95% CI	P
Total cells (mm^3)	0·81	0·58-0·92	<0·001
Neutrophils (%)	0·83	0·56-0·94	<0·001
Mononuclear cells (%)	0·77	0·44-0·92	<0·001
Monocytes (%)	0·60	0·14-0·85	0·01
Lymphocytes (%)	0·51	0·10-0·81	NS

CI Confidence interval, NS Not significant







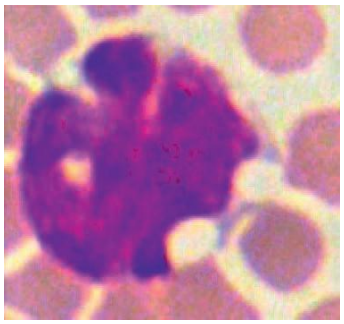


FIG 1: Morphological changes in mononuclear cells in stored CSF; after 24 hours of storage there was an increase in the number of small mononuclear cells with nuclear indentation and vacuoles. May-Grünwald Giemsa. x **1000**

DISCUSSION

There were no significant differences between the total and differential cell counts of the fresh and the stored CSF samples. In both the fresh and the stored samples, the total and differential cell counts were within the normal ranges, except for the higher proportion of monocytes and the lower proportion of lymphocytes. The concentration of protein in the fresh samples was within the normal range (Welles and others 1992, Jean and others 1997, Stocker and others 2002). The high standard deviations can be explained by the wide variations between the individual calves (Welles and others 1992, Stocker and others 2002) and the fact that the measurements
were made with a haemacytometer. There were significant positive linear correlations between the total, mononuclear, neutrophil and monocyte counts in the fresh and stored CSF, but not for the lymphocytes. The absence of a linear correlation may have been due to the cytoplasmic vacuolization and nuclear indentation observed in the stored samples.

The results of this study are encouraging. CSF analysis, which was previously difficult for bovine practitioners to perform in the field, provides a valuable diagnostic tool for assessing a neurological patient. The slight differences observed between the stored versus the fresh CSF samples did not compromise the diagnostic clinical interpretation in this series of clinically healthy calves. Further development of this method should evaluate its applicability to pathological samples, as has been done in small animal practice (Bienzle and others 2000, Fry and others 2006).

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