**Original article**

**Risk factors for blood contaminated cerebrospinal fluid collection in dogs**

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Word count:1995

**Structured Summary:**

**Objectives:** To determine the risk factors for blood contamination during cerebrospinal fluid (CSF) collection in dogs.

**Study Design and Methods:** Prospective study of 170 CSF samples. Data collected included signalment of the patient, body condition score, site of CSF collection (cerebellomedullary cistern – CMC or lumbar cistern – LC), number of attempts, clinician expertise, final diagnosis, time of day, skull formation and day of the week. Analysis of the CSF samples was then performed and the presence of blood contamination (red blood cells >500/µl) was recorded. Logistic regression was used to quantify the association of potential risk factors of the procedure. Multivariate analysis was performed on the variables that were statistically significant.

**Results**: Of the 170 CSF samples, 53% were collected from the CMC (n=90) and 47% from the LC (n=80). Blood contamination was seen in 20% (n=34) of the samples, 8.9% (n=8) in CMC and 32.5% (n=26) in LC samples. Increased odds of obtaining a contaminated CSF sample were associated with lower level of clinician expertise (odds ratio: 2.5; 95% confidence intervals: 0.9-6.7; P=0.046) and with LC vs CMC collection site (odds ratio: 8.1; 95% confidence intervals: 2.1-12.9; P=0.001).

**Clinical Significance**. There is increased likelihood of blood contamination when collecting CSF from the LC compared to the CMC site. Increased clinician experience reduced the risk of CSF blood contamination but none of the other variables examined significantly influenced this.

**Keywords: Cerebrospinal fluid (CSF); blood contamination; risk factors; lumbar cistern**

**INTRODUCTION:**

Cerebrospinal fluid (CSF) collection was first described in 1891, when it was used to relieve increased intracranial pressure in children with tuberculosis meningitis[1]. This technique has subsequently become a common diagnostic tool in the investigation of various neurological diseases in both human and veterinary medicine. The indications for CSF analysis have been reduced with the introduction of less invasive diagnostic procedures such as magnetic resonance imaging [2]. However the test still yields clinical significance for diagnosis of certain encephalopathies, myelopathies and radiculopathies[3,4].

The CSF is a relatively acellular fluid (reported reference interval for total nucleated cell counts of less than 5 per microliter) with a low protein content (<30 mg/dL on cerebellomedullary cistern [CMC] samples and <45 mg/dL on lumbar cistern [LC] samples [5]). Increased values are associated with pathology within the central nervous system where there is a compromise of the blood-brain barrier, the blood-CSF barrier or the CSF interface between the brain and the spinal cord[6].

Iatrogenic peripheral blood contamination can occur during the CSF collection procedure, typically resulting in a grossly blood-tinged fluid. Blood contamination in veterinary medicine has previously been quantified as 500 red blood cells per microliter[7,8]. The influence of blood contamination on CSF analysis is still somewhat controversial with conflicting results reported in the veterinary species. Doyle & Solano-Gallego(2009)[8] found increased protein concentration in blood contaminated CSF whilst a previous study had failed to show this[9]. Similarly, blood contamination significantly affected CSF total nucleated cell concentration [TNCC] in one study[10] but not in others[9,11, 12]. Blood contamination is also thought to affect neutrophil percentage and presence of eosinophils on cytological examination[8].In humans, blood in the CSF has been reported to alter the cell count, increase protein concentration and cause false-positive culture and cytological results, culminating in diagnostic uncertainty[13] . Various ratios have been proposed to correct the number of leucocytes and protein concentrations within the CSF in face of blood contamination[14] , but most are considered inaccurate[15] .

The risk factors for blood contamination of CSF samples following lumbar puncture in people have been reported and include race, sex, age, clinician experience and perioperative analgesia [13,16,17]. Similar information is not available in veterinary medicine therefore our goal was to try to identify patient, clinician and procedural factors associated with obtaining blood contaminated CSF samples in dogs.

**MATERIALS AND METHODS**

Aprospective study was undertaken, using data obtained from client-owned dogs undergoing CSF collection at the University of Liverpool Small Animal Teaching Hospital, from March 2016 to June 2017 (ethical approval granted for use of this data by the Ethics Committee of the University of Liverpool - VREC416). Inclusion criteria comprised dogs that required CSF analysis as part of their diagnostic investigation and for which several variables had been recorded during the collection procedure, prior to CSF analysis. Data collected included the following: age, sex, weight, body condition score, site of CSF collection, clinician experience level, number of attempts before successful collection, time of day and day of the week, final diagnosis and skull conformation. The data was further sub-categorised into dichotomous variables and categorical nominal variables. The dichotomous variables included CSF collection site (CMC or LC), clinician experience (whether a board-certified neurologist or resident in training performed the procedure), final diagnosis (grouped into inflammatory or non-inflammatory condition), time of day (the last 2 hours of the working day [3-5pm] compared to the rest of the day), number of attempts (one attempt, or, more than one attempt), body condition score (1-4 and 5-9) and skull conformation (brachycephalic or non-brachycephalic). Patients included in the inflammatory group were diagnosed with meningoencephalitis of unknown origin and steroid-responsive meningitis arteritis (CSF TNCC >5 cells/ul with and without changes on magnetic resonance imaging changes). We chose to categorise the last two hours of the working day separately as it was hypothesised that this would be the busier part of the day. The categorisation of the number of attempts was made as we hypothesised that the risk would increase after the first attempt as it would be possible to damage a vessel but not obtain CSF on the first attempt. Categorical nominal variables included days of the week (Monday-Wednesday, Thursday - Friday and weekend), age of the patient (<1, 1-2, 3-5, 6-8, 9 or greater) and weight (<10kg, 10-19.9kg, 20-29.9kg, 30-39.9kg and >40kg). Similar to what has previously been reported[7,8], a sample was considered blood contaminated when there was > 500 cells/µl. The presence of erythrophagia, haemosiderophages or haematoidin crystals (suggesting pathological rather than iatrogenic haemorrhage) was determined during cytological examination and if present, those samples were excluded from the study. The site of CSF collection was determined by the clinician in charge of each individual case, most commonly taking into account the level of the suspected pathology[Fig 1]. For those patients that required both CMC and LC samples, the LC collection was performed first. Samples were stored in a serum tube and the CSF was processed within 30-45 minutes of acquisition. Cerebrospinal fluid analysis included total TNCC and red blood cell concentration (RBC) determined by manual count (Neubauer haemocytometer), total protein (TP) concentration determined by pyrogallol red colorimetric assay, and cytologic assessment of the CSF by a board certified clinical pathologist.

All statistical analyses were performed using a standard statistical software package (SPSS: Statistical Package for the Social Sciences 22.0.1, SPSS). Regression analysis was applied to all the independent variables as a univariate analysis. Variables were considered significant if p<0.25. Multivariate analysis was performed by applying regression analysis on the independent variables that were statistically significant in the univariate analysis. The significance in the multivariate analysis was denoted if p<0.05.

**RESULTS**

A total of 166 patients were included in the study of which 170 samples were acquired. Of these samples, 53% (n=90) were taken from the CMC and 47% (n=80) from the LC. No complications associated with the CSF collection procedure were reported in any of the patients. In total, 20% (n=34) of the samples were contaminated with blood (>500 cells/ul). Blood contamination was identified in 8.9% (n=8) of the CMC samples and 32.5% (n=26) of the LC samples. The results of the univariate regression analysis are listed in *Table 1*. Following the univariate analysis, the dichotomous variables, that were statistically significant with the possibility of a contaminated outcome, were the CSF collection site [p=0.001], the level of clinician expertise [p=0.123] and the time of day [p=0.179]. The nominal variable statistically significant with the outcome of a contaminated sample were age groups 3 to less than 6 years [p=0.08] and greater than 9 years [p=0.165]. The results of the multivariate regression analysis revealed that only two variables remained statistically significant; CSF collection site (odds ratio: 8.1 ; 95% confidence intervals: 2.1-12.9; P=0.001) and level of clinician expertise (odds ratio: 2.5; 95% confidence intervals: 0.9-6.7; P=0.046).

**DISCUSSION:**

Cerebrospinal fluid analysis remains a commonly performed procedure as part of the diagnostic investigation of pathology affecting the central nervous system. Abnormalities may occur with infectious, inflammatory, neoplastic, traumatic and degenerative conditions, however, its results are rarely definitively diagnostic and most commonly only support the diagnosis[5]. The procedure is typically considered not to be associated with high risks but dogs require general anaesthesia (which comprises its own risks) and iatrogenic brainstem or spinal cord trauma[18,19] have been reported. Contraindications to perform the procedure include raised intracranial pressure, coagulopathies, atlanto-axial instability or cervical trauma and Chiari-like malformation 5. Iatrogenic presence of erythrocytes in the CSF is thought to be common in dogs with reported incidences between 12-86%, depending on the cut-off used to define blood contamination[8,9]. Our study is the first to prospectively evaluate a sample of consecutive patients undergoing CSF collection for investigation of neurological disease and we found an overall 20% incidence of blood contamination.

Causes previously identified for iatrogenic blood contamination in humans include damage to the small radicular vessels along the nerve roots as a possible cause[20] as well as trauma to the vertebral venous plexus due to overinsertion of the needle [21] The latter would generally only occur with collection at the LC site due to the procedural differences and this could explain the difference seen in this study.

Two risk factors associated with contaminated CSF samples were found in our study, namely the expertise of the person performing the procedure and whether it was obtained from the CMC or the LC. As expected, with increased experience there will inevitably be increased procedure proficiency and a similar finding has previously been reported in the human literature[17]. Regarding the collection site, it could be speculated that the different anatomy of the vasculature between both sites could be underlying the difference found in this study. More precisely, the window for not traumatising a blood vessel from the cerebellomedullary cistern is larger than that of the lumbar cistern. Also, in the majority of cases that required CMC collection, imaging of this cistern was provided, whilst this was less common for the lumbar cistern. It is possible that this resulted in a higher margin of error and increased the risk of blood contamination from vessels. Nonetheless, the use of ultrasound-assisted CSF collection has been described in humans[22] and in horses[23] and neither study identified a reduced risk of obtaining a contaminated sample.

Several variables that proved to be risk factors in the human population could not be directly translatable to our canine population. The way the procedure is performed can vary with humans, as the procedure can be undertaken with the patient conscious (with local anaesthesia), sedated or under general anaesthesia. The use of local anaesthetic in conscious patients was associated with a decreased rate in contaminated or unsuccessful lumbar taps[17].This technique was not applicable to our canine population. Risk factors have not been documented for CMC collection in humans as this is an uncommon site to obtain cerebrospinal fluid in people. It has been reported that there is an increased risk of a contamination sample in infants less than a year of age[13]. Speculative reasoning suggests an increase in technical difficulty resulting from a smaller intervertebral and a shallower depth of needle insertion to reach the cerebrospinal fluid. Such a risk factor was not evident in our canine population. Breeds of dog could account for a similar problem in terms of size-dependant acquisition of a non-contaminated CSF sample. However this was not evaluated in this study.

There are several limitations to our study. We did not control the expertise of the person assisting with the position of the patient during the collection procedure and the position itself. The positioning of the patient may have been performed by a resident in training (generally in anaesthesia or neurology) or a clinician (typically anaesthesia or neurology) and this could have possibly impacted on the results. It was also not possible to randomise the site of CSF collection as this was decided on a case specific basis, generally depending on the site of the suspected lesion (i.e. CSF collection was performed caudal to the lesion) and safety considerations. We also did not compare whether the abnormal CSF sample (abnormal white blood cell count or abnormal protein) would increase the risk of having a contaminated sample. As shown in the table, the underlying pathologies were categorised as primary inflammatory central nervous system disorders compared to non-inflammatory disease. With a greater sample size, individual conditions could possibly be compared separately. Advancement of the spinal needle with stylet, compared to without, has also been shown to be associated with a higher risk for blood contamination or unsuccessful CSF collection in children undergoing lumbar puncture[17]. This factor was not evaluated in our study, as preference for stylet in during needle advancement was unanimous in both clinicians and residents taking part in the study and further investigation of this variable may be warranted. Compared to the population enrolled in human studies[17], our sample population still remains small. However power analysis revealed a minimum sample size of 82; with an effect size of 0.3, an error probability of 0.05 and a power 0.8. Increased numbers could narrow the confidence intervals already present for the risk factors that were found, as well as identify further factors that could potentially become statistically significant. Further sub-categorising the underlying disease processes could potentially reveal other significant risk factors.

**CONCLUSION**

Our study has identified two risk factors affecting whether a CSF sample is contaminated. There is an increased likelihood [x8.1] of obtaining a contaminated CSF sample from the LC than from the CMC collection site. There is also an increased likelihood [x2.5] that a resident in training will obtain a contaminated CSF sample compared to a clinician, suggesting that an increase in expertise reduces the risk of blood contamination.

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***Table 1***: *Outcome of contamination due to independent variables.*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Factors** | **Category** | **Number of taps** | **Contaminated samples** | | **p-value** |
| **Number** | **Percentage (%)** |
| **Site** | CMC | 90 | 8 | 8.9 | **0.001** |
| LC | 80 | 26 | 32.5 |
|  |  |  |  |  |  |
| **Expertise** | Clinician | 54 | 7 | 13.3 | **0.123** |
| Resident | 116 | 27 | 23.3 |
|  |  |  |  |  |  |
| **Body condition score (/9)** | [1-4] | 79 | 14 | 17.7 | 0.440 |
| [5-9] | 91 | 20 | 22.0 | 0.486 |
|  |  |  |  |  |  |
| **Day of the week** | Monday | 36 | 6 | 16.7 | 0.853 |
| Tuesday | 25 | 4 | 16.0 | 1.000 |
| Wednesday | 33 | 9 | 27.3 | 0.732 |
| Thursday | 32 | 6 | 18.8 | 0.947 |
| Friday | 33 | 6 | 18.1 | 0.922 |
| Weekend | 11 | 2 | 18.2 | 0.887 |
|  |  |  |  |  |  |
| **Sex** | Male | 95 | 22 | 23.2 | 0.249 |
| Female | 75 | 12 | 16.0 |
|  |  |  |  |  |  |
| **Attempts** | 1 | 95 | 17 | 17.9 | 0.700 |
| >1 | 75 | 17 | 22.7 |
|  |  |  |  |  |  |
| **Diagnosis** | Primary inflammatory disease | 53 | 12 | 22.6 | 0.565 |
| other | 117 | 22 | 18.8 |
|  |  |  |  |  |  |
| **Time** | 3-5pm | 62 | 9 | 14.5 | 0.179 |
| before 3pm | 108 | 25 | 23.1 |
|  |  |  |  |  |  |
| **Age (years)** | <1 | 31 | 6 | 19.4 | 0.368 |
| [1-<3] | 40 | 5 | 12.5 | 0.483 |
| [3-<6] | 35 | 9 | 25.7 | 0.080 |
| [6- <9] | 44 | 7 | 15.9 | 0.646 |
| >9 | 19 | 6 | 31.6 | 0.165 |
|  |  |  |  |  |  |
| Weight **(kg)** | <10 | 48 | 10 | 20.8 | 0.530 |
| [10 - 19.9] | 66 | 11 | 16.7 | 0.999 |
| [20-29.9] | 28 | 8 | 28.6 | 0.999 |
| [30-39.9] | 24 | 3 | 12.5 | 0.999 |
| >40 | 4 | 0 | 0.0 | 0.999 |
|  |  |  |  |  |  |
| **Skull Conformation** | Brachycephalic | 43 | 9 | 20.9 | 0.573 |
| Non-Brachycephalic | 127 | 25 | 19.7 |