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Repeated Collection of Cerebrospinal Fluid from the Lumbosacral Region of Ewes

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ABSTRACT

***Background*:** Cerebrospinal fluid is a vital fluid from the central nervous system. Since CSF contains proteins, enzymes, hormones, neuropeptides and neurotransmitters that play critical regulatory roles in many different physiological processes, it has been extensively studied to explore different nervous disorders. Since CSF is a vital fluid from the CNS, used for clinical examination of the CNS in ruminants and other domestic species. CSF may be collected in ewes under field conditions, which allows the diagnosis of bacterial and metabolic diseases, as well as using it for cytological studies and biochemical analysis. Depending on the study, in opportunity the sampling protocol should be repeated to measure dynamic changes in the parameters selected for the analysis. Under field conditions, obtaining CSF samples from ewes is a difficult task. Thus, the aim of this work was to determine if it is possible to obtain repeated extractions of CSF by lumbosacral puncture from the same ewes under field conditions.

***Materials, Methods & Results*:** The CSF was sampled in three successive weekly collections from nine ewes sedated with ketamine. The procedure collections were made by the same trained operator, who stood behind the ewe, facing its back. Having checked that the sagital plane of the animal was perpendicular to the horizontal plane the puncture point was found by manual palpation at the slight depression between the ends of the spinous apophyses of the last lumbar and first sacral vertebrae. The wool was separated, and the area was cleansed with iodine solution. The puncture was performed with a spinal needle, after it had penetrated through the skin, the needle was pushed forward very slowly. When was listening for any vibrations ('clicks'), suggesting that the needle had crossed the dural membrane and entered into the arachnoidal space. Then, the syringe needle was withdrawn and the CSF came out slowly, either immediately or after some slow movements of the needle. If CSF did not come out, the puncture was deepened further on until the ventral arachnoidal space was reached. In the first and second collection, limpid CSF samples were obtained in all (9/9, 100%) and in 8/9 animals (89%), respectively. However, limpid CSF samples were obtained only in 4 of the animals one week later (4/9, 44 % P = 0.01). The volume of CSF extracted ranged from 0.6 to 0.9 mL/sample/animal.

***Discussion*:** The sequential collection of CSF in ewes is possible under field conditions to obtain a high percentage of samples to the along of three weekly extraction events. When only the first extraction event was considered, the sampling was totally effective even entirety of the animals. Yet by the third sampling, we obtained fewer samples than in the second event. In the present technique of repeated puncture was yielded a high efficacy in the first collection at random chosen ewes. The decrease in effectiveness was probably due to cumulative tissue damage and formation of extensive fibrous adhesions in the subarachnoidal space, which would compromise partially or totally the flow of CSF. The volume of CSF collected by ewe along the three repeated extractions did not vary, although it tended to decrease as repeated collections were performed. This tendency could also be linked with cumulative tissue damage. Nevertheless, our range of volume of CSF obtained for ewe is similar to volumes obtained in similar report. We concluded that the efficiency of weekly CSF extraction in ewes managed under field conditions decreases in the third sampling occasion.

Keywords: central nervous system, diagnostic technique, dura mater, ovine, sheep.

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INTRODUCTION

Cerebrospinal fluid (CSF) has been extensively studied to explore several different central nervous system (CNS) disorders [10]. In 1891 Quincke [3] demonstrated that CSF may be collected from the lumbar region of humans, opening new possibilities for diagnosis and therapy. In ruminants, its collection is an integral component of the clinical examination of the CNS. CSF may be collected in ewes under field conditions, allowing bacterial, metabolic, [5-8], cytological and biochemical studies [1].

Lumbosacral collection of CSF in different species includes topical preparation of the region, employment of local or general analgesia and topographical palpation to define the optimal puncture point [2,4,9]. The more commonly used sites for CSF extraction are located at different levels of the spine: either at a cranial location, i.e., the subaracnoid atlanto-occipital space, or at the lumbo-sacral region of the spine [2]. This last place does not require the employment of anesthesia [2], and is safer because the penetration to the medullar cone does not entail later complications for the animal [8].

According to needs, in occasions the sampling protocol should be repeated to measure dynamic changes in the parameters selected for the analysis [4]. However, it is not easy to maintain chronic catheters in place in extensively managed animals. Until now, according to our knowledge there are no reports of CSF repeated extractions without chronic catheterization in sheep. Therefore, this study was conducted to determine whether it is possible to obtain weekly CSF samples by lumbosacral puncture in the same ewes managed under field conditions.

MATERIALS AND METHODS

Animals and management

The work was carried out at the Campo Experimental 1 of the Facultad de Veterinaria, located in Migues, Canelones, Uruguay (34º 29' S). Nine mature ewes (5 Corriedale and 4 Milchschaff, body weight 52.0 ± 2.7 and 64.7 ± 3.2 kg, respectively), that grazed native pastures during the experiment were used.

Extraction of cerebrospinal fluid

Three cerebrospinal fluid (CSF) extractions/ewe were performed at the lumbosacral region of the spine, spaced at weekly intervals. Ketamine[1] 100 mg was administered i.v. to each sheep, and manipulations began after commencement of instability, while the ewe was standing, walking and/or in recumbency. The ewe was promptly accommodated (ventral decubitus) on a circular table (diameter = 60 cm, height = 100 cm), with four limbs loosely hanging from the table, to have a slight opening of the lumbosacral joint.

All collections were made by the same trained operator (HGQ), who stood behind the ewe, facing its back. Having checked that the sagital plane of the animal was perpendicular to the horizontal plane the puncture point was found by manual palpation at the slight depression between the ends of the spinous apophyses of the last lumbar and first sacral vertebrae. The wool was separated, and the area was cleansed with iodine solution. The puncture was performed with a spinal needle (20 G 3½ Recorder No. 8881. 230034, Sensi-Touch™ Spinal Needle Diamond Point, monoject)[2] Sherwood Medical, USA). After it had penetrated through the skin, the needle was pushed forward very slowly, listening for any vibrations ('clicks'), suggesting that the needle had crossed the dural membrane and entered into the arachnoidal space. Then, the syringe needle was withdrawn and the CSF came out slowly, either immediately or after some slow movements of the needle. If CSF did not come out, the puncture was deepened further on until the ventral arachnoidal space was reached.

Many times, probably when the needle was correctly oriented, CSF appeared without any further movement of the needle A maximum of eight punctures per ewe were performed, until either limpid or blood stained CSF was collected. The CSF was collected with a 1 mL syringe [4,9].

Data recording

Both the number of punctures required to obtain CSF, and the total volume obtained were recorded during the three CSF sampling events done to each animal. Additionally, the time employed to obtain CSF was recorded.

Data analysis

All the results are presented as means ± SD. The frequency of animals per sampling event from which it was possible to collect CSF was compared with the chi square test. The volume collected was

compared with ANOVA and the number of punctures and the different times were compared with the Kruskal-Wallis test. The differences were considered significant when $P \leq 0.05$.

RESULTS

The efficacy to obtain CSF of the lumbosacral puncture decreased from the first to the third week (Table 1). The percentage of animals in which the punctures were effective to obtain CSF was greater at the first and second weeks than in the third week of sampling ($P = 0.01$).

The number of punctures done per animal in order to obtain CSF, when considering only the animals from which samples were obtained, were not different for the first, second and third week respectively (Table 1). The volume of CSF collected when comparing the samples of the three weeks tended to decrease ($P = 0.07$).

Table 1. Frequency of ewes collected (%), mean time to obtain CSF, mean number of punctures per animal and mean CSF volume obtained (mean ± SEM), per sampling event in a flock of nine ewes sampled weekly by lumbosacral puncture.

Sampling event	Animals collected/total number of animals (%)	Time taken to obtain CSF (min)	Number of punctures/ sampled animal	Volume of CSF per animal (mL)
1st week	9/9 (100)[a]	16.0 ± 6.0[a]	2.6 ± 2.2[a]	0.9 ± 0.2[a]
2nd week	8/9 (89)[a]	15.5 ± 2.1[a]	1.4 ± 0.7[a]	0.8 ± 0.2[a]
3rd week	4/9 (44)[b]	*	1.0 ± 0.0[a]	0.6 ± 0.3[a]

For the same column: a vs b: $P < 0.01$. *: data not recorded.

DISCUSSION

It was shown that the sequential collection of CSF in ewes without catheterization is possible under field conditions, obtaining a high percentage of samples (overall, 78%) along the three weekly extraction events. When only the first extraction event was considered, the sampling was totally effective (100% of the animals), which coincides with previous results obtained by our group in other kind of experiments done under field conditions, in which only one extraction/animal was performed (100% of the sampled animals, n = 16, unpublished data). However, by the third sampling, we obtained fewer samples than in the second event. In experiments done in goats, where repeated samplings were obtained with a permanent catheter implanted in the lumbosacral region, Peregrine and Mamman [4] observed that this procedure is relatively risky, due to the use of anesthesia and traumatic installation of the device. The authors could cannulate successfully 18 of 22 goats, in contrast with the present technique of repeated puncture which yielded 100% efficacy in the first collection at random chosen ewes.

The decrease in effectiveness was probably due to cumulative tissue damage and formation of extensive fibrous adhesions in the subarachnoidal space, which would compromise partially or totally the flow of CSF. Furthermore, it has been reported in goats that tissue damage can increase the vascularization of the neighboring tissues, thus increasing the chances of contaminating the CSF samples with blood [4]. Of the four goats eliminated for the sampling of the work of Peregrine and Mamman [4], one died during the anesthetic induction and the other three goats were eliminated due to the formation of clots in the lumen of the catheters due to the trauma of the installation. In spite of the initial difficulties to install a permanent probe, once the animals are implanted successfully, there is the advantage of obtaining a bigger number of samples, either at daily or weekly intervals, which is not the case with our technique.

The volume of CSF collected/ewe along the three repeated extractions did not vary, although it tended to decrease as repeated collections were performed. This tendency could also be linked with cumulative tissue damage, as pointed out before. At any rate, our range of volume of CSF obtained/ewe is similar to volumes obtained in other reports [4,8].

CONCLUSIONS

Overall, it was concluded that although initially successful, the effectiveness of the weekly CSF collection from the lumbosacral region of the same sheep under field conditions decreased by the third week. This should be carefully considered when repeated samples are necessary for an adequate diagnosis or research sampling.

SOURCES AND MANUFACTURERS

[1]Vetanarcol König® - Buenos Aires, Argentina.

[2]Sensi-Touch™ Spinal Needle Diamond Point, Sherwood Medical, St. Louis, MO, USA.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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