Cisternal cerebrospinal fluid analysis in 24 sheep with chronic coenurosis

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Cerebrospinal Fluid (CSF),
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Pleocytosis,
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Sheep.

Summary
Coenurosis, a neurological parasitic infection of ruminants caused by the larval stage of *Taenia multiceps*, is commonly reported in Sardinia, the most representative region for ovine population in Italy. Chronic form appears as a consequence of cyst development, frequently reported in the brain and spinal cord. Diagnostic suspect of coenurosis is based on physical and neurological examination. The aim of this article is to describe physical, biochemical and cytological aspects of cisternal cerebrospinal fluid of 24 sheep with chronic coenurosis and to evaluate whether these alterations are helpful in the diagnosis of coenurosis. Cerebrospinal fluid was altered in 20 animals (83.3%). Increase of total protein was revealed in 7 animals (29.2%); an increase of total nucleated cell count was observed in 18 samples (75%). Cytological examination revealed mononuclear pleocytosis in 17 animals (70.1%). Eosinophils were observed in 16 animals in various degree (66.7%). Our results show that cerebrospinal fluid confirms signs of Central Nervous System inflammation in 20 animals out of 24 (83.3%) and in particular it was useful to identify a parasitic inflammation in 66.7% of the animals in which eosinophils were observed. Considering the results of this study, the very absence of significant neutrophilic pleocytosis could be considered useful to diagnose chronic cerebral coenurosis.

Analisi del liquido cerebrospinale di 24 ovini affetti da cenurosi cronica

Parole chiave
Coenurus cerebralis,
Liquido cefalorachidiano (LCR),
Ovini,
Pleocitosi,
Sardegna.

Riassunto
La cenurosi è un'infezione neurologica dei ruminanti causata da *Taenia multiceps* allo stadio larvale. Essa viene riportata comunemente in Sardegna, regione maggiormente rappresentativa della popolazione ovina italiana. La forma cronica si manifesta come conseguenza dello sviluppo della cisti, in particolare nel cervello e nel midollo spinale. Il sospetto diagnostico della malattia si basa sull'esame fisico e neurologico dell'animale. Questo articolo descrive le caratteristiche fisiche, biochimiche e citologiche del liquido cerebrospinale di 24 ovini affetti da cenurosi cronica e considera l'utilità di eventuali alterazioni per la diagnosi. Il liquido cerebrospinale è risultato alterato in 20 animali (83,3%). Sette dei soggetti campionati (29,2%) hanno presentato aumento delle proteine liquorali e 18 animali (75%) hanno manifestato pleocitosi. L'esame citologico ha rivelato pleocitosi mononucleare in 17 animali (70,1%). La presenza di eosinofili, in percentuali variabili, è stata osservata in 16 ovini (66,7%). Dai risultati dello studio si è potuto osservare che la valutazione del liquido cerebrospinale è stata utile per individuare un processo infiammatorio nel sistema nervoso centrale nell'83,3% degli animali. In particolare, ha permesso di sospettare un'infiammazione di tipo parassitario nel 66,7% dei casi per la presenza di granulociti eosinofili nei preparati citologici. Sulla base dei dati ottenuti, anche l'assenza di un quadro di pleocitosi neutrofilica può essere considerato utile per confermare la presenza di cenurosi cerebrale cronica.

Introduction

Coenurus cerebralis is the larval stage of *Taenia multiceps*. The adult worm inhabits the small intestine of carnivores, dog being the most frequent definitive host. The cystic larva is reported in the central nervous system of sheep and goat mostly, but it can also be found in camel, deer, pig, horse, however rarely in cattle and human (Varcasia *et al*. 2013, Yoshino *et al*. 1988).

Coenurosis occurs in 3 consecutive stages: acute, quiescent and chronic. The chronic form, commonly reported in growing sheep of 6-18 months, is caused by the development of 1 or more cysts into the brain (Scott 2012). The resulting increase of the intracranial pressure produces the typical symptoms of slowly progressive focal lesions of the brain. Symptoms vary depending on the cyst's location, size, and compression of the brain (Gul *et al*. 2007, Sharma and Chauhan 2005). According to the literature, circling is frequently towards the side of the brain in which the cyst is located (Achenef 1999). Sheep can present depression and head-pressing behaviour when cyst involves the frontal lobe of the cerebrum, loss of unilateral menace response with cyst in the contralateral hemisphere, unilateral proprioceptive deficits in the case of contralateral cerebral cyst, whereas bilateral deficits in the case of cerebellar cyst; ipsilateral head tilt with cyst in the vestibular or cerebello-vestibular pathways. Dismetria, ataxia, bilateral postural deficits and lack of menace response are typical of cerebellar lesions (Sharma and Chauhan 2005). Other reported symptoms are teeth grinding, salivation, paresis, convulsions, cerebral atrophy, thinning and morphologic changes in the cranium (Yoshino *et al*. 1988). Infected sheep usually remain isolated from the flock and show a loss of reactivity to external stimuli (Achenef *et al*. 1999, Bussell and Kinder 1997).

Clinical diagnosis of cerebral coenurosis is based on the physical and neurological examinations. It is normally supported by general information on age, breeding conditions, duration of signs and flock mortality (Komnenou *et al*. 2000).

The aim of this study was to evaluate the modifications of cisternal cerebrospinal fluid (CSF) associated to coenurosis and to confirm the utility of CSF analysis in the diagnostic scenario. Observation of CSF in sheep affected by coenurosis has been seldom reported in the literature (Doherty *et al*. 1989, Oruc and Uslu 2006, Schweizer *et al*. 2006).

Materials and methods

Between February 2010 and May 2011, 24 Sarda breed sheep with clinical signs indicative of chronic coenurosis were examined. These animals were included in a study about magnetic resonance imaging (MRI) characteristic of the brain and skull of sheep with chronic coenurosis (Manunta *et al*. 2012). Age of animals (21 ewes and 4 rams) ranged from 1 to 3 years with 25 to 33 kg body weight. All the animals received clinical and neurological examination, brain MRI and CSF analysis were also performed. Definitive diagnosis of chronic coenurosis was made by macroscopic and morphological identification of *Taenia multiceps* cyst after surgical extraction (Leske 1780).

For MRI, sheep were anesthetized and positioned in sternal recumbency. Sheep received Diazepam (0.5 mg/kg, IV) before induction of anaesthesia, which was induced with thiopenthal sodium (10 mg/kg, IV). Lidocaine (2 mg/kg, IV) was administered and sheep were orotracheally intubated. Anaesthesia was maintained with sevofluraned in oxygen delivered via a circle system designed for use with small animals. The end-tidal concentration of sevoflurane was maintained at a concentration sufficient to ensure adequate depth of anaesthesia. Saline solution (0.9% NaCl) was administered IV (5 to 10 mL/kg/h) during anaesthesia. All sheep were mechanically ventilated, and end-tidal partial pressures of carbon dioxide were maintained between 30 and 37 mm Hg (i.e. moderate hyperventilation). Immediately before extubation, lidocaine (1 mg/kg, IV) was administered to sheep to prevent an increase of cranial pressure.

Images were obtained by use of an MRI machine with a 0.23-T magnet (0.23-T MRI scanner, Paramed medical system, Genova, Italy) in the 3 planes from the atlas to the nasal cavity using a knee coil. Magnetic resonance imaging was also used to investigate the position, number, and size of cysts (expressed as cyst volume and cyst /skull volumes).

After MRI, but before surgical removal of the cyst, CSF was collected by cerebellomedullary cisternal puncture. For the collection of CSF, the patient was positioned in lateral recumbency with the skull and cervical vertebrae at the edge of the table and the neck full flexed to create a 90° angle with the cervical spine. Briefly the area overlying the Cisterna Magna was surgically prepared and puncture was performed by inserting a spinal needle (21G) (a hypodermic needle could be sufficient) at the level of the atlanto-occipital space. All surgical procedures were conducted with the use of sterile technique. Between 1 and 2 ml of CSF sample was collected in 1.5 ml polypropylene microcentrifuge tubes by free flow and processed within 20 minutes. Macroscopic appearance (colour and turbidity), specific gravity, total protein concentration (TP), total nucleated cell count (TNCC) and cytological microscopic examination of CSF were recorded for all sheep. Total protein concentration was estimated with an automated photometer (ABX Pentra 400, Horiba

Medical, Montpellier, France) by the pyrogallol red method. Cell count was performed using a Fuchs-Rosenthal chamber. A correlation between size of the cysts (volume and cyst volume-skull volume ratio) and the degree of CSF alteration (PT and TNCC) was evaluated. To determine the probability that an observed correlation occurred by chance, a two-tailed *t*-test was conducted. The following mutually exclusive hypotheses were tested: H_0: r=0 and H_1: r≠0. A correlation was considered significantly different from 0 if *p-value* was <0.05. For cytological evaluation of CSF, 2 slides for each sample were prepared using a cytospin (Shandon Cytospin 4, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and depending on the number of cells in the fluid, 200-300 µl of sample was centrifuged at 123 x g for 6 min with low acceleration at room temperature. Slides were air dried, stained with Romanowsky stain variant (Diff Quick, Dade Behring AG, Düdingen, Switzerland) and observed with an optical microscope at 10x, 40x and 100x magnification. Reference values from published data for healthy sheep were used to classify the results (Scott 2004). The degree of the increase in total protein concentration was classified as mild (67-100 mg/dL), moderate (101-200 mg/dL), or marked (>200 mg/dL). Inflammation was further classified on the basis of the TNCC, as mild (10-50 cells/mL), moderate (51-100 cells/mL), or marked (>100 cells/mL). Inflammation was also categorised on the basis of the predominant leukocyte type, as neutrophilic (>70% neutrophils), lymphocytic (>70% lymphocytes), histiocytic (>70% macrophages), mixed mononuclear (>70% lymphocytes+macrophages), and mixed (<70% lymphocytes+macrophages) (Stokol *et al*. 2009).

Results

At clinical examination, 4 ewes (Tables I and II; Id 1; 2; 20; 21) revealed chronic mastitis, disease of the feet, and suspect of lung parasites. Twenty-three out of 24 sheep had at least 1 of the following neurological signs: depression and disorientation

Table I. *Size of cyst and some parameters of Cerebrospinal fluid in 24 Sarda sheep with chronic coenurosis sampled between February 2010 and May 2011.*

Id	Cyst number	Location of cysts	C/S (%)	CV (cm^3)	MA	SG	TP (mg/dl)	TNCC
1	1	RT parietal-temporal occipital lobe	30.17	49.31	Clear	1.006	<30	29
2	3	RT frontal-parietal; frontal-parietal-occipital lobe	32.72	49.89	Clear	1.006	<30	13
3	1	RT frontal-parietal lobe	23.13	32.75	Clear	1.005	50	21
4	1	RT hemisphere	35.42	48.39	Clear	1.005	58	6
5	2	RT hemisphere	11.45	59.36	Clear	1.006	77	33
6	2	RT hemisphere	42.74	79.79	Clear	1.006	43	28
7	1	RT hemisphere	43.32	87.37	Clear	1.006	11	11
8	1	RT hemisphere	38.34	73.76	Clear	1.005	19	6.6
9	2	RT parietal-temporal-occipital lobe	26.71	36.93	Clear	1.005	15	13.4
10	1	RT frontal-parietal-occipital lobe	20.79	37.39	Clear	1.006	56	573
11	1	RT frontal-parietal lobe	35.98	55.67	Clear	1.005	20	13
12	1	RT frontal-parietal lobe	31.19	51.76	Clear	1.005	31	26.6
13	1	RT frontal-parietal lobe	39.03	70.11	pink	1.007	61	166
14	1	RT parietal-temporal-occipital lobe	34.35	70.25	Clear	1.005	<30	63
15	1	RT hemisphere	46.93	90.37	Clear	1.005	<30	17
16	1	RT hemisphere	36.07	62.04	Clear	1.006	30	87
17	1	RT hemisphere	40.64	67.42	Clear	1.006	<30	47
18	2	RT\CT left intraventricular; cerebellum	10.02	11.65	Clear	1.006	<30	3
19	1	RT temporal-parietal lobe	10.01	10.64	Clear	1.006	<30	0
20	1	RT frontal-parietal lobe	21.08	30.28	Clear	1.006	<30	6
21	1	RT parietal-temporal-occipital lobe	25.66	44.66	pink	1.005	<30	31
22	1	CT brainstem	10.25	13.46	Clear	1.005	23	1
23	1	RT parietal-occipital lobe	27.62	42.26	Clear	1.006	54	78
24	1	RT frontal parietal lobe	28.91	45.33	Clear	1.006	<30	11

Id = identification number of sheep; C/S = cyst/skull volumes; CV = cyst volume. In case of multiple cysts it indicate total volume; MA = macroscopic appearance; SG = specific gravity; TP = total protein concentration; TNCC = total nucleated cell count/miclroliter; RT = rostro-tentorial; CT = caudo-tentorial; Grey background = normal CSF

Table II. *Cytological findings in Cerebrospinal fluid in sheep with chronic coenurosis sampled between February 2010 and May 2011.*

Id	lym	Reac lym	PI	mon	Foamy cyto plasm	LP	EP	PH	MIT	eos	neutr	EP cell	MLM
1	80%	yes	-	20%	yes	yes	yes			<0.5%			
2	51.6%	-	-	46.9%	yes	-	-				1.5%		
3	30.8%	-	yes	67.8%	yes	-	-			1.4%			
4	71%	yes	-	28%	yes	-	-				1%		
5	71.1%	-	yes	26.9%	yes	yes	-			2%			
6	46.5	-	-	49.3%	yes	-	-			3.5%	0.7%		
7	48.8%	-	-	50.4%	yes	yes	-			0.8%			
8	47%	-	-	44%	yes	-	-				9%		
9	54%	yes	yes	44%	yes	yes	-		yes	1%	1%		
10	68.1%	-	-	19.4%	yes	yes	-		yes	12.5%	rare		
11	55.6%	-	yes	44.4%	yes	yes	-						
12	54.6%	yes	yes	24.8%	yes	-	-			17%	3.6%		
13	44.3%	-	yes	43.7%	yes	yes	-		yes	1.8%	10.2%	yes	yes
14	43.5%	-	-	43.5%	yes	-	-			10.8%	2.2%		
15	50%	-	-	20%	yes	-	-			2%	28%		
16	41.3%	-	yes	49.6%	yes	-	-			4.3%	4.3%	yes	yes
17	53.6%	yes	yes	36.8%	yes	yes	-	yes	yes	9.6%			
18	76%	-	-	24%		-	-						
19	-	-	-	-		-	-			-	-		
20	63.2%	-	-	36.3%	yes	-	-	yes			0.5%		
21	68.7%	-	-	26.6%	yes	-	-			3.1%	1.6%		
22	67%	-	-	33%	yes	-	-						
23	40.2%	-	-	29.9%	yes	yes	-	yes	yes	29.9%			
24	56.9%	-	-	23%	yes	-	-			6.2%	13.9%		

Id = identification number of sheep; Lym = lymphocytes; Reac lym = reactive lymphocites; PI = plasma cells; Mon = monocytes; LP = Leukophagocytosis; EP = erythrophagocytosis; PH = unidentifiable intracellular debris; MIT = mitosis; EOS = eosinophils; NEUTR = Neutrophils; EP = epithelial cells; MLM = extracellular myelin-like material; Grey background = normal CSF

(21 animals), postural abnormalities (1 animals), alterations of postural reactions (16 animals), unilateral (8 animals) or bilateral (7 animals) menace deficit, gait abnormalities (6 animals), and head turn (1 animal).

The MRI images showed 1 cyst in 19 sheep, 2 cysts in 4 sheep (Tables I and II; Id 5; 6; 9; 18) and 3 cysts in 1 sheep (Tables I and II; Id 2). Twenty-two sheep had cyst localised in rostro-tentorial (RT) position, 1 sheep had 1 cyst in caudo-tentorial (CT) position, and 1 sheep had 1 cyst in RT and 1 in CT position (Tables I and II; Id 18). The specific involvement of lobes in the brain is showed in Table I. It is noteworthy that in some cases the cyst involves the entire hemisphere, the sizes of cysts are reported in Table I. Perilesional oedema was detected in 5 ewes. Signs of haemorrhage, necrosis, atrophy and gliosis were not observed with MRI. Complete resolution of neurologic signs was observed in 22 sheep within 7 days after removal of cysts, while 2 sheep died for complications after surgery.

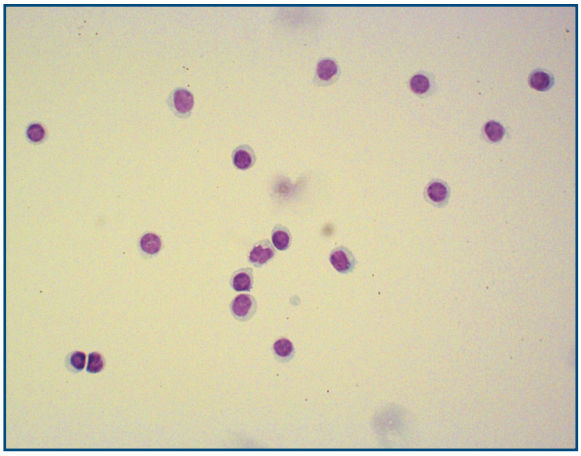

Figure 1. *Lymphocytic pleocytosis (Diff Quick 40x).*

CSF was altered in 20 animals (Tables I and II). Eighteen pathological CSFs were colourless and clear while 2 pathological CSFs showed mild pink coloration suggesting the presence of blood caused

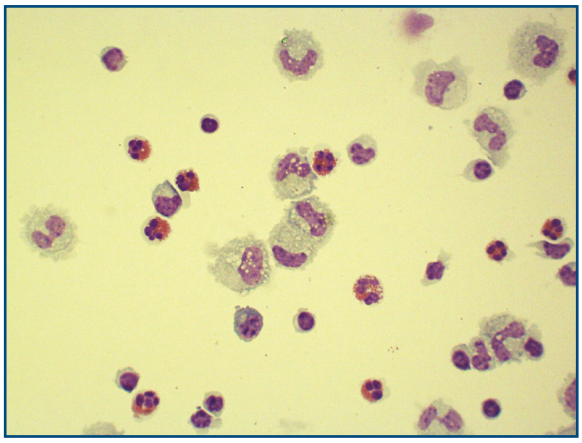

Figure 2. *Mixed mononuclear pleocytosis (70.1% lymphocytes+macrophages) with a high prevalence of eosinophils (29.9%) (Diff Quick 40x).*

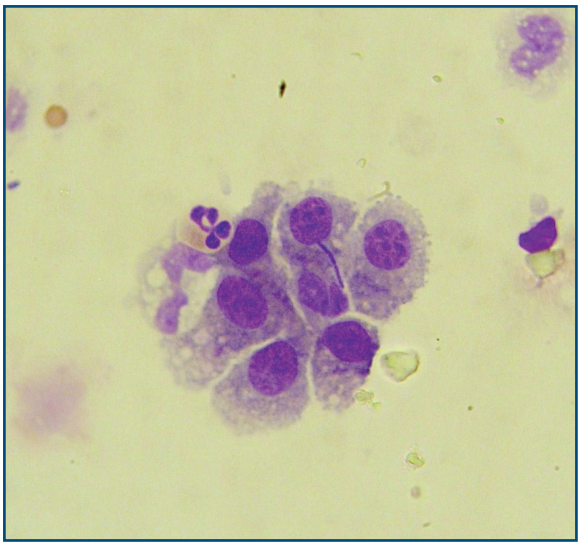

Figure 3. *Cluster of (probably epithelial) cells on-going to activation and transformation to macrophages (Diff Quick 100x).*

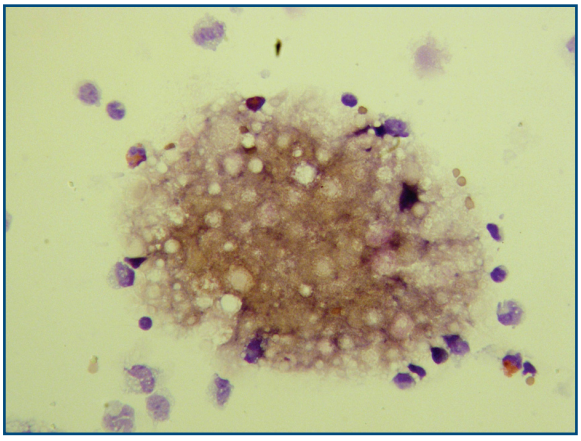

Figure 4. *Extracellular myelin-like material. It appears as variably sized aggregates of pink, foamy material, with internal circular structures that give it a honeycomb-like appearance. (Diff Quick 40x)*

by iatrogenic contamination during collection (absence of xantochromic appearance after centrifugation and absence of erythrophages/siderophages in cytological sample). All CSF had normal specific gravity value (from 1.005 to 1.007). Mild increase of TP was revealed in 7 animals (35.33 ± 3.28 mg/dl) while an increase of TNCC was observed in 18 samples (53.5 ± 23.8 cells/µl). No correlation between size of the cysts (volume and cyst volume-skull volume ratio) and the degree of CSF alteration (PT and TNCC) was observed (*p-value* > 0.05).

Mixed mononuclear pleocytosis was observed in 16 animals, lymphocytic pleocytosis was observed in 2 animals (Figure 1; Tables I and II; Id 1; 5), 1 animal showed albumin-cytologic dissociation with an increase of TP (58 mg/dl) without increase in cells (6 cells/µl) (Tables I and II; Id 4), and 1 animal showed TNCC within normal reference intervals (6.6 cells/µl) but increased percentages of neutrophils (9%) (Table II; Id 8). Eosinophils were observed in 16 animals in various degrees (6.65 ± 1.97) (Figure 2; Table II). Pleocytosis was mild in 13 animals, moderate in 3 animals and marked in 2 animals. Reactive lymphocytes with basophilic cytoplasm and/or plasmocytoid cells with more abundant basophilic cytoplasm and perinuclear clear zone were observed in 10 pathological CSFs. Monocytoid cells showed signs of intense activation and transformation in macrophages (increased size of basophilic and foamy cytoplasm, phagocytised material such as cell debris and/or cytophagia, multinucleation). Mitotic figures were occasionally observed. Uncommon elements were sporadically observed: little groups of cells ascribed as "surface epithelium" were observed in 2 samples. Cells appeared with cuboidal to columnar morphology, a wide border of pink or blue-grey cytoplasm and eccentrically located, small, round nuclei with granular to coarse chromatin (Figure 3). Two CSF showed extracellular myelin-like material. It appeared as variably sized aggregates of pink, foamy material, often with internal circular structures that give it a honeycomb-like appearance (Figure 4).

Discussion

Coenurosis is an important parasitosis of Sardinian sheep, which may cause serious economic damage in farms (Deiana 1971, Scala *et al*. 2007). Control of coenurosis can be achieved by regular anthelmintic treatment of farm dogs at 6-8 week intervals, by correct disposal of all sheep carcasses in order to avoid their dogs scavenging behaviour and by a strict control to prevent irregular slaughter. Education programs detailing these correct behaviours have

proved to be quite effective in other countries, e.g. in the United Kingdom, they allowed for stopping the sheep/dog cycle and helped reducing the incidence of coenurosis (Scott 2004). Cerebral coenurosis and the localization of the cysts can be strongly hypothesised after careful neurological examination paying special attention to general behaviour, postural tests and visual deficits (Scott 2004). However, clinical signs may be confused with other nervous conditions. It is useful to make differential diagnosis with neurological disease caused by other local space-occupying lesions in the CNS of young sheep, in particular abscesses and haemorrhage. In the absence of a focal compressive spinal cord lesion, there are no substantial differences between the composition of cisternal and lumbar CSF samples in sheep, therefore veterinarians practitioners may find useful for diagnostic purposes to collect lumbar CSF under local anaesthesia (Scott 2010). Sedation of the animal, adequate for collection of CSF both from cerebello medullary cistern or lumbosacral interspace can be made using Diazepam (0.4 mg/kg/IV bodyweight) associated with fentanyl (1-2 mcg/kg/IV). Cisternal cerebrospinal fluid analysis is an un-invasive, economic and rapid diagnostic tool that can help to confirm inflammation of central nervous system and associated with clinical examination can help to enforce suspect of parasitic infection by *Coenurus cerebralis*. There are some reports in the veterinary literature of a consistent association between an increased CSF eosinophil concentration and parasitic infection of the CNS in sheep (Doherty 1989, Lunn and Hinchcliff 1989, Schweizer 2006, Tschuor 2006). In this study, 16 sheep with coenurosis (67%) showed mononuclear pleocytosis of various degrees with presence of eosinophils, enforcing the suspect of a parasitic inflammation of CNS. The degree of eosinophilia was variable (6.65 ± 1.97). Four CSF samples were only indicative of mononuclear non-specific inflammatory condition, whereas 4 CSF samples were in normal range. This results show that the absence of abnormality in CSF or the finding of mononuclear pleocytosis do not exclude the presence of coenurus cyst.

The term "surface epithelium" is used in human medicine to describe cells in cluster or single elements, including choroid plexus, ependymal cells, endothelial cells and meningeal cells of mesenchymal origin that are found in CSF and are difficult to distinguish cytologically (Kluge *et al*. 2007). Their presence in human CSF has been recognized as either a consequence of lumbar puncture or indicators of pathologic conditions, such as trauma, inflammation or infection, that affect structures enclosing the CSF space (Kluge *et al*. 2007). When enter in the CSF, these cells may present signs of activation (indicated by separation of individual cells from cell cluster, increased polychromasia and hypercromasia in the cytoplasm) and differentiation to macrophages (Kluge *et al*. 2007).

The presence of myelin-like material in the CSF has not been described in coenurosis. This finding has been rarely reported in the relevant literature, with a few single reports in dogs, in horses with necrotizing encephalomyelitis, and in an experimental study in sheep infected by Visna (Bauer *et al*. 2006, Fallin *et al*. 1996, Mesher *et al*. 1996, Zabolotzky 2012). Initially, an underlying demyelinating or myelomalacic disease process was suspected as the cause of myelin-like material in canine CSF (Bauer *et al*. 2006, Fallin *et al*. 1996, Mesher *et al*. 1996). Nevertheless, the presence of myelin like material has been observed as an artifact of collection technique especially in lumbar samples, and in a variety of disease conditions such as idiopathic epilepsy, spondylomyelopathy, immune-mediated polyarthritis, syringomyelia (Zabolotzky 2012). Myelin in sheep with cysts of cenurosis could be associated to the necrosis of brain tissue around the cysts. Necrotic lesions around the cysts have been described by different authors (Achenef *et al*. 1999, Mouchira 2010, Nourani 2009).

Luxol fast blue staining was not carried out on CSF samples to confirm our assessment of the pink material as myelin-like in nature. However, the observed material was similar to the one previously described as myelin-like material (Freeman and Raskin 2001, Zabolotzky 2012). The absence of correlation between Total Protein or Total Nucleated Cell Count in cerebrospinal fluid with the size of the cyst or the cyst volume-skull volume ratio showed that the degree of alteration in CSF in chronic coenurosis in not dependent on the extension of the cyst in the CNS.

Conclusions

The frequency of CSF analysis in disease investigation may decrease as CT scans or MRI expands in veterinary medicine as it has in human medicine. Since these advanced diagnostic modalities are not easily deployed in farm animal practice, CSF analysis associated with physical examination remains a simple and economic diagnostic method that provides important assistance in establishing a diagnosis of CNS inflammation. Such a method could help to suspect parasitic infection, such as coenurosis, when certain alterations, like for example the presence of eosinophils, are shown. Even if it could be considered an "old" test, CSF diagnostic utility will potentially increase with new observations and additional assays, such as direct detection of some microorganisms with PCRs methods.

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