

### **Unusual findings in ovine cerebrospinal fluid**

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**Signalment:** 4 year old, female, Texel cross ewe.

**History:** The ewe was a homebred breeding ewe on a farm in the Scottish Borders. The flock was composed of 530 ewes and 150 ewe hogs which were housed from January to April. The ewes had been dosed for liver fluke in the previous autumn but not treated for worms, and had no worm treatment at lambing. Nematode parasites were effectively controlled by integrated evasive/anthelmintic strategies and fluke was controlled with 2 or 3 treatments per year.

The farmer noticed the ewe was recumbent in the field. This was the only animal affected at that time but several ewes had died over the grazing season, on the same field, and these occurrences had not been investigated. This field is situated by a river in the middle of a large village, and is used by many members of the public for walking their dogs.

The ewe was first examined on farm on the 7<sup>th</sup> October 2009.

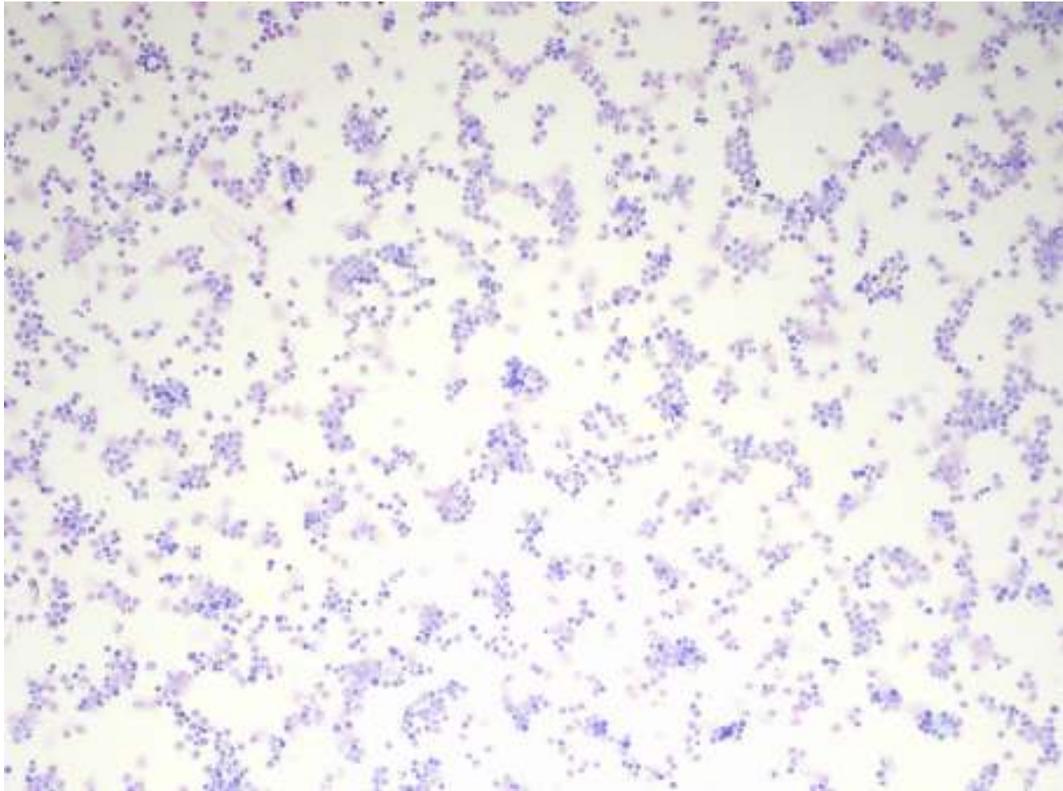
**Clinical findings:** The ewe was in good body condition, recumbent, weak and uncoordinated. She could stand only when assisted and preferentially laid on her right side. She demonstrated mild hyperaesthesia and dorsiflexion of the neck. There was traumatic corneal damage to the right eye.

Neurological examination demonstrated that spinal limb withdrawal reflexes and proprioceptive reflexes were present but difficult to assess. The menace reflex and pupillary light reflex were positive bilaterally, and there was no nystagmus or strabismus.

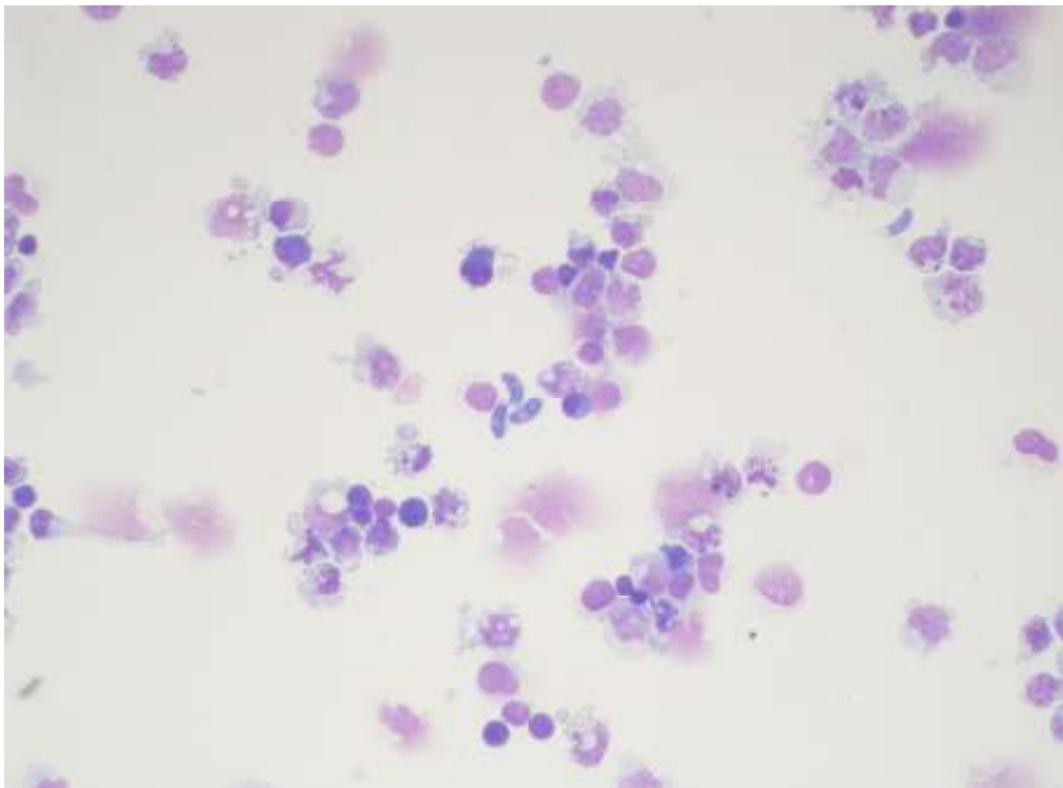
The ewe was treated with dexamethasone, vitamin B1 and penicillin but as the signs were not entirely typical of cerebrocorticonecrosis and in view of the field history, a CSF sample from the lumbosacral space was collected to investigate other possible causes.

#### **CSF analysis**

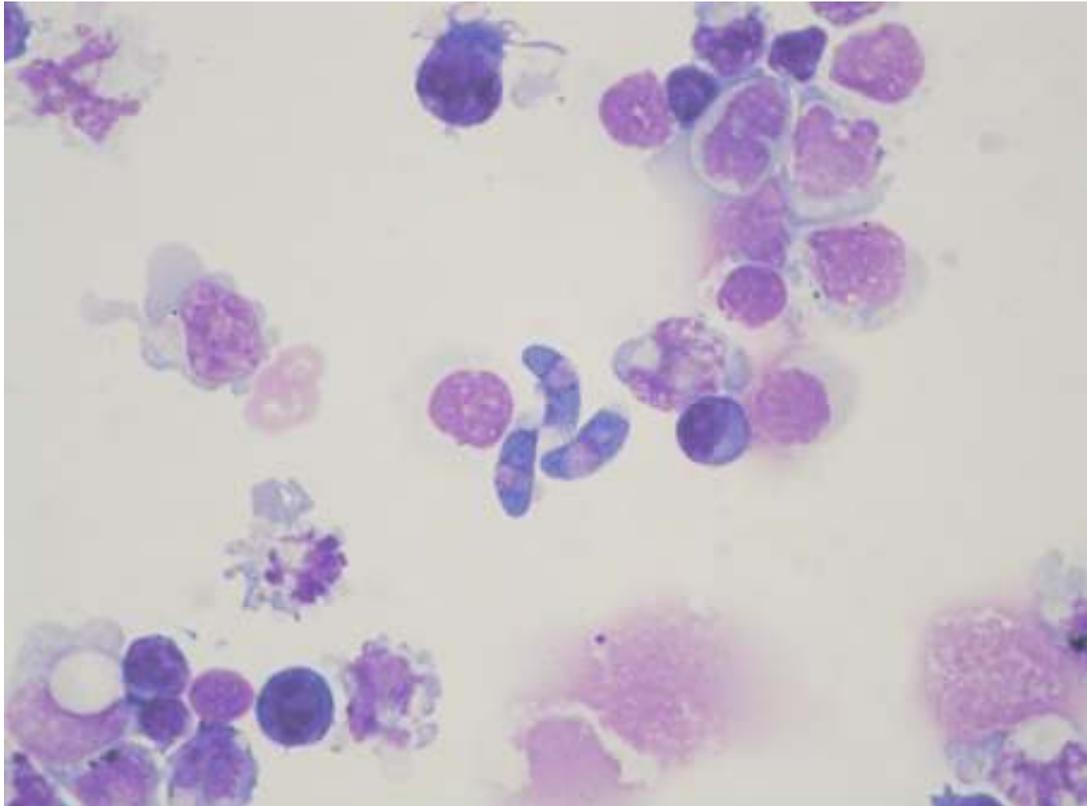
		Sheep reference intervals
<b>Appearance</b>	Slightly turbid	Clear
<b>Specific gravity</b>	1.012	<1010
<b>Total protein</b>	2.19 g/l	<0.4 g/l
<b>Total cell count</b>	$0.679 \times 10^9/l$	$<0.01 \times 10^9/l$



**Figure 1** Cytospin of 100µL from CSF. May-Grünwald Giemsa stained, x100.



**Figure 2** Cytospin of 100µL from CSF. May-Grünwald Giemsa stained, x400.



**Figure 3** Cytospin of 100 $\mu$ L from CSF. May-Grünwald Giemsa stained, x1000 oil.

**Questions:**

What is your preliminary diagnosis?

Which other tests would you carry out to obtain a definitive diagnosis?

## **Further investigation and discussion**

### ***Cytological interpretation of the CSF***

The cytopspins have a clear background. The nucleated cell count is markedly increased, and they consist of a mixed population of 65% macrophages, 15% small to medium lymphocytes and 20% non-degenerate neutrophils with occasional plasmacytoid lymphocytes (darker basophilic cytoplasm with perinuclear clear zones) and plasma cells. The macrophages are mainly activated (abundant basophilic cytoplasm with variably sized non-staining vacuoles). Rare eosinophils are seen. Scattered around are very small numbers of ellipsoid organisms (~10-12 x 3-5 µm), with a grossly granular basophilic cytoplasm with a characteristic clear area in one pole and an eosinophilic round eccentric area (nucleus) closer to the other pole. No bacteria or other aetiological agents are seen. The diagnosis was severe mixed cell pleocytosis with protozoa (*Sarcocystis* sp., suspected).

The ewe did not respond to treatment and was euthanased and presented for post mortem examination on 13 October 09.

### ***Post mortem examination findings***

Gross examination identified bilateral, regionally extensive opacity of the corneas (presumptive keratitis), mild multifocal melanosis on tongue, myocardium and brain (non-pathological breed-related changes), mild congestive splenomegaly and mild congestion of the lungs (likely agonal changes). There was no gross evidence of pathological changes in the oesophagus or any other muscle and brain and spinal cord were grossly unremarkable.

### ***Histopathological findings***

Sections of oesophagus, right ventricle, left ventricle and diaphragm were examined. In all these sections variable numbers (2-5 in the oesophagus and 20-50 in the right ventricles) of intrasarcolemmal round cysts containing a variable number of tightly packed, hyperbasophilic comma-shaped organisms, 15-30µm long (*Sarcocystis* cysts, presumptive). There was no associated inflammatory infiltration and these tissues were otherwise histologically unremarkable.

A section of spinal cord at the level of C3 was examined and had minimal multifocal perivascular accumulation of lymphocytes (perivascular cuffing), more frequent in the grey matter. Neuronal bodies were swollen with no associated gliosis. This was considered an artefact. A section from the right frontal cortex was also examined, and it was histologically unremarkable.

The final histopathological diagnosis was intra-sarcolemmal parasitic cysts, *Sarcocystis* sp. presumptive, in oesophagus, left and right ventricle and diaphragm.

The inflammation observed in the grey matter of spinal cord (C3) was mild but it was possible that other areas which were not sampled were more severely affected. It was not possible to discount *Sarcocystis* sp. infection of the CNS with the sections available and further histological sectioning of brain and spinal cord were recommended.

### ***Discussion***

The main differential diagnoses at the time of presentation were cerebrocorticonecrosis, listeriosis, louping ill, acute sarcocystis, acute coenurosis and lead poisoning. The presence of zoites in the CSF supported the diagnosis of a protozoal infection.

Protozoal infections that can occur in sheep (phylum Apicomplexa, family Sarcocystidae) comprise *Toxoplasma gondi*, *Neospora caninum* and *Sarcocystis* sp.. *Toxoplasma* and occasionally *Neospora* usually only cause abortions, without other clinical signs. There are four reported species of *Sarcocystis* in sheep. *S. ovifelis* (formerly *gigantea*) and *S. medusiformis* are transmitted by cats (definitive host) and develop into macroscopically visible cysts in sheep, while *S. ovicanis* (formerly *tenella*) and *S. arieticanis* are transmitted by canids and develop into microscopic cysts.

Of these four species, only *S. ovicanis* has been reported to cause neurological signs due to penetration and encystment of zoites (from 2<sup>nd</sup> and 3<sup>rd</sup> generation meronts) into neurons and glial cells, in addition to widespread muscle parasitosis.

Clinical signs of acute sarcocystosis depends on the dose of ingested sporocysts and the immune status of the host, and anaemia, anorexia, fever, weight loss, lethargy and abortion are the most common signs. When the nervous system is involved, signs include muscle weakness and ataxia of variable severity, with hindlimb paresis that can progress to recumbency.

On gross examination, there are few gross lesions, consisting of small areas of pallor in muscles and discoloured foci in the white or grey matter of fixed spinal cord. Histopathological examination shows cysts in many striated muscles, and if the CNS is involved, the brain and especially the spinal cord show non-suppurative encephalomyelitis, characterised by perivascular cuffing and gliosis, with areas of oedema and necrosis. These lesions are associated with the presence of meront bodies, however, those are rarely detected in histological preparations. In the case of CNS involvement, there may be other concomitant inflammatory lesions, due to the widespread presence of the parasite, such as hepatitis, glomerulitis, retinitis and non-suppurative interstitial pneumonia associated with meront bodies. Usually, even severe muscle infestations are well tolerated. In fact inflammatory reactions in muscle are rarely observed and consist of foci of inflammation associated with degenerate *Sarcocystis*.

In this case, considering the recumbency, the presence of central nervous system signs and the findings in the CSF, a diagnosis of sarcocystosis was considered most likely. Adding the results of the post mortem examination and of the histopathology, the likely sarcocystis involved was *S. ovicanis*.

Molecular analysis was performed in order to characterise the protozoa. The most successful method for *Sarcocystis* species has been the analysis of variable region of ribosomal 18S RNA genes. DNA was extracted from the CSF and from paraffin wax-embedded left ventricle using the DNeasy<sup>®</sup> Blood and tissue Kit (Qiagen, Alameda, CA), according to the manufacturer's instructions. Sequences from *S. tenella* (Genebank: L24383.1), *S. arieticanis* (Genebank: L24382.1), *S. gigantea* (Genebank: L24384.1), *Toxoplasma gondi* (Genebank: L24381.1) and *Neospora caninum* (Genebank: L24380.1) were aligned using BioEdit sequence alignment editor V7.0.9.0 and new primers were designed using the online tool Primer3Plus (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>). The CSF failed to amplify the PCR product, although it was positive for the presence of a sheep housekeeping gene (RW1). A PCR product was obtained from the right ventricle and was, as expected, approximately 400bp. This was sent for sequencing at The GenePool (Ashworth Laboratories, University of Edinburgh). This sequence had 97% homology to the 18S rRNA gene of *Sarcocystis capracanis* (GeneBank: L75472.1).

*S. capracanis* is the most pathogenic and prevalent *Sarcocystis* species in goats. In experimental infections by Unterholzner in 1983 (cited by Nigro *et al*, 1991) it was reported that sheep inoculated with sporocysts of *S. capracanis* did not become infected demonstrating that although this species is closely related to *S. ovicanis* they were two separate species with different host specificity. *S. capracanis* is able to replicate in liver, kidney and brain of affected goat, as does *S. ovicanis* in sheep.

The results of our study showed that this sheep was infected with *S. capracanis*. Since, it was not possible to amplify a *Sarcocystis* sequence from the CSF, we cannot exclude that the organism seen in the CSF was a different organism, or a different *Sarcocystis* sp. It is also possible that the sheep was co-infected with two different organisms, but this is considered unlikely, because resistance to infection usually develops making a second infection, with systemic multiplication of the organism, less likely.

These results demonstrate that the host specificity of *S. capracanis* may not be as strict as previously demonstrated and further studies of experimental transmission should be considered. It is also possible that these two organisms are not two different species but different genetic strains of a single species.

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