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Short Communication

Identification of *Sarcocystis capracanis* in cerebrospinal fluid from sheep with neurological disease

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ABSTRACT

Protozoal merozoites were identified in the cerebrospinal fluid of two sheep with neurological disease in the UK. Polymerase chain reaction (PCR) identified the merozoites as *Sarcocystis capracanis*, a common protozoal pathogen of goats. This is the first report of this species infecting sheep and may represent an aberrant infection with sheep acting as dead end hosts, or alternatively could indicate that sheep are able to act as intermediate hosts for *S. capracanis*, widening the previously reported host range of this pathogen. It is possible that *S. capracanis* is a previously unrecognised cause of ovine protozoal meningoencephalitis (OPM) in the UK.

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1. Introduction

Sarcocystis species are apicomplexan protozoa with an obligatory heteroxenous life cycle, which alternates between a herbivorous or omnivorous intermediate host (IH) and a carnivorous definitive host (DH) (Dubey et al., 1989a; Fayer, 2004; Lindsay et al., 1995). There are approximately 200 species of *Sarcocystis* infecting a wide range of vertebrates (Tenter, 1995) and the number of species capable of acting as an IH for a particular sarcocystis species can be very few or many. The most common pathological changes associated with sarcocystis in IH are tissues cysts containing bradyzoites within muscle, although encephalitis is occasionally associated with sarcocystis in sheep (*Sarcocystis ovicanis*) and horses (*Sarcocystis neurona*) (Van Vleet and Valentine, 2007). Humans act as IH for two species of sarcocystis – *Sarcocystis hominis* and *Sarcocystis suihominis*, with infections often associated with eating uncooked beef and pork, respectively.

Four species of *Sarcocystis* commonly infect sheep: *Sarcocystis ovifelis* (formerly *gigantea*) and *Sarcocystis medusiformis* are transmitted by felids (DH) and develop into macroscopically visible cysts in sheep (IH), while *S. ovicanis* (formerly *tenella*) and *Sarcocystis arieticanis* are transmitted by canids (DH) and develop into microscopic cysts in sheep (IH) (Dubey et al., 1989a). *S. ovifelis* and *S. medusiformis* are considered of low pathogenicity (Heckeroth and Tenter, 1999; Tenter 1995). *S. ovicanis* can cause anorexia, fever, decreased weight gain, anemia, and death in lambs and has been associated with neurological disease, known as ovine protozoal myeloencephalitis (OPM), in adult sheep (Caldow et al., 2000; Dubey et al., 1989b; Henderson et al., 1997; Jeffrey et al., 1988; Morgan et al., 1984; O'Toole et al., 1993). Recently other species of *Sarcocystis* have been reported to occur in sheep including *S. gracilis*-like (Giannetto et al., 2005) and *Sarcocystis mihoensis* (Saito et al., 1997).

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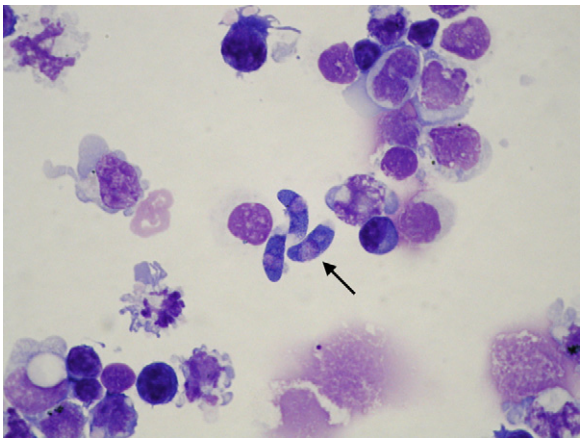

Fig. 1. Digital image of cerebrospinal fluid (May-Grünwald stain) of case A showing a mixed cell population and a group of three slightly curved parasitic merozoites (arrow).

Sarcocystis capracanis utilises the dog as DH and goat as IH. Tissue cysts are commonly found in the muscle of goats and the species considered of low pathogenicity in natural infections. Infection by *S. capracanis* has not been reported previously in sheep.

The advent of the polymerase chain reaction (PCR) has enabled accurate and rapid species-specific identification of *Sarcocystis* and differentiation from other apicomplexan protozoa such as *Toxoplasma* and *Neospora*. In this report we have used PCR to speciate protozoal organisms found in the cerebrospinal fluid (CSF) of two adult sheep with neurological disease.

2. Case A

In October 2009 in south west Scotland a 4 year old female Texel cross ewe was presented for veterinary attention with neurological abnormalities including recumbency, weakness, ataxia, mild hyperaesthesia and dorsiflexion of the neck. The ewe was the only animal in the group affected, but several cohorts had previously died over the grazing season on the same field without further investigation. The ewe was treated with dexamethasone, vitamin B1 and penicillin and a cerebrospinal fluid (CSF) sample from the lumbosacral space was collected. The CSF cytology indicated a severe mixed cell pleocytosis with presence of rare ellipsoid and slightly curved structures of approximately 4–6 μm in width and 12–16 μm in length (Fig. 1). These had a grossly granular basophilic cytoplasm with a characteristic non-stained area in one pole and an eosinophilic round eccentric area closer to the other pole. These were interpreted as apicomplexan protozoal zoites, possibly of *Sarcocystis*, *Toxoplasma* or *Neospora* sp.

The ewe did not respond to treatment, was euthanased and a post mortem examination was performed. Gross examination identified bilateral, regionally extensive opacity of the corneas (suspected keratitis). Microscopic examination of sections of oesophagus, right and left ventricles and diaphragm revealed 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 coma-shaped organisms, 15–30 μm long, suspected to be *Sarcocystis* species (Fig. 2). There was no associated inflammatory infiltration and the 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 frequently in the grey matter. A section from the right frontal cortex was also examined and was histologically unremarkable.

The final histopathological diagnosis was (1) intrasarcoplasmic parasitic cysts (suspected to be *Sarcocystis* sp.) in the oesophagus, left and right cardiac ventricles and diaphragm and (2) minimal, multifocal, lymphocytic, perivascular myelitis.

Molecular analysis was performed in order to characterize the protozoa. The most successful method for *Sarcocystis* species identification has been the analysis of variable regions of ribosomal 18S RNA genes (Heckeroth and Tenter, 1999; Tenter et al., 1992; Yang et al., 2001). DNA was extracted from the CSF and from paraffin wax-embedded right ventricle using the DNeasy® Blood and tissue Kit (Qiagen, Alameda, CA), according to the

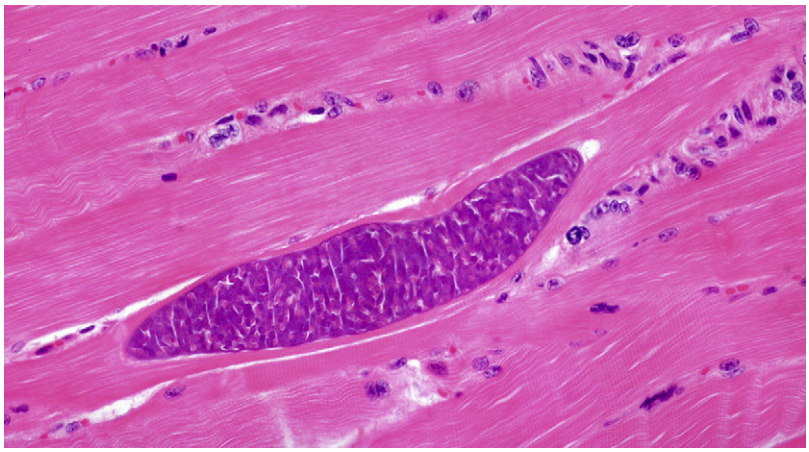

Fig. 2. Digital image of haematoxylin and eosin stained section of muscle from case 1 showing a typical S. *arcocystis* cyst within the sarcoplasm.

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