Case 5

PRIMARY CENTRAL NERVOUS SYSTEM PROTOTHECOSIS IN A DOG

Authors: Jason Stayt, Mellora Sharman, Amanda Paul, Peter Irwin, and Tibor Gaál

Department of Veterinary Clinical Pathology, School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch, Western Australia, Australia.

Signalment: 8 year old, spayed female Australian Cattle dog cross

History: The dog presented to the Murdoch University Veterinary Teaching Hospital with a 48 hour history of ataxia and mild obtundation and 24 hours of inappetance and a soft cough.

Clinical examination findings:
On physical examination the dog was obtunded but responsive and moderately ataxic. Neurological examination demonstrated a left head tilt, reduced gag reflex, right-sided proprioception deficits, bilaterally increased hindlimb reflexes and increased forelimb muscle rigidity. Focal alveolar infiltrates consistent with mild aspiration pneumonia were observed on thoracic radiography and CT demonstrated mild dilation of the 4th ventricle.

Laboratory data
Routine haematology and serum biochemistry were performed by the referring veterinarian and no significant changes were detected. Urinalysis revealed minimally concentrated urine (urine specific gravity 1.020) with no other abnormalities.
CSF Analysis:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference interval (where applicable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total volume (ml)</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Turbidity</td>
<td>nil</td>
<td></td>
</tr>
<tr>
<td>Sample colour</td>
<td>pink</td>
<td></td>
</tr>
<tr>
<td>Protein (g/L)</td>
<td>18.57</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>RBC (x 10^9/L)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>WBC (x 10^9/L)</td>
<td>2.03</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

Cytologic examination:

Cell count (200 cells);
- Neutrophils 5%
- Lymphocytes 32%
- Macrophages 14%
- Eosinophils 49%

Cytospin preparations were highly cellular and well preserved with a clear background containing occasional red blood cells and low numbers of round to oval capsulated organisms measuring approximately 7-11μm, surrounded by a thin clear capsule measuring approximately 0.5μm (Figure 1). The organisms typically had a basophilic granular cytoplasm although some appeared to contain variably sized, metachromatic staining globules. A mixed inflammatory cell population was also found that was predominantly composed of eosinophils with lesser numbers of lymphocytes, macrophages, and occasional neutrophils. The macrophages were markedly activated and demonstrated recent erythrophagia and cytophagia. Some macrophages also contained phagocytosed organisms with similar morphologic features to those observed free in the background (Figure 2). Occasional binucleate macrophages, rare mast cells, and rare plasma cells were also present.
**Figure 1:** Cytospin preparation of CSF. Note the marked eosinophilic pleocytosis. (Wright’s; scale bar 10μm)

**Figure 2:** Cytospin preparation of CSF showing the organism phagocytosed by a macrophage (Wright’s; scale bar 10μm)

**Interpretation:**
Marked eosinophilic inflammation with organisms consistent with *Prototheca sp.*
Clinical Progression and repeated CSF results:
A diagnosis of central nervous system protothecosis was made and treatment with several antifungal medications including fluconazole, amphotericin B, and terbinafine, and judicious doses of corticosteroids (prednisolone) was commenced. At three weeks the dog was neurologically normal, but three months after commencing therapy was presented with neurological signs similar to those observed prior. Also noted at this time was significant spinal pain which was most severe in the cervical region and characterised by reluctance to lift the neck. Repeat CSF analysis revealed an eosinophilic pleocytosis, however no organisms were detected. Relapse of CNS protothecosis was considered most likely and it was assumed that the neck pain was neuropathic in its origin. Previous treatment continued with the addition of an aminoglycoside antibiotic (genatmicin) and analgesic medications, including a GABA analogue (gabapentin) and an opioid (tramadol). Clinical signs initially improved but progressive deterioration occurred until 6.5 months post diagnosis at which time euthanasia was elected.

Necropsy examination:
The most significant changes were limited to the brain and meninges. Diffusely, the meninges were moderately thickened and contained multifocal to coalescing areas of haemorrhage. The blood vessels of the leptomeninges were moderately hyperaemic (Figure 3). Upon sectioning the brain, the lateral ventricle of the right cerebral hemisphere was moderately dilated.

Histologic examination:
Histological examination of the cerebral cortex revealed multifocal infiltrates of lymphocytes and plasma cells that were mainly centred on choroidal blood vessels. Multifocal inflammatory cell infiltrates composed of macrophages, lymphocytes and plasma cells, and occasional eosinophils were present within the neuropil adjacent to the choroid plexus of the lateral ventricles (Figure 4). The meninges were diffusely thickened by increased numbers of plump fibroblasts and blood vessels were hyperaemic and surrounded by infiltrates of lymphocytes and plasma cells. Periodic acid-Schiff staining demonstrated the presence ovoid, magenta staining organisms within areas of inflammation (Figure 5).
Figure 3: Photomicraph of the brain in situ.

Figure 4: Cerebral cortex and lateral ventricle. Multifocal mononuclear inflammatory cell infiltrates can be seen within the choroid and in the adjacent parenchyma (H&E; scale bar 20μm).
Figure 5: PAS staining *Prototheca* sp present amongst the predominantly plasma cell infiltrate (PAS, scale bar 10μm)

Microbiology:
Culture of CSF on Sabouraud’s dextrose agar at 25°C and subsequent sugar assimilation tests identified *Prototheca zopfii* (Figure 6).

Figure 6: Cultured organisms (Lactophenol blue; scale bar 5μm)
Discussion:
Protothecosis is a rare disease caused by members of the achlorophyllous algae of the genus Prototheca. It is presently classified belonging to the family Chlorellacae which are ubiquitous in nature. There are currently five species assigned to the genus Prototheca including Prototheca wickerhamii, P. zopfii, P. stagnora, P. ulmea, and P. blaschkeae sp. Of these five species, P. zopfii, P. wickerhamii and P. blaschkeae are generally reported to cause disease in humans and animals.1-8

The organisms are unicellular, spherical to ovoid, and range in size from 3 to 30μm in diameter. They are distinguished from other algae, such as Chlorella by their lack of chloroplasts and differ from fungi by the lack of glucosamine in their cells walls. They reproduce asexually via a process of cytoplasmic cleavage to produce between 2 and 20 endospores. The endospores are released from their sporangia (mother cells) upon rupture of their cell walls.1,2

The pathogenesis of canine protothecosis is uncertain however the ingestion of Prototheca and involvement of the gastrointestinal tract is usually reported.3 In a recent review of canine protothecosis in 17 Australian dogs, most had signs referable to colitis.4 Cutaneous infection is the most common route of infection in humans and mainly occurs through traumatic inoculation of the algae or contamination of an open wound.4 Cutaneous infections in dogs occurs less commonly.5 Disseminated infection is common in the dog and often involves several organ systems including the eye, kidney, heart, central nervous system, and bone.4,5,6 The prognosis for any dog with protothecosis and which dissemination is evident is grave.3,4

To our knowledge, primary infection of the central nervous system has not been previously reported and the detection of Prototheca organisms in the cerebrospinal fluid was diagnostic. In our case, the clinical signs were specific to the central nervous system and clinical signs referable to other body systems were not found. The finding of mild pneumonia in this dog was likely secondary to aspiration pneumonia due to neurologic dysfunction. Moreover, there were no previous reports of gastrointestinal signs or ill health provided by the owner. This was supported by the normal histologic findings in tissues other than the brain. A possible explanation for the inoculation of P. zopfii into the brain could include trauma, either external or internal via the nasal cavity, however again, there was no gross or histologic evidence for this. This case demonstrates the possibility of a primary infection of the central nervous system by Prototheca sp, without clinical or histologic evidence of disseminated disease.

Acknowledgements:
The authors wish to thank Mr Gary Allen, Department of Veterinary Clinical Pathology, School of Veterinary and Biomedical Sciences, Murdoch University, and PathWest Laboratory Medicine, Western Australia, for assistance with microbiological culture and identification.
References