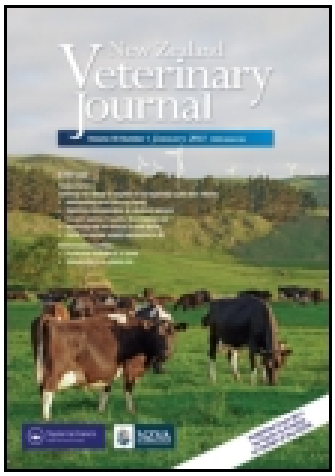

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Progressive ataxia and seizures in a Cocker Spaniel: a new type of neurodegenerative disease with novel intra-neuronal inclusions

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Clinical Communication

Progressive ataxia and seizures in a Cocker Spaniel: a new type of neurodegenerative disease with novel intra-neuronal inclusions

RD Jolly*§, I Schraa† and TR Halsey*

Abstract

AIMS: To describe the histopathological lesions of a new canine disease characterised by progressive ataxia, head tremor and seizures, and to deduce the cause of the lesions.

METHODS: Formalin-fixed tissues were processed into paraffin wax and epoxy resin for light and transmission electron microscopy of variously stained tissue sections.

RESULTS: Significant lesions relevant to the disease were found only in the brain. They consisted of hypoplasia of the cerebellum and the presence of large pale inclusions in the perikaryon of neurons in the neocortex and in macrophages. The inclusion material was not compartmentalised and did not stain for carbohydrate, mucopolysaccharide or lipid. This material displaced nuclei to the periphery of the cells where they were seen as basophilic distorted crescent-shaped structures.

CONCLUSIONS: The inclusions were probably made of polymerised protein similar, though not identical, to those of Pick, Lewy and Collins bodies that characterise a variety of chronic neurodegenerative diseases of humans. A genetic basis to this disease was considered probable.

KEY WORDS: *Dog, ataxia, neurodegeneration, intra-neuronal inclusions, protein*

Introduction

Progressive neurological disease in young dogs with symmetry of signs and involving several neural systems is highly suggestive of an inherited metabolic defect, particularly if this includes changes in learned behaviour or temperament. This report concerns such a case, which involved a male Cocker Spaniel dog purchased at 3 months of age. Within a few days of purchase, the new owners noted he had a clumsy hindlimb gait which was, at first, attributed to him being a puppy. This became increasingly worse and, with the onset of head tremor, the dog was presented for veterinary examination.

Case History and Clinical Findings

The dog was subjected to clinical examination on a number of occasions over 11 months. On initial examination at 4 months of age, generalised motor seizures, head tremor and distinct hindlimb ataxia were evident. Hepatic encephalopathy was discounted as a likely cause on the basis of serum biochemistry, and the dog was placed on 30 mg phenobarbitone twice daily. Despite this, neurological signs became progressively worse in ensuing weeks, to a stage when the dog had 10–15 episodes of seizure activity per day that would last up to 15 sec. He also began to snap or bite when handled. At 8 months of age, medication was increased to 60 mg of phenobarbitone twice daily and 400 mg of potassium bromide once daily in an attempt to control the tremors and seizures.

By 8 months of age, there was also hypermetria of the hindlimbs associated with reduced conscious proprioception, in addition to hindlimb ataxia. Sciatic and patellar reflexes were exaggerated. There was an inconsistent menace response but vision appeared intact and normal pupillary light reflexes were normal.

Results of haemogram and cerebrospinal fluid (CSF) examinations were normal but the electrolyte panel showed an elevated chloride concentration of 129 mmol/l (normal range 98–107 mmol/l). A bowel biopsy was negative for lipofuscin-like pigments on histopathological examination.

The owners declined further diagnostic tests such as computerised tomography and magnetic resonance imaging or brain biopsy, and elected to continue conservative treatment, as the frequency of seizures stabilised following increased anticonvulsant dosages. However, by 15 months of age, seizure activity became more frequent and did not respond to increased medication. An acute onset of vomitting occurred associated with abdominal pain and distension. Exploratory laparotomy revealed a severe necrotising pancreatitis with secondary peritonitis. Following surgery the dog was maintained on palliative treatment of pentobarbitone and intravenous fluids but died shortly afterwards.

The body was placed in a freezer for several hours before being removed and submitted for necropsy, 15 hours after death; complete freezing did not occur. Selected tissues, including the bowel biopsy taken at 6 months of age, were fixed in 10% formol saline, processed into wax, and sections stained with haematoxylin and eosin (H&E), periodic acid Schiff (PAS), luxol fast blue, Sudan black, toluidine blue, and alcian blue methods. Formalin-fixed frozen sections were stained with H&E, PAS and Sudan black methods. Additional brain neocortex was post-fixed in 1% osmium tetroxide and processed into epoxy resin. "Thick" sections were stained with toluidine blue for light microscopy,

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CSF	Cerebrospinal fluid
H&E	Haematoxylin and eosin
PAS	Periodic acid Schiff

and "thin" sections using the lead citrate/uranyl acetate method for transmission electron microscopy.

Pathology

Gross pathology

There was severe necrotising pancreatitis with local fat necrosis, and a fibrinous peritonitis. The cerebellum was estimated to be approximately two thirds of normal size.

Histopathology

With the exception of lesions associated with the pancreas, histopathological changes were limited to the brain, in which there was thrombosis of many blood vessels and some haemorrhage. In some gyri of the neocortex there were swollen hydropic astrocytes with pyknotic nuclei, but this was not a generalised lesion. There were also some superficial freezing artefacts. The architecture of the cerebellum was relatively normal other than for some slight segmental thinning of the granular layer. At the tips of three gyri of the posterior vermis there was a near absence of granular cells (Figure 1).

Many neurons in the neocortex, particularly in the parietal area, contained large pale inclusions filling much of the cytoplasm and displacing normal cytoplasmic structures and the nucleus to the periphery of the cell (Figure 2). The nucleus was often grossly distorted, appearing as a thin basophilic crescent-like structure at one pole of the cell. The pale inclusions appeared opaque, sometimes slightly basophilic, and frequently showed "chatter" marks from the microtome knife (Figure 3a). They did not stain with any of the methods used nor did they fluoresce under ultraviolet light or rotate polarised light. There were also many distended cells, interpreted to be macrophages, in which the nuclei were displaced to the periphery of the cell and, like those in similarly affected neurons, these were also pyknotic and crescent-shaped. Although these cells frequently appeared empty, others contained opaque material with knife "chatter" (Figure 3b), or stringy remnant material. Some of these cells were seen in close approximation to neurons in which inclusions were evident, but they were not seen adjacent to blood vessels, within perivascular spaces or within the leptomeninges. Subjective assessment of the density of neurons in the cerebral cortex suggested a loss of neurons.

Electron microscopy

Transmission electron microscopy confirmed displacement of normal cell structures (Figure 4). The inclusion material was largely electron-lucent but contained a network of fine, amorphous, electron-dense material, and many electron-dense particles interpreted to be ribosomes or degenerated ribosomes (Figure 3c).

Discussion

The hindlimb ataxia and hypermetria with normal hindlimb strength suggested cerebellar disease. The inconsistent menace response most likely reflected the sedative effects of anticonvulsant therapy, as vision and pupillary light reflexes appeared normal. Behavioural changes and seizure activity indicated cerebral disease. The age of onset and progressive nature of clinical signs strongly suggested a degenerative central nervous disease, possibly a lysosomal storage disease. The bowel biopsy was performed to investigate the possibility of "ceroid-lipofuscinosis with brown bowel syndrome", a rare storage disease of Cocker Spaniels but usually seen in older animals (Jolly et al 1994). Although this case was negative, a similar case had recently been diagnosed at the Massey University Veterinary Teaching Hospital using such means (HM Burbidge[1], pers. comm.) and provided the rationale for this diagnostic procedure. The elevated serum chloride concentration was interpreted to be the result of the potassium bromide this dog was receiving. Although an ion-specific electrode was used for the measurement of chloride, this methodology has been reported to give falsely high chloride values due to the electrode's inability to fully discriminate chloride from other halide ions such as bromide (Trepanier 1995).

The pancreatic necrosis, peritonitis and shock were the immediate cause of death and were considered unrelated to the underlying neurological disease. The microscopic vascular lesions were those of disseminated intravascular coagulation which, together with the hydropic astrocytes, probably reflected disturbances in blood clotting and ionic and fluid exchanges associated with the terminal disease.

The hypoplastic cerebellum noted both grossly and histologically explains the clinical signs of cerebellar disease. The severe hypoplastic lesions noted at the tips of three gyri, one of which is depicted in Figure 1, were not considered artefacts of sectioning. The large pale intra-cytoplasmic neuronal inclusions probably reflect the neuronal dysfunction responsible for signs that were interpreted as being due to cerebral disease. The relationship between these two distinct changes is uncertain. The opaqueness of the inclusion material and knife "chatter" was strongly reminiscent of that seen in colloid of thyroid follicles, suggesting a similar consistency. That it was relatively solid is also indicated by flattening of the nuclei and displacement of organelles. There was no bilayer membrane evident by transmission electron microscopy between the accumulated material and cytoplasm containing the main cell organelles, indicating that the accumulated material was not compartmentalised. Flattening of the nucleus is an unusual lesion, even in lysosomal storage diseases in which nuclei may also be displaced to the periphery of the cell, but still appear rounded.

The material within cells that were considered to be macrophages was similar, though not identical, to that within neurons and was probably derived from phagocytosis of neuronal debris including the inclusion material. The fact that they sometimes appeared empty, or partly empty, suggested that the accumulated material may have been partly digested and, consequently, dissolved during the preparation of paraffin sections. Such macrophages would be expected to occur near blood vessels or within perivascular spaces, but they were not. If the intracellular material was relatively solid, as suggested, their migratory ability may have been compromised. An alternative interpretation is that they were microsatellite cells.

The histopathology and ultrastructure of inclusion material in

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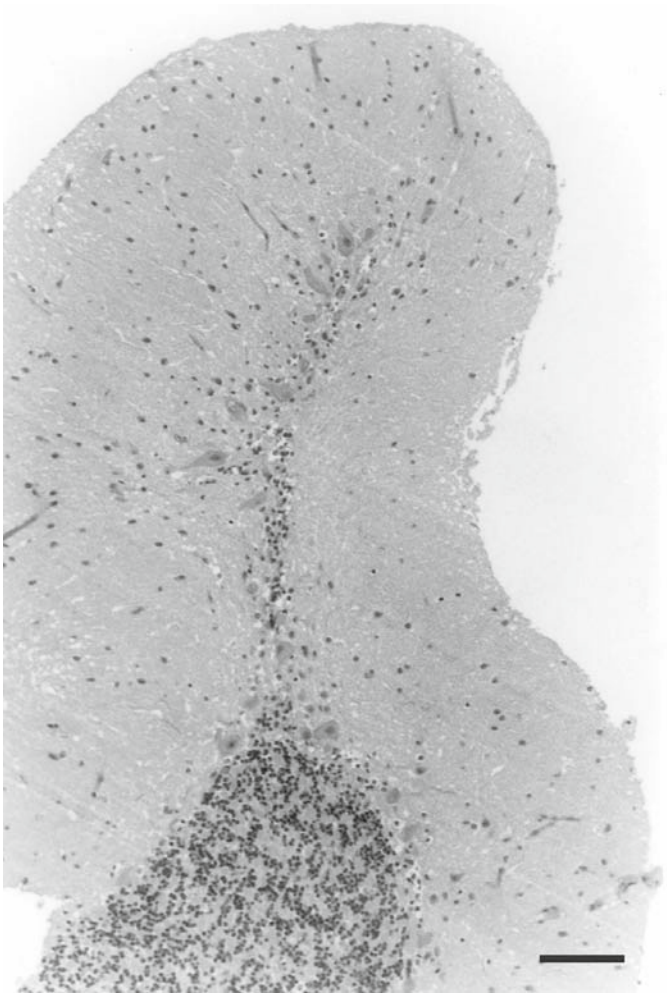

Figure 1. Light photomicrograph of the tip of a cerebellar folium showing near absence of granular cells, but presence of Purkinje cells (H&E, bar=9.1 µm).

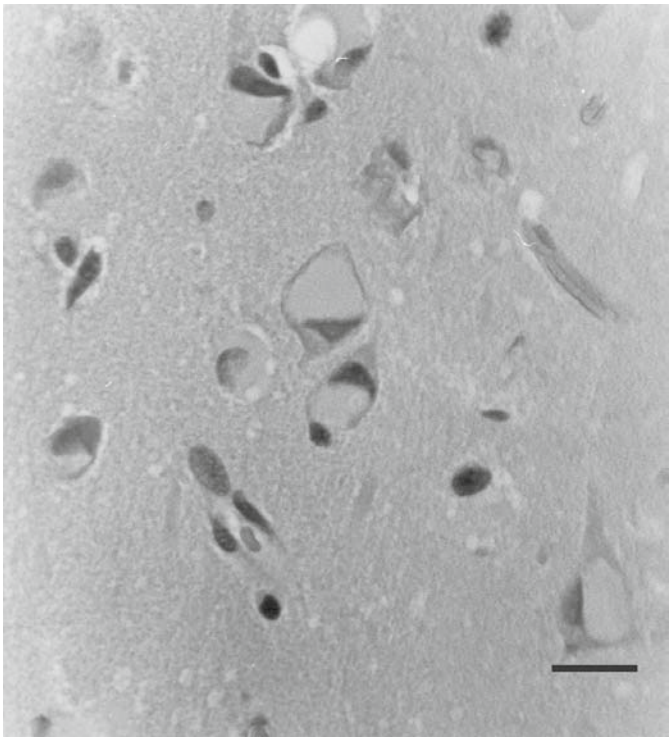

Figure 2. Light photomicrograph of cerebral cortex showing a group of neurons with large pale intracytoplasmic inclusions that have distorted the nuclei and displaced them to the periphery of the cells (H&E, bar=3.7 µm).

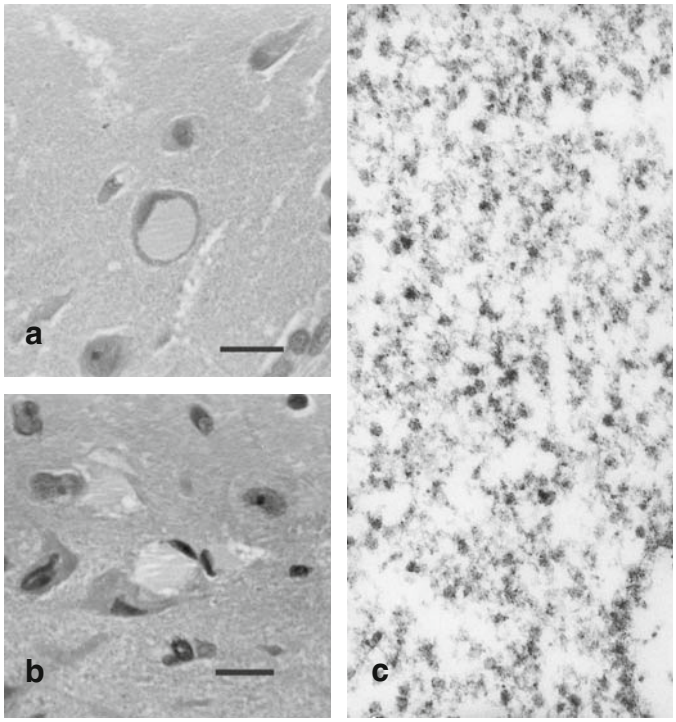

Figure 3. (a) Light photomicrograph of the cerebral cortex depicting a neuron (similar to those in Figure 2) in which the accumulated material shows knife "chatter" marks (H&E, bar=3.7 µm). (b) A light photomicrograph of a cell interpreted as a macrophage, showing similar accumulated material with knife "chatter" marks (H&E, bar= 3.7 µm). (c) Transmission electron micrograph of accumulated material in a neuron of the neocortex which shows as a loose network of light-staining amorphous □occules which contrast with dark-staining granular material (72,000x).

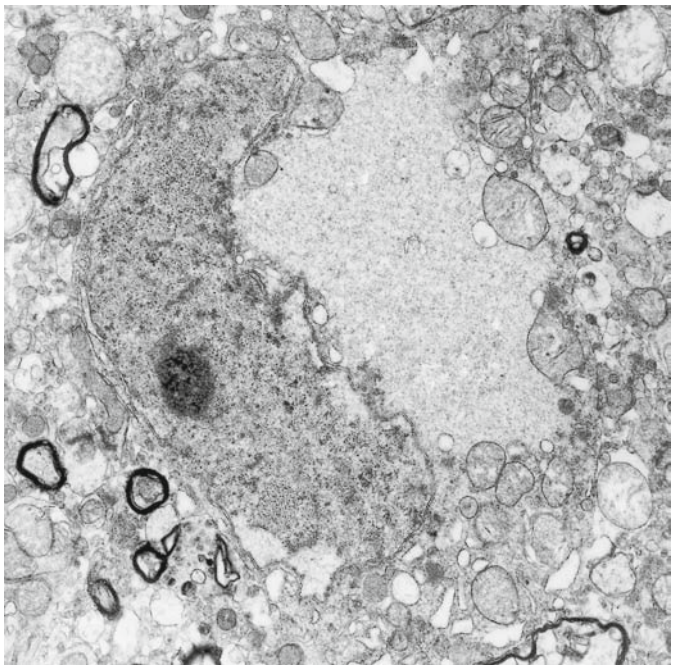

Figure 4. Transmission electron micrograph of a neuron in the neocortex showing the material that has accumulated and displaced the nucleus and cytoplasmic organelles to the periphery of the cell. This material is not compartmentalised (7,800x).

neurons is highly suggestive of intracytoplasmic accumulation of poorly soluble polymerised protein. The slight basophilia sometimes noted using light microscopy was likely due to the presence of ribosomes, as structures resembling ribosomes were evident in these cells on electron microscopy. There are similarities, but also differences, to other neuronal cytoplasmic inclusions of specific accumulated proteins associated with chronic neurodegenerative diseases of humans. These include Pick bodies in Pick and related diseases, in which the protein is Tau; Lewy bodies in Parkinson's and related diseases, in which the protein is α-synuclein (Goedert 1999); Collins bodies in a familial encephalopathy, in which the protein is neuroserpin (Davis et al 1999ab) and; hyaline bodies in familial amytrophic lateral sclerosis, in which the protein is that of copper/zinc superoxide dismutase (Kakizuka 1998). Pick disease is just one of many neurodegenerative "tauopathies" (Lee et al 2001).

In the present disease, the putative accumulating protein is unknown. It may have been synthesised in the cytoplasm because it appears not to be glycoslated (i.e. was PAS-negative) and because of its apparent association with ribososmes. In contrast to these inclusions, Collins bodies are PAS-positive and consist of the glycosylated protein, neuroserpin. Whereas more than one Pick, Lewy or Collins body may be seen within a single neuron, only one body was evident per neuron in the syndrome described here, and these were less discrete than inclusion bodies seen in these other diseases. A further group of protein-accumulating neurodegenerative diseases is made up of those that accumulate subunit-c of mitochondrial ATP synthase, albeit as multiple smaller lysosomal bodies. These are most, but not all, of the ceroid-lipofuscinoses, a complex group of storage diseases of humans and animals known under the umbrella eponym of Batten disease (Goebel et al 1999; Jolly et al 1999). Other neurodegenerative diseases in which large neuronal bodies occur that superficially resemble those seen in this Cocker Spaniel and the other chronic neuropathies discussed above, are those of Lafora body and Bielschwsky body diseases. In contrast, these bodies are mainly made of PAS-positive polyglycans and have low protein content (Cavanagh 1999).

The cause of the neurological disease in this Cocker Spaniel is unknown, but a genetic basis is thought most likely. As this appeared to be an isolated case and the parents were allegedly normal, a recessive mode of inheritance would be expected. However, familial forms of Pick's, Parkinson's and neuroserpin accumulating diseases tend to be dominantly-inherited traits. If this was so for the disease in the Cocker Spaniel, then a new mutation would be probable.

In conclusion, a new type of neurodegenerative disease characterised by progressive ataxia, head tremor and seizures, and novel intra-neuronal inclusion bodies was diagnosed in a 15-month-old Cocker Spaniel dog. The inclusion bodies were probably made of polymerised protein similar, though not identical, to those of Pick, Lewy and Collins bodies that characterise a variety of chronic neurodegenerative diseases of humans. A genetic basis to this disease was considered probable.

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