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Progressive encephalomyelopathy and cerebellar degeneration in a captive-bred snow leopard (*Uncia uncia*)

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PROGRESSIVE encephalomyelopathy with cerebellar degeneration has been described in captive cheetahs (Palmer and others 2001) and in young domestic cats (Palmer and Cavanagh 1995). This case report describes the clinical and histopathological findings in a very similar condition affecting a young snow leopard (*Uncia uncia*) that had been born in a zoological park in eastern England as part of the globally coordinated breeding programme for this critically endangered species.

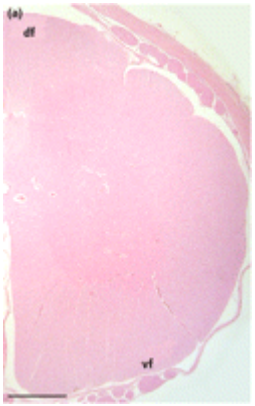
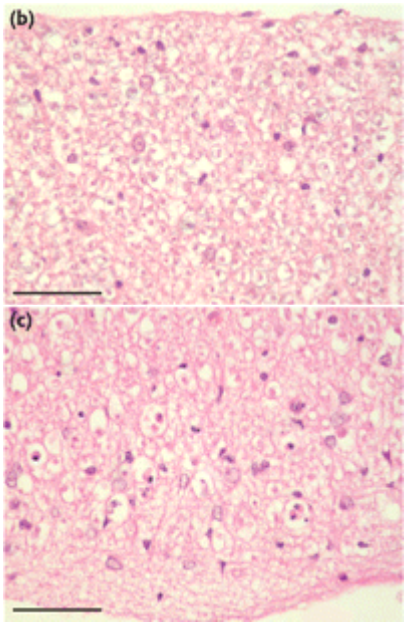


FIG 1 : Transverse hemisection from the lumbar enlargement of the spinal cord of a young snow leopard (*Uncia uncia*). (a) Regions of subpial white matter from the ventral funiculus (vf) and dorsal funiculus (df). Bar=1 mm. (b) Dorsal funiculus and (c) ventral funiculus, both illustrated at higher magnification. Dilated myelin sheaths with myelophages are present in the ventral funiculus, while axons in the dorsal funiculus are unremarkable. Bars=50 µm. Haematoxylin and eosin



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The affected animal was one of a litter of two male cubs. It was initially presented at the age of six weeks with progressive signs of incoordination and ataxia, although it was still eating, snarling and biting without difficulty. In retrospect, its keepers noted that it had always been slightly 'floppy'. By three months of age, the animal showed increasing incoordination of the hindlimbs, and two weeks later it was unable to support its weight on its hindlimbs or control their movement. The animal weighed 12 kg and radiographic examination revealed no abnormalities of the vertebral column. The animal was euthanased by intravenous injection of pentobarbitone sodium.

A complete postmortem examination was performed immediately after death. Other than agonal pulmonary congestion, no macroscopic abnormalities were observed. The brain, spinal cord and vertebral column were grossly unremarkable. The brain and spinal cord, and a range of other tissues were removed and fixed in 10 per cent formal buffered saline. The non-neurological tissues were processed routinely to paraffin wax and 5 µm sections were stained with haematoxylin and eosin. The brain and spinal cord were processed using an extended protocol for neurological tissues, and 8 µm sections were stained with haematoxylin and eosin. Transverse sections of the brain were examined at the level of the frontal lobe, diencephalon, midbrain, pons, cerebellum and medulla. Sections of the spinal cord were taken at the cervicomedullary junction, the cervical enlargement, mid-thorax, lumbar enlargement and sacrum. Peripheral nerves from the cauda equina, dorsal root ganglia, diaphragm and appendicular

striated muscle were examined. Non-neurological tissues sampled included the trachea, lung, heart, aorta, kidney, spleen, lymph node, thymus, thyroid, adrenal, salivary gland, small and large intestines and pancreas.

Other than agonal pulmonary congestion and oedema, histopathological abnormalities were confined to the central nervous system. In the spinal cord, Wallerian-type degeneration occurred in white matter tracts in all funiculi except for the dorsal columns, which were almost completely spared. The degeneration was broadly bilaterally symmetrical, extended the whole length of the spinal cord, and was most severe towards the periphery, subjacent to the pia mater (Fig 1). Axonal degeneration was of typical Wallerian type, characterised by the presence of numerous widely dilated myelin sheaths, many of which contained myelophages (Fig 2); it was accompanied by gliosis. These changes were most prominent in the ventral columns and in the dorsal spinocerebellar tracts.

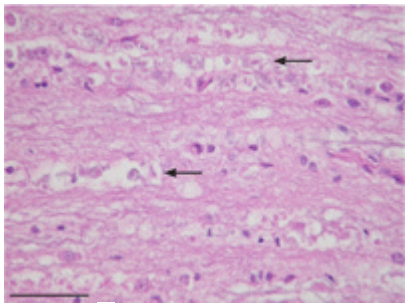

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FIG 2 : Longitudinal section through the ventral column of the spinal cord of a young snow leopard (*Uncia uncia*), just below the pial surface (at the bottom of the figure), showing dilated myelin sheaths (arrows) containing myelophages, typical of Wallerian-type degeneration. Haematoxylin and eosin. Bar=50 µm

In the cerebellum, changes were most pronounced in the cortical layers of the vermis. There was marked depletion of Purkinje cells, with some of the remaining cells displaying chromatolysis-like changes, cell shrinkage and occasional vacuolation (Figs 3, 4). The molecular layer was equivocally thinned in some regions, and occasional focal microglial proliferations were present (Fig 4), in addition to clusters of hypertrophied astrocytes elsewhere. Modest gliosis was present in the cerebellar medullary substance, but Wallerian-type degeneration was not observed.

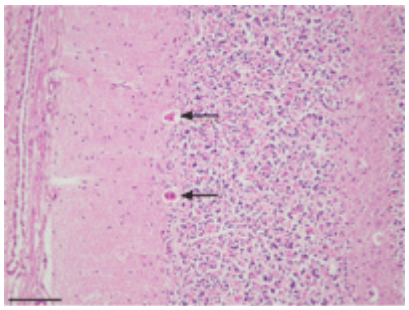

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FIG 3 : Cerebellar cortex of a young snow leopard (*Uncia uncia*), showing a reduced number of Purkinje cells. Small numbers of remaining Purkinje cells (arrows) are necrotic. Haematoxylin and eosin. Bar=100 µm

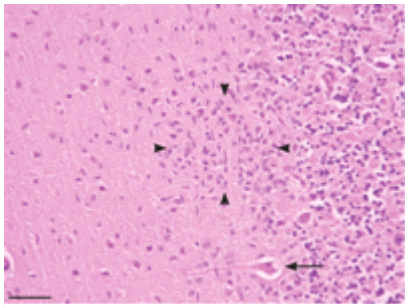

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FIG 4 : Cerebellar cortex of a young snow leopard (*Uncia uncia*), showing proliferation of rod cells (microglia [arrowheads]) in the molecular layer of the cerebellum. A shrunken vacuolated Purkinje cell (arrow) is present against a background of Purkinje cell depletion. Haematoxylin and eosin. Bar=50 µm

To investigate the possibility of a viral aetiopathogene sis for the disease seen in the snow leopard, immunolabelling of cerebellum was undertaken against antigens of feline coronavirus (FCoV), feline calicivirus (FCV), feline herpesvirus type 1 (FHV-1) and feline panleukopenia virus (FPLV) using standard protocols for domestic felid tissues (Kipar and others 1998, 2001). The brain of an unaffected snow leopard cub and domestic cat tissues were used as controls. In the case of FCoV and FCV labelling, occasional mild non-specific labelling of white matter axons in the cerebellum was observed. Labelling for FHV-1 and FPLV were uniformly negative. Subsequently, DNA was extracted from sections of the paraffin-embedded tissues using standard protocols (QIAamp DNA Mini Kit; Qiagen, Qiagen DNeasy Tissue Kit; Qiagen, Nucleon HT DNA extraction system; Tepnel Life Sciences) and subjected to PCR amplification using standard protocols and primers specific for FPLV (Schatzberg and others 2003). Replicate samples were negative for FPLV DNA for all three protocols used, while known positive feline

control tissues subjected to identical extraction and amplification procedures gave amplicons of appropriate size.

The affected animal was one of a litter of two male cubs born to a female that had previously produced normal off-spring from the same mating. The female had been vaccinated against FPLV, FHV-1, FCV and chlamydophilosis using inactivated feline vaccines (Fevaxyn iCHPChlam; Fort Dodge Animal Health), and had received the most recent booster seven months before the birth of the cubs. Routine parasitic control using ivermectin had been undertaken before the pregnancy. Contraceptive doses of melengestrol acetate (1000 mg, in the form of a slow-release capsule) had been administered several years previously, and the capsule had been removed two years before the pregnancy. The parents' diet was predominantly poultry, supplemented with rabbit, pheasant and occasionally horsemeat. The littermate of the affected animal had been markedly underweight at the age of three weeks and was removed for attempted hand rearing. It died within a few hours, and complete postmortem examination supported by immunohistochemistry revealed the presence of severe subacute suppurative leptomeningitis of presumptive bacterial origin, involving the cranial spinal cord and brainstem. No pathology was present in the other organs, and no central nervous system changes comparable to those of its sibling were present at a retrospective review.

Hindlimb ataxia attributable to spinal cord degeneration has been reported previously in snow leopards. In the first report (Haltia and Wahlberg 1984), six cubs, in two litters from the same dam at Helsinki Zoo, developed progressive hindlimb paresis starting from the ages of two to nine months. Two of these animals were examined histologically; both exhibited extensive myelin loss in the lateral and ventral columns, accompanied by a lesser degree of axonal degeneration and astroglial reaction. The dorsal columns were largely spared. Slight evidence of secondary Wallerian-type degeneration was noted in the brainstem. There are further reports of a condition with similar clinical and pathological features affecting snow leopard cubs born in Swiss and French zoos between 1997 and 2003 (Robert and others 2004). Two further neurodegenerative conditions have also been reported in snow leopards. Leukoencephalopathy was described in a two-year-old male snow leopard that developed progressive hindlimb ataxia (Junge and others 1986). That was characterised histologically by extensive myelin vacuolation and Wallerian-type degeneration that was most pronounced in lumbar and sacral segments of the spinal cord, but was also present more cranially and in the brainstem. Perivascular cuffs of lymphocytes, macrophages and plasma cells were also present, involving the cerebrum in which myelin changes were described as less prominent. No aetiological agent was identified and the condition was ascribed to primary axonal degeneration with secondary demyelination. Chromatolysis of proprioceptive neurons, predominantly in the thoracic nucleus, was described in France (Robert and others 2003). Cubs presented with progressive head or body tremors and paresis.

The case described here has similarities to the cases described by Haltia and Wahlberg (1984) and Robert and others (2004). The age of onset and clinical presentation are similar, as are the distribution and character of the non-inflammatory lesions of Wallerian-type degeneration, confined largely to the ventral and lateral columns. However, minimal changes were noted in the brains of the cubs described by Haltia and Wahlberg (1984) and Robert and others (2004). The Purkinje cell depletion and cerebellar gliosis seem to have been described only in the present case. It is thus unclear whether each of these cases is unique or part of a spectrum of a single disease condition. The pathological changes in the present case are similar to those described in progressive encephalomyelopathy and cerebellar degeneration in captive cheetahs (Palmer and others 2001) and in encephalomyelopathy of young cats (Palmer and Cavanagh 1995). There is a similar degree of variability in the descriptions of the pathology underlying hindlimb ataxia in cheetahs (Walzer and Kubber-Heiss 1995, Walzer and others 1998, 2003, Palmer and others 2001), and may partly explain why investigations have so far failed to identify a definitive aetiology (Callanan and others 1999). One factor frequently postulated to promote the disease in cheetahs is loss of genetic polymorphism. However, the environment may be the key factor in how the disease develops, as captive and free-ranging cheetahs of equivalent genotypes have exhibited different disease profiles in response to similar challenges (Munson and others 2005). A genetic study also failed to establish a relationship between neurodegenerative disease in cheetahs and alterations in the mitochondrial genome (Burger and others 2004). Even with mating carried out according to studbook recommendations, as was the case here, the genetic ancestry of snow leopards is narrow. Preliminary pedigree analysis (Robert and others 2004) indicates that common ancestors appear in the lineages of both affected and unaffected animals. Thus additional, extrinsic, rather than genetic, factors may underlie the propensity for both snow leopards and cheetahs to develop unusual diseases, such as hepatic veno-occlusive disease (Van Den Ingh and others 1981, Munson and Worley 1991) and encephalomyelopathy. Feeding a diet of unsupplemented poultry was implicated in the cases described by Robert and others (2004). In the animal reported here, the diet of the parents was also predominantly, although not exclusively, poultry. Recent descriptions of leukoencephalomyelopathy in specific pathogen-free cats fed γ-irradiated food (Cassidy and others 2007) have suggested a role for subtle micronutrient deficiencies in such pathology. Additional aetiologies that have been suggested for encephalomyelopathies of felids include exposure to toxins, and viral infections. FCoV and FHV-1 have been isolated from the brains of a subset of cheetah cases (Walzer and others 1998), but a robust causal relationship has not been demonstrated in further studies (Shibly and others 2006). In the present case, a combination of immunohistochemical and molecular approaches failed to find evidence of involvement of FCoV, FHV-1, FPLV or FCV.

In conclusion, this case may be part of a spectrum of spinal cord degenerative disease in captive snow leopards described previously. The distinctive histological features suggest that it may be more appropriately classified as an

example of the progressive encephalomyelopathy with cerebellar degeneration previously reported in captive cheetahs and domestic cats. At present the cause remains unknown.

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