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IDIOPATHIC ACUTE ONSET MYELOPATHY IN CHEETAH (*ACINONYX JUBATUS*) CUBS

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Key words: Cheetah, *Acinonyx jubatus*, demyelination, CNS, copper, myelopathy, neurologic disease, pathology.

INTRODUCTION

Numerous cases of ataxia, hind limb paresis, and paralysis have occurred in cheetah (*Acinonyx jubatus*) cubs over the past 10 yr within the European Endangered Species Program (EEP) population. In reports made to the International Cheetah Studbook keeper, various facilities acknowledge hind limb paresis as the cause of death in related juvenile animals.¹⁴ These apparent cases have occurred in focal clusters. Data obtained by questionnaire among EEP cheetah holders (Walzer, unpubl. data) indicate that cases have occurred in 12 locations in mainland Europe, two in the British Isles, one in Namibia, and one in Dubai. Hind limb paresis in cubs is the most important medical factor limiting the growth of the EEP cheetah population, and it is the major veterinary concern of the European felid taxon advisory group.

Ataxia in cheetahs was first described in South Africa.⁷ The disease was linked to copper deficiency, although no specific copper level analysis was carried out. Several years later, two European litters in the Netherlands developed progressive hind limb weakness and ataxia at 18–26 wk of age. Pronounced Wallerian degeneration of the spinal dorsal

and ventral roots was noted at necropsy, with afferent and efferent nerve degeneration. Copper levels in the liver and kidneys were low, and the condition, as in the South African report, was ascribed to copper deficiency.³¹

A progressive ataxia with discrete onset was subsequently reported from a zoo in eastern Austria.²⁷ The five related 7- to 10-yr-old adult animals progressed from paresis to final paralysis in 2 yr. A massive, bilaterally symmetric, primary demyelination of the ventral and lateral columns of the spinal cord white matter, predominantly in the descending tracts of the thoracolumbar spinal column, was observed. Hematology, blood chemistry, serology, and cerebrospinal fluid (CSF) values were normal, and the cause of the myelopathy was not determined.

A litter of five cubs (three males and two females) was born at the Nürnberg Zoo in October 1994. One female cub was hand-raised after its second day. At 6 wk of age, the parent-raised cubs developed mild ocular discharge, which progressed to ulcerative corneal lesions in one cub. After ocular treatment, the latter animal was returned to its enclosure where it developed spontaneous hind limb ataxia. By the end of January 1995, the other three parent-raised cubs also exhibited varying degrees of hind limb ataxia and paresis (Gaukler, pers. comm.).²⁸ After euthanasia, histopathology revealed spinal cord demyelination with axonal swelling and concomitant astrogliosis that was most pronounced in the sulcomarginal and spinocerebellar tracts. Furthermore, lymphomonocytic perivascular cuffs, discrete neuronal necrosis, and gliosis were found in the rhombencephalon. Immunohistochemical examinations for canine distemper virus (CDV) and

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Table 1. Origin, age, sex, and disease onset of the cheetah cubs examined.^a

Litter	Cub (number/identity)	Sex	Origin	Birth date	FHV-1 ^b	Ataxia onset	Death date	Ataxia ^c
1	1/FD90	F	Salzburg	25 May 1996	17 Aug 1996	28 Aug 1996	11 Nov 1996	
1	2/7D85	F	Salzburg	25 May 1996	17 Aug 1996	11 Sep 1996	NA	present
2	3/14E5	M	Salzburg	27 Oct 1996	10 Dec 1996	16 Dec 1996	NA	present
2	4/3C37	F	Salzburg	27 Oct 1996	10 Dec 1996	16 Dec 1996	NA	
2	5/3CFC	F	Salzburg	27 Oct 1996	10 Dec 1996	16 Dec 1996	NA	
2	6/FCDD	M	Salzburg	27 Oct 1996	10 Dec 1996	16 Dec 1996	NA	present
3	7/CF67	F	Salzburg	19 Sep 1997	20 Mar 1998	04 Apr 1998	24 Nov 1998	
3	8/0EE4	F	Salzburg	19 Sep 1997	20 Mar 1998	04 Apr 1998	24 Aug 1998	
	9/Fota 1	UNK	Fota	01 Jun 1997	01 Aug 1997	14 Aug 1997	21 Aug 1997	

^a FHV-1 = feline herpesvirus 1; NA = not applicable; UNK = unknown.

^b Nasal-ocular symptoms of an FHV-1 infection first recorded.

^c Ataxia still persists—animal not euthanatized.

Aujeszky's disease had negative results.¹⁰ The hand-raised littermate never developed clinical disease and died at 2 yr of age of an unrelated gastrointestinal foreign body. There were no central nervous system (CNS) lesions in this cub (Hännichen, pers. comm.).²⁸

A workshop on cheetah cub ataxia took place in 1999 in Ireland, where ataxia was reported in seven out of 37 litters at the Fota Wildlife Park in Ireland, beginning at 2–6 mo of age. Morbidity was 25–100%. Ataxia onset was preceded by serous ocular and nasal discharges that progressed to severe chemosis and mucopurulent discharge.⁹ Ocular swabs did not reveal any infectious agents.² Initial ataxia progressed rapidly to paresis of varying severity. In advanced cases forelimb involvement and a head tremor were noted. With the exception of one litter, the signs occurred within 4 wk after a vaccination regimen with a modified live vaccine against feline herpesvirus (FHV-1), feline calicivirus (FCV), and feline parvovirus (FPV). Wallerian degeneration within the spinal cord in conjunction with cerebellar degeneration was found in these cubs.²⁰

This article presents clinical onset and progression, diagnostic approaches, therapeutic trials, and pathologic findings encountered in acute onset cheetah cub ataxia at Zoo Salzburg in Austria and in one animal provided by Fota Wild Animal Park.

MATERIALS AND METHODS

Case histories

Eight cheetah cubs from three litters at Zoo Salzburg, Austria, and one cub born in Fota Wildlife Park, Ireland, were involved (Table 1). The Irish cub was included in this study because it was euthanatized at the author's request to investigate and

demonstrate the early lesions observed in this syndrome.

All cheetahs in Salzburg were kept in grassy enclosures of 6,000 m² with several heated pens. The animals were fed a diet of beef, rabbit, and chicken supplemented periodically with commercially available vitamin–mineral tablets (VMP[®], Pfizer Corp., Karlsruhe, D-76032 Germany). The cheetahs were vaccinated annually using an inactivated FHV-1, FCV, and FPV vaccine (Felimun[®], Veterinaria AG, Zurich, CH-8045 Switzerland). Adult cheetahs were dewormed periodically with mebendazole (15 mg/kg: two for 2 days; Mebenvet[®], Janssen, Beerse, Belgium) or ivermectin (0.25 mg/kg; Ivomec[®], Merck, Sharpe & Dohme, Haarlem, NL-2031 The Netherlands) after fecal examination. All cubs were dam reared in outside enclosures.

Two cubs in litter 1 were born in May 1996 from dam 1921 (International Studbook ID) and sire 1740. After deworming with pyrantel pamoate (Banminth[®], Pfizer Corp., Vienna, A-1220 Austria; 40 mg/kg) at 4 wk of age, the cubs were vaccinated at 6 wk of age using Felimun[®]. The dam began sneezing when the cubs were 11 wk old, and the cubs began sneezing several days later. Mild serous nasal and bilateral ocular discharges and conjunctivitis developed during the next few days, the cubs' appetite decreased, and they appeared listless. The ocular lesions of cub 2 progressed, with the development of unilateral purulent discharge and corneal ulceration. Ocular and nasal swabs and scrapings for bacterial and viral examinations as well as blood samples for hematology, blood chemistry, and serology were collected. Because of the unilateral distribution of the lesions, chlamydial infection was presumed and topical treatment was ini-

tiated with tetracycline (Terramycin® AS, Pfizer Corp., Austria). However, swabs and scrapings were negative for *Chlamydomphila*, but viral culture revealed FHV-1. Therapy was changed to topical Vidarabin (Vidarabin Thilo®, Agepha GesmbH, Vienna, A-1150 Austria; q.i.d.) and subconjunctival injections of prednisolone (Solu-Dacortin®, Merck, Darmstadt, D-64293 Germany; 10 mg/eye) to reduce swelling and pain. Both cubs also received two doses of a paraimmunity inducer consisting of inactivated parapox ovis (Baypamun® HK, Bayer AG, Leverkusen, D-51368 Germany). Eleven days after the onset of the ocular lesions, cub 1 was hand captured and restrained for additional blood sampling. Thirty minutes after the procedure, it spontaneously developed hind limb ataxia and paresis, showing a swaying, high footing gait. It would fall over laterally while turning, followed by short bouts of hind limb dragging. Traumatic insult as a result of the hand capture was diagnosed. The animal was placed on tolfenamine (Tolfedine®, Vetoquinol, Lure, F-70204 France; 4 mg/kg, i.m.) and observed. Because the cub did not improve, it was anesthetized with ketamine (Ketamidor®, Richter Pharma GesmbH, Wels, A-4600 Austria; 10 mg/kg) and xylazine (Rompun® TS, Bayer AG; 2 mg/kg) so that more extensive diagnostic procedures could be performed. When the cub was returned to its enclosure 45 min later, the littermate (cub 2) had also become ataxic and parietic. Both cubs and the dam received a CuSO_4 (50 mg, s.i.d., p.o.) supplementation. The cubs were treated with flunixin meglumine (Finadyne®, Essex-Chemie, Luzern, CH-6002 Switzerland; 1.1 mg/kg, p.o., s.i.d.), 2.2 mg/kg vitamin B₁, 2.2 mg/kg vitamin B₆, and 0.022 mg/kg vitamin B₁₂ i.m., s.i.d., for 1 wk (Neurobion®, Merck), alpha-tocopherol 7 mg/kg/wk, and selenium 0.025 mg/kg/wk i.m. (Selen-E-sol forte®, Richter Pharma GesmbH). The condition of the cubs did not deteriorate during the next few weeks, with cub 2 improving progressively, although clinical condition could not be correlated with the therapeutic regimen. Hind limb muscle atrophy became apparent in both cubs. Cub 1 did not improve, and it was euthanatized with an i.v. overdose of pentobarbital sodium at 6.5 mo of age. Cheetah 2 remains alive, and it has been ataxic without disease progression for the past 5 yr (Vitaud, pers. comm.).

The second litter of four cubs (cubs 3, 4, 5, and 6) was born in October 1996 from dam 2196 and sire 2819. At 6 wk of age, sneezing was noted in the dam and subsequently in the cubs. In December 1996, one cub suddenly collapsed and dragged its hind limbs while under video surveillance. Within 1 hr, two other cubs exhibited varying degrees of

ataxia and paresis. Two animals appeared markedly depressed and pyrexia (39–40.2°C). Blood samples and conjunctival and nasal swabs were taken. Treatment of all four animals was immediately started with depot ampicillin i.m. (Albipenal® Depot, Intervet GmbH, Vienna, A-1210 Austria; 100 mg, q.o.d.), acyclovir (Zovirax®, Glaxo-Wellcome GmbH, Vienna, A-1140 Austria; 100 mg, p.o., b.i.d.), parapox ovis paraimmunity inducer (Baypamun HK®, Bayer AG; 1 ml, s.q., s.i.d.), and vitamin B complex (Neurobion®, Merck). Despite a short period of improvement, three cubs (cubs 3, 4, and 6) developed mild spontaneous ataxia, preceded by sneezing and nasal-ocular discharge. Treatment with acyclovir (100 mg, p.o., s.i.d.) and Baypamun® (1 ml, s.c., s.i.d.) was immediately reinstated, and in cubs 5 and 6, in which ataxia persisted for more than 8 hr, prednisolone (Solu-Dacortin®, Merck; 150 mg, i.v.) was administered. Blood samples plus nasal and conjunctival swabs for virologic examination were repeatedly taken. In all animals, ataxia completely resolved after 56 hr. Cheetah 3 retains occasional mild ataxia, whereas cheetah 6 developed ataxia in September 2001 and remains ataxic.

The third litter of four cubs was born in September 1997 from dam 1921 and sire 1740. The dam crushed two cubs in the first 2 days. At 2 wk of age and every 2 wk thereafter for 6 wk the remaining two cubs (cubs 7 and 8) were treated prophylactically with serum neutralizing antibodies against FPV, FHV-1, and FCV (Serocat®, Rhone Merieux GmbH, Laupheim, D-88471 Germany) and were vaccinated subsequently three times with inactivated FPV, FHV-1, and FCV vaccine (Felimun®, Veterinaria AG). When the cubs were 6 mo of age they began sneezing. The dam began sneezing at the same time. Treatment of both cubs with serum neutralizing antibodies against FPV, FHV-1, FCV, and the paraimmunity inducer Baypamun® was started and continued for 5 days. As sneezing resolved, the cubs were removed from their enclosure and put on exhibit. Three hours later, both cubs were found ataxic and parietic. Treatment with prednisolone (Solu-Dacortin®, Merck; 150 mg, i.v., s.i.d.), acyclovir (200 mg, p.o., b.i.d.), and depot ampicillin was started. Cub 7 improved markedly within the next 24 hr, whereas cub 8 improved slowly over the period of several weeks and was finally able to walk around its enclosure. At 8 mo of age, cub 8 demonstrated another bout of paresis and became recumbent. Its condition did not markedly improve, so it was anesthetized, sampled, and evaluated using magnetic resonance imaging (MRI) before being euthanatized with an i.v. overdose of

pentobarbital sodium. Cub 7 experienced a further onset of ataxia and paresis at 11 mo of age and was euthanatized at 14 mo of age after sampling and MRI examinations.

Cub 9 provided by Fota Wildlife Park was born in a litter of five cubs. At 8 wk of age the animal demonstrated sneezing and mild ocular discharge. Two weeks later it became ataxic and was euthanatized at 11 wk of age in Ireland at the author's request.

Viral isolation and serologic, histopathologic, immunohistochemical, and in-situ polymerase chain reaction investigations

Four cubs (cubs 1, 7, 8, and 9) were necropsied, and samples of a large range of neural and extra-neural tissues were fixed in 5% neutral buffered formalin. Tissue blocks were embedded in paraffin, sectioned at 4 μ m, and stained with hematoxylin and eosin, Luxol fast blue (LFB), and Nissl's stains. In addition, immunohistochemistry using the avidin-biotin complex method was applied on brain and spinal cord tissues using antibodies against glial fibrillary acidic protein (GFAP), neurofilament, *Toxoplasma gondii*, *Neospora caninum*, *Encephalitozoon cuniculi*, rabies virus, Aujeszky's disease virus, Borna disease virus (BDV), FHV-1, FPV, and CDV. For electron microscopy, cubes of the spinal cord of cub 1 were fixed in glutaraldehyde and osmium tetroxide and embedded in Agar 100 resin® (Agar Scientific Ltd., Essex, CM2 48DA, U.K.). Ultrathin sections were stained with uranyl acetate and lead citrate according to standard techniques and examined with a Zeiss EM 900 transmission electron microscope. A polymerase chain reaction (PCR) method for FHV-1 was established. The target sequences for selection of oligonucleotide primers were derived from published sequences of the FHV-1 thymidine kinase (tk) gene¹⁷ and the FHV-1 glycoprotein I (gI) genes.²⁴ Primers were designed (Primer Designer®, version 3.0, Scientific and Educational Software, Durham, North Carolina 27722-2045, USA) as published previously and were predicted to amplify 306-bp (FHV-1-tk) and 765-bp (FHV-1-gI) products.²⁵ Polymerase chain reaction was performed using a commercially available PCR kit (PCR DIG Probe Synthesis Kit, Boehringer Mannheim, Mannheim, D-68298 Germany) with 2 μ l of extracted sample and primer concentration of 20 μ M. The PCR parameters used were identical to those used in the domestic cat.²⁵ A previously isolated FHV-1 strain, propagated on Crandell feline kidney (CrFK) cells, served as a positive control, and DNA extracted from mock-infected CrFK cells and water were used as negative con-

trols. The reaction products were separated on 1% agarose gel, stained with ethidium bromide, and examined under ultraviolet light.

In addition to standard serum biochemical analysis, serum samples from the ataxic cheetahs and control cheetahs were examined for copper content. Copper is released in a 4.7 buffer system from ceruloplasmin, to which copper is bound, and then quantitatively reduced to cuprous state. The Cu⁺ forms with specific complexant 3,5 DiBr-PAESA, a stable colored complex; the color intensity measured with a Cobas Mira Plus Analyzer (Roche, F. Hoffmann-La Roche Ltd., Basel, CH-4070 Switzerland) is proportional to the amount of copper in the sample. To aid in the interpretation of serum copper levels, serum samples from five domestic dogs and five domestic cats were chosen at random in the same laboratory and processed in a similar manner as the cheetah serum samples.

For evaluation of liver copper levels, formalin-fixed samples from 13 ataxic cheetahs (Zoo Salzburg, Herberstein Zoo,²⁷ Nürnberg Zoo,¹⁰ and La Palmyre Zoo, France), nine cheetahs without CNS disease, three domestic dogs, three domestic cats, and one wild lynx (*Lynx lynx*) were examined. Liver tissue (150–200 mg), 4 ml of 65% nitric acid, and 3 ml of 30% hydrogen peroxide were put in a Teflon pressure bomb and heated using microwaves for 30 min at 195°C and 15-bar pressure. The resulting clear fluid was diluted with 20 ml of deionized water and examined by inductively coupled plasma optical emission spectroscopy and atom absorption spectrometry. In addition, the formalin-fixed sample was examined to exclude this substance as a source of copper. In six cases, samples were examined twice to evaluate the robustness of the test system. Standard bovine liver reference material (1577b, National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8562, USA) served as an additional control.

RESULTS

The results of the neurologic examination in the individual cubs were very similar and, therefore, are presented in common (Table 2). Neurologic examination of the eight affected cubs indicated an upper motor neuron lesion, characterized by the spinal reflexes summarized in Table 2. Furthermore, this neurologic diagnosis was reinforced by the slow onset and whole-limb distribution of muscle atrophy.

Hematology and blood chemistry results from 43 blood samples did not reveal any deviation from normal.¹² Serum electrophoresis studies were also normal. Serologic examinations revealed elevated

Table 2. Summary of the spinal reflexes examined in eight ataxic cheetah cubs.^a

Muscle tone	Quadriceps	Cranial tibial	Extensor radialis	Triceps	Crossed extens	Withdrawal PI	Withdrawal T1	Perineal
+2/+3	+4	+3	+2/+3	+2/+3	elicited	+1/+2	+1	+1/+2

^a +1 = Decreased; +2 = normal; +3 = exaggerated; +4 = very exaggerated or clonus; PI = pelvic; T1 = thoracic limb.

to extremely elevated antibody titers against FHV-1, persisting during the entire study period or life of most of the cubs. Besides feline infectious peritonitis (FIP) titers (negative—1:100, with the exception of one sample from cub 8 with a titer of 1:1,600), no antibodies against feline leukemia virus, feline immunodeficiency virus (enzyme-linked immunosorbent assay), Borna virus disease (BVD), encephalomyocarditis virus, tick-borne encephalitis virus, mucosal disease complex virus (six samples), Teschen-Talfan disease virus (enterovirus; four samples), *Listeria monocytogenes*, and *Chlamydo-*

phila psittaci were found. Antibody titers (IgG) for *Toxoplasma gondii* were elevated in numerous samples, but the elevations were not considered significant. Further serologic results are summarized in Table 3.

Similarly, CSF appeared normal. Silver stain and immune agarose electrophoresis of five CSF and serum samples to detect oligoclonal banding were negative.

Serum copper values ranged from 6 to 22 $\mu\text{mol/L}$ in litter 1 and 3.9 to 26 $\mu\text{mol/L}$ in litter 2, revealing no significant difference between the ataxic

Table 3. Summary of serologic tests carried out in the eight cheetah cubs.^a

Cub ID	Date	FIP (INDIR.IF)	CDV (INDIR.IF)	FHV (INDIR.IF)	FHV (SN)	PLV (INDIR.IF)	FCV (INDIR.IF)
1	10 Sep 1996	100	20	640	NA	40	NA
1	26 Sep 1996	neg	neg	5,000	NA	NA	NA
1	16 Oct 1996	neg	neg	5,000	NA	NA	NA
2	10 Aug 1996	neg	10	1,280	NA	20	NA
2	26 Sep 1996	neg	neg	1,280	NA	NA	NA
2	16 Oct 1996	neg	20	1,280	NA	NA	NA
3	16 Dec 1996	neg	10	320	NA	neg	80
3	27 Dec 1996	10	neg	160	5,000	20	40
3	05 Feb 1997	neg	neg	10	NA	20	40
3	27 Feb 1997	10	neg	20,000	10,000	80	neg
4	16 Dec 1996	neg	neg	320	NA	neg	80
4	27 Dec 1996	100	neg	40	NA	neg	10
5	16 Dec 1996	10	neg	320	2,500	40	20
5	27 Dec 1996	neg	neg	320	5,000	10	10
5	05 Feb 1997	neg	neg	neg	NA	40	10
6	16 Dec 1996	10	neg	80	NA	neg	40
6	27 Dec 1996	10	neg	neg	NA	10	neg
6	05 Feb 1997	10	neg	10	NA	10	10
6	27 Feb 1997	100	neg	10,000	2,500	20	10
6	17 Mar 1997	10	neg	5,000	5,000	40	10
7	03 Jul 1998	100	10	neg	NA	20	80
7	20 Jul 1998	NA	10	neg	NA	neg	20
7	29 Sep 1998	NA	40	10	NA	20	20
7	24 Nov 1998	100	20	1,280	NA	neg	320
8	04 Apr 1998	100	neg	neg	NA	40	neg
8	20 Jul 1998	neg	neg	neg	NA	10	320
8	24 Sep 1998	1,600	80	>1,280	NA	320	160

^a NA = not applicable, test not carried out; neg = negative; INDIR.IF = indirect immunofluorescence; SN = serum neutralizing; CDV = canine distemper virus; FHV = feline herpesvirus; FCV = feline calicivirus; PLV = feline panleucopenia virus; FIP = feline infectious peritonitis.

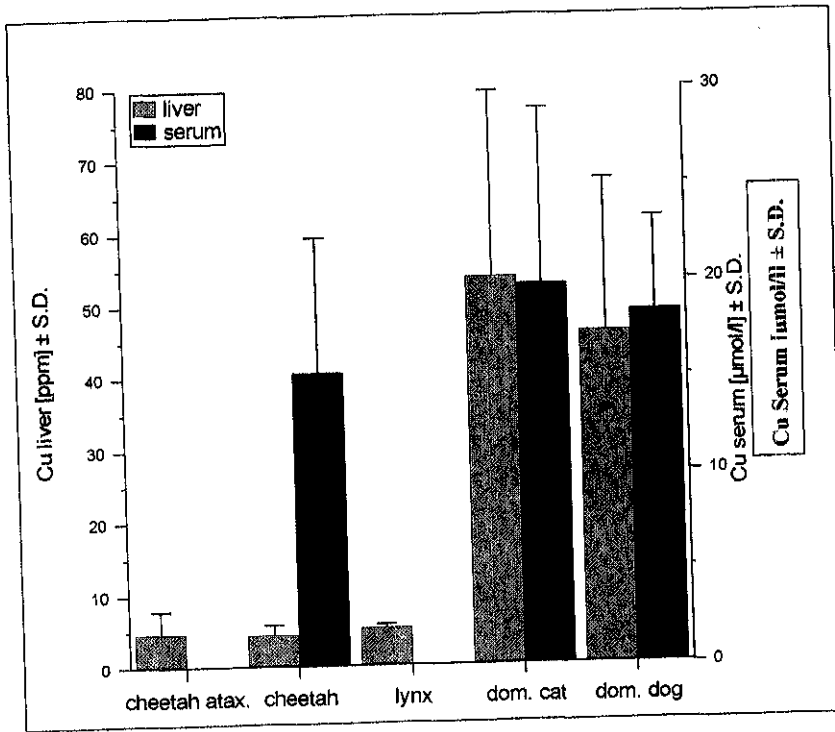


Figure 1. Comparative serum and liver copper values in control cheetahs, ataxic cheetahs, wild lynx, and domestic dog and cat.

cheetahs ($15.2 \pm 7 \mu\text{mol/L}$) and the domestic controls (dogs: $18.3 \pm 5 \mu\text{mol/L}$; cats: $19.7 \pm 9 \mu\text{mol/L}$; analysis of variance [ANOVA]: $P < 0.01$).

There was no significant difference in liver copper levels between ataxic cheetahs ($4.6 \pm 3 \text{ ppm}$) and cheetahs without CNS disease ($4.3 \pm 1.5 \text{ ppm}$; ANOVA: $P < 0.01$) or between cheetahs ($5.2 \pm 0.6 \text{ ppm}$) and a wild lynx. Liver copper values for domestic cats ($53.6 \pm 25.8 \text{ ppm}$) and dogs ($45.9 \pm 21.3 \text{ ppm}$) were not significantly different, but they were significantly higher than values for the cheetah and the single lynx (ANOVA: $P < 0.01$; Fig. 1).

Nasal and ocular swabs, inoculated on CrFK cell cultures, showed a marked cytolitic, plaque-forming cytopathic effect (CPE) within 1–4 days, considered characteristic for herpesvirus infections; furthermore, the CPE was inhibited by prior incubation with FHV-1 antiserum, and electron microscopy yielded herpesvirus particles within the supernatant. Polymerase chain reaction with FHV-tk and FHV-gI primers resulted in amplification products of the expected size of 306 and 765 bp, respectively. Polymerase chain reaction products were sequenced by using an ABI Prism 310 genetic analyzer (Perkin-Elmer, Norwalk, Connecticut 06835, USA) and compared with already published

sequences, yielding 99% homology on an amino acid level to FHV-1. A cross-neutralization test using FHV-1 isolates from a domestic cat and a cheetah as well as the corresponding antisera yielded no significant titer differences.

Neither radiographic and myelographic examinations, carried out in cheetahs 1–6, nor T1-weighted MRI in three ataxic cheetah cubs revealed any deviation from the norm. A paramagnetic contrast medium, gadolinium diethylenetriamine pentaacetic acid (0.1 mmol/kg), was also used. No lesions or deviations from the healthy control animal were determined.

At necropsy, ascarids were present in the small intestine of cubs 1 and 8. Furthermore, the hind limbs of cub 8 were severely atrophied, whereas the other cheetahs showed no gross lesions. Extraneural histology revealed discrete purulent pneumonia in cub 1 and discrete nephrocalcinosis in cub 7. Cheetah 8 revealed severe purulent tracheitis, discrete nephrocalcinosis, demodicosis, and mild enteritis due to ascaridosis as well as severe osteoporosis of both humeri. Histology of the CNS in all cubs revealed similar lesions in the spinal cord that differed only in intensity. In general, myelinated fibers in the lateral and ventral funiculi were affected, whereas the dorsal columns and proper

fascicles were largely spared with the exception of cub 7. The bilaterally symmetric lesions were most prominent within the thoracolumbar segments and consisted of ballooning of myelin sheaths, which contained mostly preserved axons and occasionally swollen or degenerate macrophages (myelinophages). In all but one cub (cub 9, Fota), florid astrogliosis in the lateral funiculi was associated with the myelin sheath vacuolation. No lesions were observed in the spinal cord gray matter or in the ventral and dorsal nerve roots.

Cub 9 from Fota Wild Animal Park, euthanatized 1 wk after the onset of ataxia, had the least pronounced lesions. Only mild ballooning of several myelin sheaths—containing intact axons and only rarely swollen axons or myelinophages—was obvious in the ascending pathways of the thoracic spinal cord and, to a lesser degree, in the ascending and descending pathways of the lumbar regions—excluding the dorsal funiculi (fasciculus gracilis and fasciculus cuneatus). The cervical spinal cord showed only discrete inflammation of the leptomeninges. Alterations of the brain were confined to the cerebellum. Besides glial shrubbery within the molecular layer, mild loss of Purkinje cells and some ectopic Purkinje cells was noticed.

Cub 1, euthanatized 2.5 mo after the onset of ataxia, had more pronounced lesions. Mild to moderate ballooning of numerous myelin sheaths in the lateral and ventral funiculi, confined to the ascending and descending tracts, was observed. Ballooning of some myelin sheaths in the dorsal funiculi was only evident in the lumbar spinal cord. Most vacuolated myelin sheaths contained intact axons, and only occasional myelinophages were found. In addition, the ascending tracts of the lateral funiculi contained massive fibrillary astrogliosis, confirmed by LFB staining (Fig. 2b) and GFAP (Fig. 2c) immunohistochemistry. Besides mild perivascular lymphocytic cuffing in pons and the medulla oblongata, the brain revealed no abnormalities.

Similar lesions were present in the spinal cord of cub 8, euthanatized 4.5 mo after the onset of ataxia, except that the number of distended myelin sheaths, the size of the vacuoles, and the number of myelinophages were slightly increased when compared with cub 1. In addition, focal microglia was found unilaterally in the lateral funiculus of the lumbar segment. In the brain, degenerative lesions were obvious in cerebellum and pons, where several distended myelin sheaths containing myelinophages were found in some foliae and the caudal peduncles of the cerebellum. Focal perivascular lymphocytic cuffings were found in the cerebral cortex and the leptomeninges.

Cub 7 (Fig. 2a), euthanatized 7.5 mo after the onset of ataxia, had the most severe lesions. All lateral and ventral ascending and descending tracts throughout the spinal cord were involved, as were the dorsal funiculi and the proper fascicles, although to a lesser degree. In the ventral and lateral funiculi, tremendous vacuolation of the white matter tracts was observed because of enormously distended myelin sheaths, many of them containing myelinophages. Furthermore, focal mild fibrillary and gemistocytic astrogliosis was found within the white matter. In the brain, ballooning of myelin sheaths was observed in the central white matter, the caudal peduncles of the cerebellum, and in some white matter tracts of the pons, accompanied by mild astrogliosis.

Using Nissl's stain, no neuronal abnormalities were found throughout the CNS. In addition, neither immunohistochemistry using antibodies against the neurofilament (Fig. 2d) nor electron microscopy indicated primary axonal degeneration. Electron microscopy of the spinal cord in cub 1 revealed cleavages and discontinuous separation of myelin lamellae surrounding intact axons as well as an increased number of unmyelinated axons and numerous astrocytic fibres (Fig. 3). Peripheral nerves from two cubs were studied: the sciatic nerve of cub 8 revealed focal discrete Wallerian degeneration, whereas no abnormalities were detectable in cub 7.

Indications of toxoplasmosis, neosporosis, encephalitozoonosis, rabies, Aujeszky's disease, and Borna disease could not be found within any brain and spinal cord using immunohistochemistry and no evidence of infection with FHV-1, FCV, FPV, and CDV was obtained.

Polymerase chain reaction, as described above, using FHV-tk primers and DNA templates derived from formalin-fixed and paraffin-embedded brain and spinal cord tissue from cub 1 proved negative, as did the samples inoculated on CrFK cell cultures, where no CPE could be observed.

DISCUSSION

The clinical onset of this disease appears to be extremely rapid.⁹ Cubs of all three litters became ataxic within 3 hr—sometimes spontaneously. Stressful events such as hand capture or placement in a new enclosure can apparently precipitate the disease.³¹ The initial case at the Nürnberg Zoo occurred after transport (Gaukler, pers. comm.).

In the cases of ataxia reported in Nürnberg, Fota, as well as in our cases in Salzburg, nasal and ocular discharges preceded ataxia, as did ulcerative corneal disease in several instances. In our cases, atax-

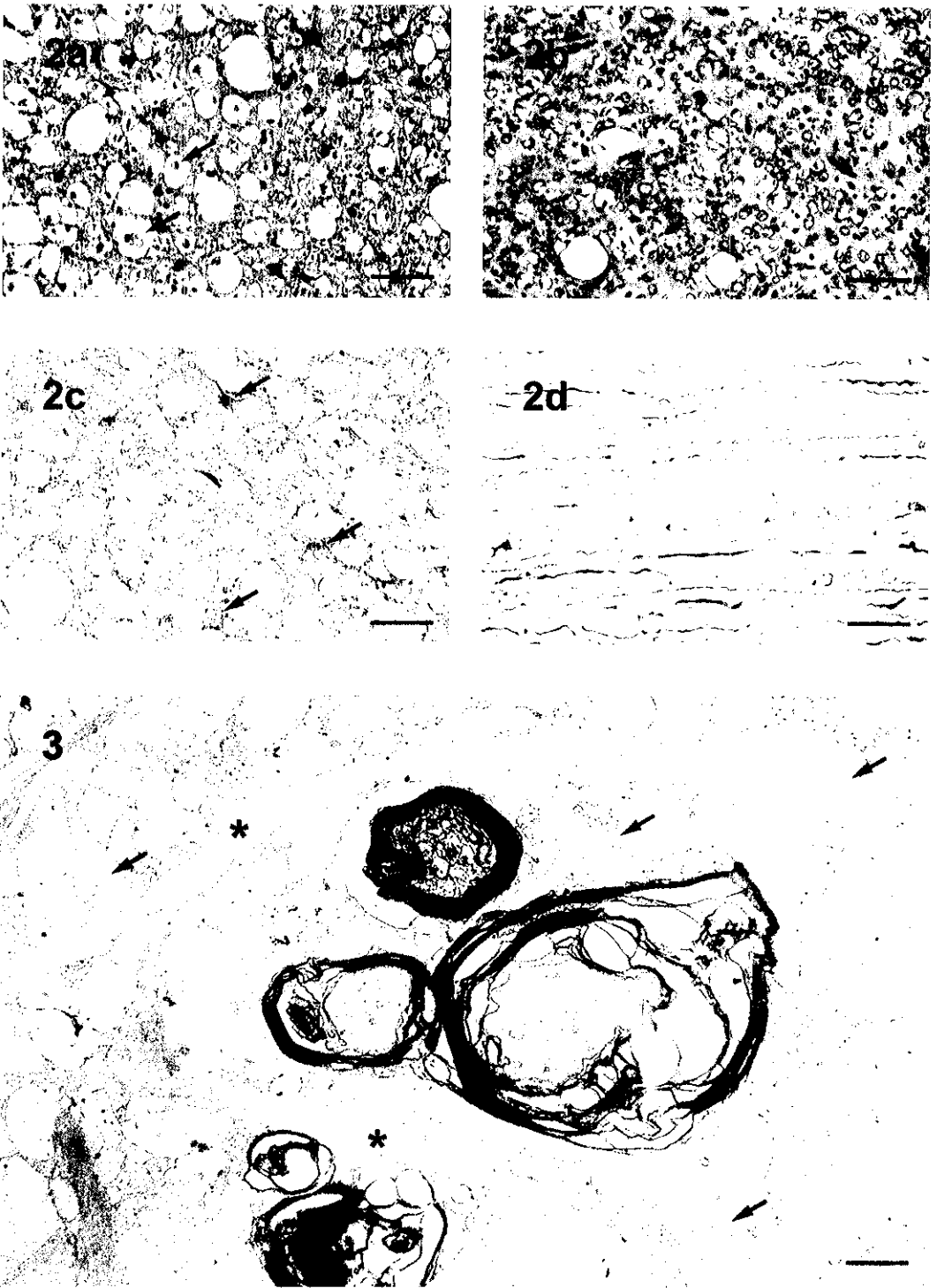


Figure 2. Histologic sections of spinal cord white matter tracts. **a.** Ballooning of myelin sheaths containing intact axons or myelinophages (arrows) and reactive fibrillary astrogliosis; H&E, bar = 50 μ m. **b.** Severe astrogliosis and several distended myelin sheaths; LFB (luxol fast blue stain), bar = 50 μ m. **c.** Tremendous vacuolation of white matter tract with numerous astrocytes (arrows); GFAP (glial fibrillary acidic protein stain), bar = 50 μ m. **d.** Longitudinal section showing intact axons surrounded by distended myelin sheaths; NF (neurofilament immuno staining), bar = 100 μ m.

Figure 3. Electron microscopy of spinal cord ascending tract, showing some intact myelinated axons with splicing of myelin sheaths as well as numerous unmyelinated axons (arrows) and astrocytic fibres (asterisks); bar = 3 μ m.

ia was recognized 6–15 days after the nasal and ocular disease. In our three Salzburg litters, the causative agent was FHV-1. Serologic screening confirmed the presence of high levels of antibodies, up to titers of 1:10,000, against FHV-1 in all cubs. The one oropharyngeal swab examined for FHV-1 during the Fota episode was negative, as was one ocular swab examined for *Chlamydophila* by PCR.² No causative agents were apparently identified in the Nürnberg cases. Although all serologic examinations for FHV-1 in the Fota cases were reportedly negative,^{2,20} serum samples from a litter of ataxic cubs from Fota subsequently showed extremely high FHV-1 titers (>1:1,280) (Walzer, unpubl. data). This disparity needs further investigation. Furthermore, the failure of the one hand-raised Nürnberg cub to develop clinical disease and its lack of CNS lesions at 2 yr of age and the development of both in its dam-raised littermates (see Introduction) are interesting. We believe that FHV-1 may play an important role in cheetah cub ataxia. Additional RNA-PCR methodology may be necessary, along with a shorter interval between disease onset and necropsy, to identify FHV-1 within the spinal cord in future cases. Additional study of FHV-1 as a possible causal factor in the cheetah cub ataxia is needed.

Bilateral symmetric myelin degeneration of the spinal cord was observed in all necropsied Salzburg cheetah cubs. Lesions were distributed throughout the entire length of the spinal cord in the three cheetahs in which clinical signs persisted for 2.5–7 mo. Such segmental distribution of lesions has been described previously,^{23,31} although lesions in adult cheetahs were most prominent in the thoracolumbar region and decreased in severity cranially and caudally.²⁷

Severity of the lesions appears to be related to the duration of the clinical signs.²⁰ In cub 9, clinical signs persisted for only 7 days, and the histologic lesions were very discrete but more or less identical. Furthermore, cub 9, with only 1 wk between ataxia onset and necropsy, had not only the mildest lesions but also mild inflammation of the leptomeninges, thereby suggesting that the degenerative myelin lesions follow infectious inflammatory insult.

Ballooning of myelin sheaths surrounding mostly preserved axons characterized the myelopathy found in the four ataxic cheetah cubs necropsied. None of the spheroids that usually characterize acute axonal degeneration were found, and electron microscopy confirmed the intact nature of the axons. Ultrastructural results should be interpreted cautiously, however, because artifactual splicing of

myelin sheaths is possible.⁵ However, the intact unmyelinated axons and the lack of features typical of early axonal degeneration (proliferation of tubular and vesicular elements of the agranular endoplasmic reticulum or swelling of axonal mitochondria) corroborate the suspicion that the axons were not affected primarily.⁵ Demyelination has been considered secondary to axonal degeneration, however.²⁰ Although myelin loss in the affected cheetahs exceeded axonal degeneration, their disease should be distinguished from such primary demyelinating diseases such as multiple sclerosis.^{1,22} The initial lack of perivenous initial lesions is the most important feature that distinguishes the cheetah disease from demyelinating disease, according to human medical criteria.²² Additional histopathologic studies are needed to characterize the type of myelin degeneration in this cheetah disease, however.

Similar disorders, in which myelin loss exceeds axonal loss, include subacute combined degeneration due to vitamin B12 deficiency and myelopathies of unknown pathogenesis in cats and several breeds of dogs.^{11,26} The distribution pattern of lesions and funicular involvement differ in these conditions and in the cheetah disease, and peripheral nerves are not affected in the ataxic cheetahs. In the Afghan Hound,⁸ Poodle,¹⁵ and Siberian Husky,⁴ hereditary factors may affect the development of similar lesions, as they may in the cheetah, which appears to be depauperate in genetic variation.^{18,19} Whereas a genetic component to disease predisposition and response cannot be ruled out in the animals affected in Salzburg, Fota, and Nürnberg, the patterns of incidence do not, however, indicate a major genetic basis for this disease.⁶

With the exception of two very discrete focal areas of perivascular cuffing in the brainstem of cub 1, no inflammatory lesions were found in the brains of our affected Salzburg cheetahs. Perivascular cuffing in the brainstem was described in two of the 10 cheetahs from Fota and in all four cases from Nürnberg.^{10,20} Although swelling of the Purkinje cell cytoplasm, shrinkage and displacement of nuclei, and cell death were described previously in eight of the 10 cheetahs in Ireland, the only cub to reveal similar lesions was cub 9 from Fota.²⁰ Nevertheless, mild demyelination of white matter in the rhombencephalon was noted in two cubs. It is difficult to reconcile these neuropathologic lesions to a single etiologic entity.²⁰ However, although the descriptions of the types of myelin loss in the other Fota cases and in the European cases other than our own differ, we believe that the cases are caused by the same entity.

The significance of copper level in serum and liver with regard to this disease process is uncertain. Serum copper values varied widely ($15.2 \pm 7 \mu\text{mol/L}$), with no significant difference between levels in the ataxic cheetahs and in the domestic control dogs ($18.3 \pm 5 \mu\text{mol/L}$) and cats ($19.7 \pm 9 \mu\text{mol/L}$) (ANOVA: $P < 0.01$). Copper deficiency was initially suspected in the Nürnberg cases based on numerous serum samples. Although potentially low serum copper levels ($2.4\text{--}7.0 \mu\text{mol/L}$) were found, increased levels of $15.7\text{--}70 \mu\text{mol/L}$ after p.o. and i.v. copper sulphate application were not accompanied by improved clinical signs. No correlation between the incidence of ataxia and serum copper concentration was reported in the Fota cases,⁹ and no association was noted by us. We found no difference in liver copper levels between ataxic cheetahs and cheetahs that had died of other causes; the investigators in the Netherlands³¹ and in Nürnberg did not find any as well (Haenichen, pers. comm.).

The clinical signs and the distribution of the spinal cord lesions in the spinal cord resembled those seen with copper deficiency, also called enzootic ataxia, in sheep and goats,^{3,30} although there were significant histologic differences. Cerebellar cortical degeneration and hypoplasia have been reported in lambs and goat kids affected by copper deficiency.³⁰ Furthermore, in enzootic ataxia, cavitation of the cerebral white matter is regularly reported.^{3,23} In addition, we did not see any peripheral nerve lesions in the cheetah cubs, as reported in goat kids with clinical hypotonia and hyporeflexia associated with copper deficiency.³⁰ Although demyelination in conjunction with copper deficiency in lambs and goat kids is usually considered to be secondary, or Wallerian, it may be primary and accompanied secondarily by axonal loss.^{3,13,23,30} Clearly, myelin loss such as that which was visible in the cheetah cubs is difficult to classify. We believe that although copper levels did not directly influence the onset or clinical progression of disease in the cheetahs, they may have been part of a multifactorial etiology, such as has been proposed for a swaybacklike disease in red deer (*Cervus elaphus*).²⁹ Normal copper liver levels clearly need additional study.

Although ataxia similar to what we saw has not been reported in cheetah cubs in North America, another white matter disease, leukoencephalopathy that is characterized by reactive astrocytosis in the cerebral white matter with symmetric degeneration and necrosis, has been seen in these animals.¹⁶ Together with other cheetah diseases, these two white matter diseases have negatively affected the global

captive population of this species, and they may compromise sustainable breeding efforts.

A number of human and animal disorders that feature white matter demyelination have been described with various possible etiologies, including viral, genetic, autoimmune, nutritional–metabolic, toxic, and physical factors. The causes of several demyelinating disorders of dogs and cats remain unclear.^{21,26} Morphologic changes seen with these disorders resemble those seen in the ataxic cheetah cubs. Molecular biological techniques will be necessary to identify mechanisms that may interfere with the production or structural integrity (or both) of myelin.

Furthermore, analytic and epidemiologic investigations of the environmental status of captive cheetahs, e.g., nutrition and standard medication, may provide a clue to the pathogenesis of this disease entity.

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