CHAPTER 4 : NEURONOPATHIES AND AXONOPATHIES

Chapter 4.1

CLASSIFICATION

This group of conditions is characterised by selective non-inflammatory, neuronal degeneration involving either neurons in their entirety (neuronopathies) or axons in a more restricted manner (axonopathies). Division between these two categories may be difficult purely on morphological grounds as the axon is a dependant part of the neuron [1]. Jubb & Huxtable subclassify these conditions into three categories according to the distribution of the lesions in the central and peripheral nervous system:

- central neuronopathies and axonopathies (e.g. organomercurial poisoning, congenital axonopathy in Holstein-Friesian calves and the axonal dystrophies),
- central and peripheral neuronopathies and axonopathies (e.g. organophosphate poisoning, neonatal copper deficiency and neurodegeneration of Horned Hereford calves), and
- peripheral axonopathies (uncommon and mostly reported in the horse e.g. equine laryngeal hemiplegia and equine stringhalt)

Reference
ACUTE ASPERGILLUS CLAVATUS POISONING IN CATTLE: LIGHT MICROSCOPICAL AND ULTRASTRUCTURAL LESIONS IN THE SPINAL CORD

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Abstract

Lesions in three adult cattle with acute *Aspergillus clavatus* intoxication were studied. Animals were fed a ration that included grain sorghum meal, a by-product from a malt-producing factory, from which *A. clavatus* was cultured. Affected cattle were killed 2-7 days after displaying clinical signs consistent with intoxication. Light microscopical findings were chromatolysis and vacuolation of neurons particularly in the ventral horns of the spinal cord and in selective brain stem nuclei and Wallerian degeneration in the dorsal, lateral and ventral funiculi of the spinal cord, ventral nerve roots, dorsal root ganglia and, to a lesser extent, the peripheral nerves. Nuclei in the brain stem exhibiting chromatolytic changes included the red, ambiguus, lateral and medial vestibular nuclei, spinal tract nucleus of the trigeminal nerve and the dorsal nucleus of the vagus nerve. Ultrastructurally, progressive loss of granular endoplasmic reticulum and free ribosomes occurred and the cytoplasm contained vacuoles possibly originating from Golgi complexes. Changes in mitochondria were non-specific. The nature and distribution of the neuronal chromatolytic changes and nerve fibre degeneration in *A. clavatus* neuromycotoxicosis suggest a toxic neuronopathy/axonopathy with primary injury to the neuron manifested by chromatolysis and with secondary or concurrent axonal degeneration.

Key words: *Aspergillus clavatus*, motor neurons, chromatolysis, Wallerian degeneration, toxic neuronopathy/axonopathy, cattle, grain sorghum meal
**Introduction**

The saprophytic fungus *Aspergillus clavatus* may contaminate cereals and cereal by-products and intoxication of cattle may occur following ingestion of infected feed. Outbreaks of poisoning with the fungus have been reported in cattle that ingested sprouted wheat [12], malt sprouts [11], malt culms [7], sprouted barley grains [16,17], sprouted maize [13] and sorghum beer residues [14]. Sorghum beer is a traditional drink in southern Africa and its residues are sometimes used as a feed supplement for cattle [12].

There are no consistent gross lesions in *A. clavatus* poisoning in ruminants, although degeneration and necrosis of muscles in the hindquarters may occur in cattle [14]. Histological lesions in most cases comprised neuronal chromatolysis in the ventral horns of the spinal cord, spinal ganglia and selected brain nuclei [13,14]. Wallerian degeneration was described in the spinal cord in sheep fed contaminated malt culm [7]. A chronic neurological syndrome in 76/100 cattle that received sorghum beer residues was reported in South Africa [21]. *Aspergillus clavatus* was implicated as the most likely cause. Light microscopy revealed neuronal chromatolysis and widespread Wallerian degeneration in the spinal cord, spinal nerve roots and peripheral nerves. A primary axonopathy with secondary myelin loss was proposed [21].

An outbreak of neuromycotoxicosis attributed to *A. clavatus* provided an opportunity to study the acute lesions with special reference to ultrastructural changes in the spinal cord in detail.

**History of outbreak**

A farmer in the Nylstroom district of the Northern Province in South Africa fed a ration consisting of concentrate, chicken litter, roughage and grain sorghum meal to a herd of 150 South Devon cattle. The meal was a by-product from a malt-producing factory. Within a period of 6 days and due to no apparent reason, twenty-three cattle of varying ages (bulls, cows and heifers) developed nervous signs such as muscle tremors, hypersensitivity, paresis of the hind legs and knuckling over of the fetlocks. In some animals these clinical signs became more severe with exercise. Others manifested opisthotonus or kicking movements while recumbent, and paralysis. One cow died within one day of showing clinical signs. *Aspergillus clavatus* was isolated from the feed.

**Material and methods**

The pathology in three affected cattle was studied. Animals comprised a heifer (Bovine No. 1) and a bull (Bovine No. 2) that were killed 2-4 days after clinical signs were noted by the owner and a 7-months-old heifer calf (Bovine No. 3) that was killed seven days following the onset of clinical signs. Animals were
Chapter 4.2

euthanised by an intravenous overdose of pentobarbiturate. Necropsies were performed and a range of specimens including the entire brain and spinal cord, peripheral nerves (sciatic and femoral nerves) were collected and fixed in 10% neutral buffered formalin. The dura mater of the spinal cord was incised to allow rapid penetration of fixatives. Paraffin sections of these tissues were prepared and stained with haematoxylin and eosin (HE) according to standard procedures for light microscopy. Selected sections of medulla oblongata and spinal cord (cervical, thoracic and lumbosacral portions) were stained with luxol fast blue/periodic acid-Schiff/haematoxylin (LFB/PAS/H) and luxol fast blue/Holmes (LFB/H).

For transmission electron microscopy, specimens of the ventral horn grey matter of the lumbosacral portion of the spinal cord were collected from the three cattle. Tissues were fixed by immersion in 2.5% gluteraldehyde in 0.1 M sodium cacodylate buffer within 20 min of euthanasia. Specimens were post-fixed in osmium tetroxide. Semithin sections were stained with toluidine blue for tissue orientation and ultrathin sections were stained with uranyl acetate and lead citrate and studied with a transmission electron microscope.

Results

Gross lesions

In one case (Bovine No. 2) paleness of some muscle groups of the hindquarter especially the vastus muscles, were noted.

Microscopic lesions

In all three animals, lesions were of similar nature and distribution and were most pronounced in the spinal cord, comprising degeneration of neurons and Wallerian degeneration with a bilateral distribution. Affected neurons were seen throughout the entire spinal cord, including the cervical and lumbar intumescenses, but were most common in the lumbar part. Individual and groups of neurons in the ventral horns often displayed central chromatolysis, were swollen, had rounded contours and exhibited central loss of Nissl granules and cytoplasmic pallor (Fig. 1). The central cytoplasm sometimes appeared granular or finely vacuolated leaving a thin rim of eosinophilic cytoplasm beneath the plasmalemma. In several affected neurons their nuclei were displaced peripherally and the chromatic dispersed. Karyorrhexis was occasionally noticed. A few nerve cell bodies stained strongly eosinophilic in which the nuclei were absent. The neuropil of the spinal cord gray matter revealed swollen axons, focal mild gliosis and satellitosis. Chromatolytic neurons and focal gliosis were also demonstrated in the dorsal root ganglia in the cervical and lumbar portions (Fig. 2).

Scattered individual myelinated fibres in the dorsal, lateral and ventral funiculi of the spinal cord showed Wallerian degeneration, with no selective tract involvement. In longitudinal sections of the cord there was fragmentation of indi-
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Fig. 1: Spinal cord. Chromatolysis and eccentric nuclei in ventral horn neurons. HE.

Fig. 2: Dorsal root ganglion. Note chromatolysis of neurons and gliosis. HE

Fig. 3: Ventral nerve roots, lumbar spinal cord. There is myelin swelling of scattered nerve fibres. HE.

Fig. 4: Peripheral nerve (sciatic nerve). Digestion chamber (arrow) containing macrophages indicating Wallerian degeneration. HE

Fig. 5: Brain stem. Note prominent central chromatolysis and vacuolation of neurons. HE.

Fig. 6: Brain stem. Spongy change in the dorsal nucleus of the vagus nerve. HE.
individual axons and ballooning of myelin sheaths forming digestion chambers occasionally occupied by macrophages with pyknotic nuclei. These macrophages contained small amounts of luxol fast blue-positive and PAS-positive material.

The ventral and, to a lesser extent, the dorsal nerve roots revealed a small number of swollen and degenerated myelin sheaths and focal gliosis (Fig. 3). Chains of digestion chambers containing myelin debris, degenerated axons and macrophages were seen in the peripheral nerves. These changes were more prominent in proximal than in distal portions of the nerves (Fig. 4).

Swollen, markedly chromatolytic neurons with cytoplasmic vacuolation were demonstrated in selected nuclei of the brain stem and medulla oblongata including the red nucleus, ambiguous nucleus, lateral and medial vestibular nuclei, spinal tract nucleus of the trigeminal nerve and the dorsal nucleus of the vagus nerve (Fig. 5). Multifocal myelin swelling and Wallerian degeneration in adjacent white matter tracts was occasionally noted (Fig. 6).

Histology of affected muscles of Bovine Nos. 2 and 3 confirmed degeneration, necrosis and fragmentation of individual muscle fibres.

Ultrastructural lesions

In neurons with chromatolysis, the central cytoplasm exhibited loss of granular endoplasmic reticulum (ER) and free ribosomes and contained increased numbers of mitochondria and numerous short tubular profiles (Figs. 7, 8). The peripheral cytoplasm often had a granular appearance and was denuded of organelles (Figs. 7, 8) while in other neurons the granular ER and clusters of ribosomes were preserved at the periphery of the cell bodies or around an eccentrically placed nucleus. Some cells contained tubules, dense bodies and densely packed membranes and vacuoles possibly originating from Golgi complexes (Figs. 9, 10). Larger membrane-bound cytoplasmic vacuoles which were either empty or contained fine membranous material were present in a small number of neurons. A few of these larger vacuoles were coated by electron-dense amorphous material (Fig. 11). There was vacuolation and loss of cristae in mitochondria but they were not significantly enlarged. Normal concentrations of neurofilaments surrounding organelles were noted in affected neurons (Fig. 9). In nuclei that were eccentrically displaced, their outer contours were irregular and some nuclei were pyknotic. Axosomatic synapses were preserved.

In contrast to neurons showing chromatolysis, the cytoplasm of a few degenerated neurons was markedly condensed and was devoid of nuclei (Fig. 12). The cytoplasm was dark-staining and contained slightly swollen mitochondria, clumped ribosomes and vacuolated Golgi complexes.

Swollen axons in regions of affected nerve cell bodies contained axoplasmic debris and degenerated organelles consistent with Wallerian degeneration.
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Fig. 7: Transmission electron micrograph. Spinal cord. The central cytoplasm is pale, depleted of Nissl substance and contains increased numbers of mitochondria. The peripheral cytoplasm is granular and the nucleus eccentric. Bar = 5 µm.

Fig. 8: Transmission electron micrograph. Spinal cord. Higher magnification to illustrate paleness of central cytoplasm and loss of organelles from the peripheral cytoplasm. Bar = 3 µm.

Fig. 9: Transmission electron micrograph. Spinal cord. The cytoplasm of a chromatolytic neuron contains vacuoles, mitochondria, dense bodies and bundles of neurofilaments (arrow). Bar = 3 µm.

Fig. 10: Transmission electron micrograph. Spinal cord. In this neuron, prominent vacuolation of the cytoplasm is seen. Bar = 2.5 µm.

Fig. 11: Transmission electron micrograph. Spinal cord. The central cytoplasm reveals the presence of clusters of free ribosomes and several large vacuoles sometimes coated by electron-dense material (arrow). Bar = 5 µm.
Discussion

In this study of acute *A. clavatus* intoxication of cattle, consistent light microscopic findings were chromatolysis of neurons in the ventral horns of the spinal cord and in selective brain stem nuclei. In addition, Wallerian degeneration in the dorsal, lateral and ventral funiculi of the cord, ventral nerve roots, dorsal root ganglia and peripheral nerves was noted.

Neuronal chromatolysis has been documented previously as the predominant change in *A. clavatus* poisoning in cattle fed mouldy sorghum beer residue (‘maroek’) [14] and sprouting maize [13] in South Africa. Clinical signs and lesions displayed by cattle and sheep in this mycotoxicosis may vary between outbreaks, which may be related to dose levels or different toxic metabolites that are produced by the fungus under different circumstances [12]. *Aspergillus clavatus* is known to produce several mycotoxins such as patulin, cytochalasin E, escladiol and two tremorgenic metabolites, namely, tryptoquivalone and tryptoquivaline [3,4,8,19]. Sheep that had been fed infected malt culms developed neuropathology closely resembling the lesions in this study [7], while Shlosberg and others reported central chromatolysis of neurons restricted to the hippocampus and medulla in sheep that were fed a ration that included sprouted barley grains, a waste product from a malt extract [16,17]. In this outbreak the mortality was exceptionally high with 96% of 168 adult sheep dying over a period five months despite discontinuation of feeding the infected ration four days after the onset of clinical signs in the flock. Neuropathological changes were not reported in the cases from France and Bulgaria, while cerebrocortical haemorrhage and malacia but no neuronal lesions have been documented in cattle from China [7].

Chromatolysis of neurons in the spinal cord in the three acutely intoxicated cattle in our study had a bilateral distribution and occurred concurrently with scattered Wallerian degeneration in the spinal cord white matter, dorsal root ganglia and peripheral nerves. We propose a toxic neuronopathy/axonopathy with primary injury to the neuron manifested by chromatolysis, and with secondary or concurrent axonal degeneration. Widespread degeneration and loss of axons and neuronal chromatolysis in the spinal cord was previously reported in cattle exposed to sorghum beer residues [21]. The condition most likely represented chronic *A. clavatus* poisoning and a primary axonopathy was proposed [21]. The nervous lesions of *A. clavatus* poisoning resemble those described in swayback and enzootic ataxia [1,24] and in ‘valsiekte’ (literally translated as falling disease), a nervous disorder of Dorper or Dorper cross-bred lambs in South Africa of presumably toxic aetiology [22]. In copper deficiency it has been proposed that primary injury to the cell body of neurons impedes the ability of these nerve cells to sustain their axons [6]. The pathogenesis of chromatolysis in copper deficiency
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may relate to depletion of the mitochondrial respiratory chain enzyme cytochrome oxidase or other copper-containing enzymes, or may reflect injury of the nervous system by oxygen radicals [18]. Wallerian degeneration in peripheral nerves in our study was more prominent in proximal than in distal portions of the nerve making a 'dying-back' neuropathy unlikely. Neuronal chromatolysis is also described in motor neuron disease and dysautonomia in a variety of domestic animals [9,18].

Ultrastructurally, changes in the chromatolytic neurons closely resembled those reported in copper deficiency in lambs [5] and in the axonal reaction, a retrograde response of neuronal cell bodies to axonal injury [2,15,20]. There are, however, some differences. In enzootic ataxia, mitochondria in affected neurons showed profound lesions characterised by swelling, agglutination of the cristae and the formation of unusually dense granular matrix [5]. These changes were not demonstrated in A. clavatus intoxication. In the axonal reaction, an increase in the number of mitochondria or a redistribution and hypertrophy of mitochondria have been reported [2,15,20]. We could not demonstrate synaptic stripping of affected neurons by glial cells as is frequently demonstrated in the axonal reaction [2], but this may be due to the acute nature of the lesions in our cases. Axo-somatic synapses in chromatolytic neurons were also preserved in swayback in lambs [5].

Chromatolysis of neurons in the brain stem nuclei such as the red and lateral vestibular nuclei might be explained by a direct toxic effect on the perikaryon or retrograde axonal reaction. Affected brain stem nuclei also included the spinal tract nucleus of the trigeminal nerve and the dorsal nucleus of the vagus nerve. The distribution of these lesions is important from a diagnostic viewpoint since spongiform changes in the neuropil and neuronal vacuolation of these nuclei occur commonly in the brains of cattle with bovine spongiform encephalopathy (BSE) [23]. Lesions due to A. clavatus poisoning in the brain stem should also be differentiated from idiopathic brain stem neuronal chromatolysis and hippocampal sclerosis that was reported in clinically suspect cases of BSE in the United Kingdom [10]. In this condition, neuronal chromatolysis, microvacuolation and necrosis in several brain stem nuclei especially the vestibular nuclear complex, the red nucleus and the dorsal vagal nucleus were noted.

Acknowledgements

The authors are grateful to Mr J Putterill for technical support.

References


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24. Wouda W, Borst GHA, Gruys E (1986) Delayed swayback in goat kids, a study of 23 cases. Vet Q 8:45-56
SPINAL CORD DEGENERATION IN ADULT DAIRY COWS ASSOCIATED WITH THE FEEDING OF SORGHUM BEER RESIDUES

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Journal of the South African Veterinary Association 65:184-188 (1994) (reprinted with permission)
Chapter 4.3

Abstract
A neurological syndrome in a herd of Friesland cows (n=100) on a diet including sorghum beer residues is described. Over a period of 8 months, 76 cows developed weakness in the hindquarters, progressively worsening ataxia and, eventual, paralysis and permanent recumbency. The course of the disease varied from 2-8 weeks. The lesions were significant in the cows necropsied and included extensive, bilaterally symmetrical dilation of myelin sheaths, axonal swelling and loss with Wallerian degeneration, and depletion of myelin in both ascending and descending tract at all levels of the spinal cord. Focal neuronal degeneration in the spinal cord grey matter and dorsal root ganglia, and focal loss of axons and ovoid formation in the spinal nerve roots and ischiatic nerves were also evident. The pathogenesis of the spinal cord lesions appears to involve a primary axonopathy with secondary myelin loss. The epidemiology, clinical signs and pathology suggest that the disease was associated with the fungus *Aspergillus clavatus*.

Key words: spinal cord degeneration, axonopathy, demyelination, cattle, sorghum beer residues, *Aspergillus clavatus*
'Maroek' intoxication

Introduction

Non-inflammatory degeneration of the spinal cord is an uncommon finding in ruminants in South Africa. It has been described in cattle, sheep and goats in poisoning with the plant *Helichrysum argrosphaerum* [1] (JJ van der Lugt, 1993, unpublished observations), overdosage with closantel in sheep and goats (JJ van der Lugt, 1993 unpublished observations) and rafoxanide in sheep [12], and 'valsiekte' [17], diplodiosis (L Prozesky, Onderstepoort Veterinary Institute, 1993, unpublished observations) and copper deficiency in lambs [6]. In cattle, spinal cord degeneration has also been reported in poisoning with *Ficus cordata* subsp. *salicifolia* [10].

The saprophytic fungus, *Aspergillus clavatus*, is known to cause degeneration and necrosis of large neurons in selective areas of the brain and spinal cord in cattle [8,9]. Multifocal areas of mild microcavitation and associated axonal degeneration were described in some of the affected animals [9]. In this paper we report on a neurological syndrome in adult cattle characterised histologically by degeneration and loss of axons and depletion of myelin of the spinal cord, presumably caused by *A. clavatus*.

History

Nervous signs occurred in 76 out of 100 Friesland cows in lactation and aged between 3 and 8 years, near Potchefstroom in the Western Transvaal. Initially, weakness of the hind legs, lateral swaying of the hindquarters in an effort to maintain balance, and a stiff-legged gait were noticed. Clinical signs were often not evident when the cows were at rest, but became apparent when they were chased. These clinical signs were invariably progressive, and ataxia, paralysis and permanent recumbency often followed knuckling over at the fetlocks and dragging of the hind legs. Several cows assumed a dog-sitting position, being unable to rise on their hind limbs. Most showed a loss of condition and milk production ceased completely. Affected animals remained alert with a good appetite prior to becoming recumbent. The period from the appearance of clinical signs to recumbency varied between 2 to 8 weeks. No muscle tremors or hypersensitivity were noted. Seventy-six cows became affected over a period of eight months and, apart from two animals submitted for necropsy, all were eventually destroyed. No clinical signs were present in dry cows and heifers.

The cattle were kept on the veld and were kraaled at night where they had access to babala (*Pennisetum glaucum*), lucerne (*Medicago sativa*), midmar grass (*Lolium multiflorum*) (when available) and a mixture of wheat, maize grit and sorghum beer residue. The diet was supplemented with salt, bone meal and phosphate. The sorghum beer residue, obtained from a beer company in a dry and unfermented form, was stored in metal drums prior to being fed to the cows. The same ration, without sorghum beer residue, was given to the dry cows and heifers.
Chapter 4.3

Materials and Methods

Pathology

Two, 5-7-year-old, recumbent cows were submitted for examination and were euthanased by intravenous injection of pentobarbitone sodium. At necropsy, the entire brain and spinal cord, portions of the ischiatic nerve and a range of tissue specimens were fixed by immersion in 10% buffered formalin for light microscopy. The dura mater of the spinal cord was incised to allow rapid penetration of fixative. Coronal sections were made of the brain, while the spinal cord was sectioned at C3, C7, T5, T13, L2 and L5, both transversely and longitudinally to include the corresponding dorsal root ganglia. Tissues were routinely prepared and stained with haematoxylin and eosin (HE) and selected sections of the spinal cord with luxol fast blue Holmes (LFB/H) and luxol fast blue periodic acid-Schiff haematoxylin (LFB/PAS/H).

Toxicology

Specimens of brain, liver and fat from one cow were tested for pesticides containing organophosphorous or hydrocarbon compounds. From both animals, the liver was analysed for copper, and the brain and spinal cord for lead and mercury, respectively. Heparinised blood from one of the cows submitted for necropsy as well as from 5 other affected cows was analysed for cholinesterase activity.

Results

Pathology

No specific gross lesions were seen. Microscopical changes in both animals were similar and comprised severe, extensive, bilaterally symmetrical nerve fibre degeneration resembling Wallerian degeneration in both ascending and descending white matter tracts at all levels of the spinal cord, particularly in the thoracic and lumbar portions. In each spinal cord segment the lesions were most pronounced in the lateral and ventral funiculi, particularly beneath the dorsal spinal nerve rootlet and on either side of the ventral fissure with relative sparing of the dorsal funiculi and those areas immediately adjacent to the grey matter (Fig. 1). In the affected areas, myelin sheaths were dilated, giving the white matter a vacuolar appearance (Fig.2). The majority of ballooned sheaths were empty or occasionally contained swollen axons or large, ovoid structures designated as spheroids (Fig. 3). Axonal spheroids stained lightly eosinophilic with HE and had a finely granular appearance. Several sheaths contained tissue debris or foamy macrophages which usually had pycnotic nuclei and phagocytosed LFB-positive or PAS-positive material in their cytoplasm (Fig.3). In the most severely affected funiculi, a mild
Fig. 1: Transverse section of thoracic spinal cord. There is extensive vacuolation and myelin depletion of the lateral and ventral funiculi with relative sparing of the dorsal funiculi and white matter adjacent to the grey matter. Luxol fast blue periodic acid-Schiff haematoxylin.

to moderate deficiency of stainable myelin was demonstrated in sections stained with LFB. In longitudinal sections stained with LFB/H, ‘digestion chambers’ were observed as ballooning ellipsoids along the course of axons. These ellipsoids contained fragmented and focally swollen axons and myelin debris, or were empty (Fig.4).

Reactive astrocytes were present in affected funiculi and some contained intracytoplasmic, granular PAS-positive material. In the lateral funiculi just beneath the point of entry of the dorsal root, a few perivascular lymphocytic infiltrates were seen (Fig.4).

A few chromatolytic neurons and axonal spheroids as well as mild multifocal gliosis were present in the spinal cord grey matter. There was, however, no apparent reduction in the number of neurons. Lesions in the spinal nerve roots and peripheral nerves comprised degeneration of single nerve fibres with loss of axons and formation of myelin ovoids containing axonal fragments. Chromatolysis, cytoplasmic vacuolation and apparent loss of single neurons, focal accumulations
Fig. 2: Higher magnification of the peripheral white matter in the ventral funiculi of the thoracic spinal cord. Note balloononed myelin sheaths giving the white matter a vacuolar appearance. Luxol fast blue periodic acid-Schiff haematoxylin.

Fig. 3: Dilated myelin sheaths are empty or contain spheroids (arrow) or tissue debris. Lumbar spinal cord. Luxol fast blue periodic acid-Schiff haematoxylin.

Fig. 4: Thoracic spinal cord; lateral funiculus beneath the dorsal spinal nerve rootlet demonstrating Wallerian degeneration. There are multiple ellipsoids along the course of axons which contain macrophages and myelin debris, or are empty. Luxol fast blue periodic acid-Schiff haematoxylin.

Fig. 5: Dorsal root ganglia to illustrate central chromatolysis of a neuron and perineuronal infiltrations of lymphocytes. HE.

of lymphocytes, often in a perineuronal location, and satellitosis were seen in the dorsal root ganglia (Fig. 5).

In the medulla oblongata and cerebellar white matter there was mild, focal axonal and myelin degeneration, but no apparent neuronal changes.

No significant pathological changes were observed in sections of other organs and tissues.
Toxicology

No organophosphorous or hydrocarbon compounds could be demonstrated in the specimens. The concentrations of copper, lead and mercury in the tissues were within normal values, and normal cholinesterase activity was detected in specimens of heparinised blood.

Discussion

The noteworthy features of the pathology in the two necropsied cases were the nature and severity of the spinal cord lesions. These differed from those reported previously in adult cattle in South Africa. The degeneration and loss of axons and the paucity of normal axon profiles in the most severely affected funiculi are suggestive of a primary axonopathy with secondary myelin breakdown. Neuronal chromatolysis in the spinal grey matter and spinal root ganglia and axonal spheroids, although not conspicuous, are also consistent with distal axonopathy [6].

The cause of the syndrome was not determined. The absence of clinical signs in the heifers and dry cows which did not receive sorghum beer residue, the extended course of the disease and the non-inflammatory nature of the spinal cord lesions suggested a toxic aetiology associated with feeding of the sorghum beer residue. Intoxication by a metabolise or metabolises of *A. clavatus* was regarded as the most likely diagnosis. Poisoning by *A. clavatus* occurs sporadically in South Africa and is diagnosed on circumstantial evidence such as clinical signs, histological evidence of degeneration and necrosis of certain groups of neurons in the nervous system and the ingestion of feed infected with the fungus [7]. *A. clavatus* has been associated with intoxication in cattle grazing sorghum beer residues, sprouted wheat and malt sprouts as well as in cattle and sheep given malt culms, a distillery by-product [3,5,8,9,11,16]. Recently, the fungus was incriminated in the death of sheep fed sprouted barley grains [15]. The herd was examined at the end of the outbreak, and as the incriminated residue had been consumed, no mycological and toxicological examinations were undertaken.

In the present outbreak, clinical signs included a stiff-legged gait progressing to ataxia and eventual permanent recumbency and death, as reported in previous outbreaks of the intoxication [8,9]. In contrast to previous observations, hypersensitivity and muscle tremors were not observed in the present cases. Gilmour *et al* [3] described pronounced spinal cord degeneration in sheep, similar to the lesions seen in the present cases, while cerebrocortical haemorrhage and malacia were reported in an outbreak of the disease in China [5]. It has been suggested that clinical and pathological differences in *A. clavatus* poisoning may be dose dependent or be related to the production of different toxins by the fungus [3]. The particular toxin of the fungus incriminated in the toxicosis however, remains unidentified [7,13]. The pronounced nervous lesions of *A. clavatus*
poisoning distinguishes it from other tremorgenic conditions such as those induced by ergots and endophytes.

The pathogenesis of the nervous lesions associated with *A. clavatus* needs to be defined. Toxic injury to motor neurons was suggested as the probable cause of neuronal chromatolysis in cases of this intoxication [8]. Gilmour et al [3] concluded that the lesions caused by the fungus may be explained by primary axonal injury. The findings of this study are in agreement with the latter observations.

The nature and distribution of the spinal cord lesions have many similarities to enzootic ataxia in lambs and goat kids [6] and 'valsiekte' of sheep in South Africa [17]. Copper deficiency in cattle has only rarely been incriminated in a syndrome comparable to enzootic ataxia in sheep [6,14], and the normal levels of copper in the livers of the two animals further militate against copper deficiency as a possible cause of the syndrome. 'Valsiekte', a nervous condition often associated with 'kaalsiekte' of newborn lambs (*Chrysocoma tenuifolia* poisoning), is not known to affect cattle [7].

Some organophosphorous compounds cause delayed neurotoxicity with axonal degeneration of the long descending and ascending spinal tracts [2,6]. No such compounds were used on the farm and the negative toxicology and normal activity of cholinesterase excluded this differential diagnosis.

Spongy degeneration of the spinal cord white matter with or without evidence of demyelination has also been reported in cattle after poisoning by the plants *Helichrysum argyrosphaerum* [1] (JJ van der Lugt, 1993, unpublished observations), *Ficus cordata* subsp. *salicifolia* [10] and *Cycas media* (*Zamia staggers*) [4], and in diplodiosis in lambs (L Prozesky Onderstepoort Veterinary Institute 1993 unpublished observations). The cows in the present outbreak had no access to these plants or maize infected with *Diplodia maydis*, and *Zamia staggers* has not been diagnosed in South Africa. Status spongiosis of the central nervous system has also been described in hexachlorophene toxicity [6], but the herd was not exposed to this compound.

**Acknowledgements**

We wish to thank the staff of the Sections of Toxicology and Pathology for technical assistance and especially Mr JP Putterill, Mrs L Limper, Mr AH Loock and Prof JAW Coetzer for their assistance and advice. We also gratefully acknowledge Dr DH Gould for reviewing representative slides and for his valuable advice.

**References**