THE CLINICOPATHOLOGY AND PATHOLOGY OF SELECTIVE TOXICOSES AND STORAGE DISEASES OF THE NERVOUS SYSTEM OF RUMINANTS IN SOUTHERN AFRICA

De klinische verschijnselen en pathologie van selectieve toxicoses
en stapelingsziekten van het zenuwstelsel van herkauwers
in zuidelijk Afrika

(met een samenvatting in het Nederlands)

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Aan Adee
En aan mijn ouders

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CHAPTER 1 : GENERAL INTRODUCTION

Background

Southern Africa is well known for the diversity and beauty of its flora. Ironically, about 600 plants in the subcontinent are toxic and a large proportion of these are potentially poisonous for livestock [10,29,30,35]. In most of the Region (Fig. 1), livestock is traditionally kept under extensive conditions on veld that is frequently denuded by drought, burning and overstocking. Under these circumstances, animals may be forced to eat poisonous plants that they would normally avoid. Devastating outbreaks of poisoning have been reported under such conditions. During 1926 and 1927 for example, more than 600 000 sheep were affected by plant-induced photosensitization in the Northern Cape Province, while in the period 1969 to 1970 up to 70 000 sheep died of photosensitivity in the Middelburg district of the same province [10]. In addition to plant poisonings, mycotoxicoses such as diplodiosis have also been recognised as of economic importance for livestock production in Southern Africa [10,11].

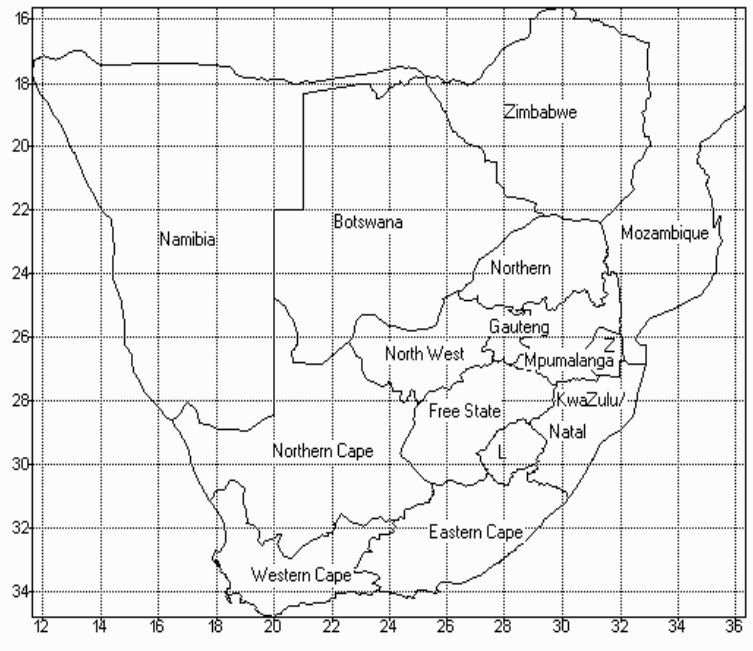

Fig. 1: Countries within the southern African Region and provinces of South Africa (L = Lesotho, Z = Zwaziland)

In a recent study, the economic importance of plant poisonings and mycotoxicoses in livestock in South Africa was estimated [13]. The annual total

cost of these intoxications to the livestock industry was conservatively estimated at ZAR 104 million. The figure did not include hidden losses such as diminished production, reproductive failure, and the cost of not utilising toxic pastures and the fall in price of infested land. Specific data for neighbouring countries is to the best of our knowledge not available.

Kellerman and others [13] determined the six most important plant poisonings and mycotoxicoses in ruminants in South Africa (Table 1). Poisoning by plants containing cardiac glycosides caused the greatest economic loss in cattle. In sheep and goats, the hepatogenous photosensitivity diseases ‘geeldikkop’ and ‘dikoor’ collectively were regarded as the most important.

The detection, isolation and identification of toxic compounds biosynthesised by plants and fungi is an important research field in veterinary toxicology. Investigations into the biochemistry of these toxins are essential to elucidate mechanisms of action, define toxin-induced effects, develop analytical assays, and assist in the design of management protocols to control and prevent poisonings. These preventative measures are based upon accurate prediction of the variation in toxin level associated with factors such as plant species, growth stage, and environmental change [5]. With these aims in mind, research in South Africa and more specifically at the Onderstepoort Veterinary Institute and Faculty of Veterinary Science, University of Pretoria, Onderstepoort has mainly focused on the most important plant toxicoses and mycotoxicoses that occur in the subcontinent (Table 1). It is therefore not surprising that toxic natural products of all except two of these plants and fungi listed in Table 1, have been isolated and characterised by South African scientists.

A number of poisonous plants and fungi may cause nervous signs and death in ruminants in southern Africa. With the exception of diplodiosis, these poisonings are not of major economic importance and occur sporadically in most instances. These intoxications are often associated with particular feeding practices, and sometimes occur on farms in remote areas where veterinary assistance is not freely available and case material difficult to obtain for study. An example of such rare neurological condition is poisoning by the plant *Helichrysum argyrosphaerum* in sheep and goats. Outbreaks of intoxication have been restricted to Namibia and occurred in South Africa for the first time after abnormally high rainfall ended a 9-year period of drought in the North West Province (Fig. 1).

The toxic principle(s) of most of these neurotoxicoses have not been determined and chemical analysis to achieve a diagnosis is not available. Their diagnosis therefore, is based on a detailed history, characteristic clinical signs, identification of toxic plants that have been consumed or specific epidemiological factors on the property, and especially the recognition of histological lesions, if present, in the nervous system. Detailed descriptions of the histological and ultrastructural lesions of most of these neurotoxicoses are lacking [10].

Table 1: The six most important plant poisonings and mycotoxicoses in South Africa in cattle and small stock [13].

Name of intoxication and incriminated plant(s)/fungus (in brackets)	**Toxin(s)**
Cattle	
1. Acute poisoning with cardiac glycoside-containing plants: 'Tulp' (*Homeria* and *Moraea* spp.), 'Slangkop' (*Urginea* spp.) and 'Witstorm' (*Thesium lineatum*)	1. Non-cumulative bufadienolides [7,17,18,19,26,27,31,32,33]
2. Seneciosis (*Senecio* spp., esp. *S. latifolius* and *S. retrorsus*)	2. Pyrrolizidine alkaloids [20,34]
3. 'Gifblaar' poisoning (*Dichapetalum cymosum*)	3. Monofluoroacetate [21]
4. 'Gousiekte' (*Pachystigma*, *Fadogia*, and *Pavetta* spp.)	4. Pavetamine [8,9,28]
5. *Lantana* poisoning (*L. camara*)	5. Pentacyclic triterpenes [14,15,16]
6. Diplodiosis (fungus *Stenocarpella maydis*)	6. Unknown [10]
Sheep and goats	
1. 'Geeldikkop' (*Tribulus terrestris*) and 'Dikoor' or *Panicum* photosensitivity (*Panicum* spp.)	1. Steroidal saponins in *T. terrestris* [12,24,25] and possibly in *Panicum* [22,23]
2. 'Vermeersiekte' (*Geigeria* spp.)	2. Sesquiterpene lactones [1,6]
3. Acute intoxication with cardiac glycoside-containing plants e.g. 'tulp' (*Homeria* and *Moraea* spp.), 'slangkop' (*Urginea* spp.) and 'Witstorm' (*Thesium lineatum*); and chronic poisoning with cardiac glycoside-containing plants or 'krimpsiekte' (*Cotyledon*, *Kalanchoe* and *Tylecodon* spp.)	3. Non-cumulative bufadienolides in 'tulp' and 'slangkop' [7,17,18,19,26,27,31,32,33] and cumulative bufadienolides in 'krimpsiekte'-containing plants [2,3]
4. Seneciosis (*Senecio* spp. esp. *S. latifolius* and *S. retrorsus*)	4. Pyrrolizidine alkaloids [20,34]
5. 'Gousiekte' (*Pachystigma*, *Fadogia*, and *Pavetta* spp.)	5. Pavetamine [8,9,28]
6. Diplodiosis (fungus *Stenocarpella maydis*)	6. Unknown [10]

Aim and outline of this study

In this study the clinical signs and pathology of five plant poisonings and a mycotoxicosis affecting the nervous system of domestic ruminants in southern Africa are described. For comparative purposes, an inherited storage disease (β-mannosidosis) and a drug-induced neurotoxicosis (closantel overdosage) are also presented. A common feature in the nervous tissue of these conditions is some degree of myelin and/or cellular vacuolation. Case material of these conditions was collected during outbreaks of disease and, in three of the poisonings, also during subsequent feeding trials with toxic plant material.

The study had three aims in mind:

- To document the pathology of those conditions where detailed descriptions were lacking,
- To study the appearance and/or pattern of the lesions from a differential diagnosis perspective and
- To consider the mechanisms underlying the nervous lesions.

The conditions in this thesis are grouped into three categories:

- Lysosomal storage diseases (Chapter 2.1),
- Myelinopathies (Chapter 3.1) and
- Neuronopathies and axonopathies (Chapter 4.1).

Although most lysosomal storage diseases in animals are inherited and characterised by the accumulation of sphingolipids, glycolipids, oligosaccharides, or mucopolysaccharides within lysosomes in multiple cell types, a few are induced by the ingestion of toxic plants. A novel lysosomal storage disease in goats caused by *Ipomoea carnea* is reported (Chapter 2.2). β-Mannosidosis in Hereford calves is documented, a breed not previously known to be affected by this inherited disorder (Chapter 2.3). The ultrastructural lesions and lectin histochemistry in *Solanum kwebense* poisoning in cattle, a plant toxicosis characterised by the development of lesions in the cerebellum suggestive for a storage disease, are also described (Chapter 2.4).

Four myelinopathies are described in this thesis:

- *Helichrysum argyrosphaerum* poisoning which induces blindness, nervous signs, status spongiosis, optic neuropathy and retinal degeneration in small stock (Chapter 3.2),
- Closantel intoxication in small stock (Chapter 3.3),
- A novel toxicosis causing blindness and myelin vacuolation following exposure to *Ornithogalum prasinum* and *O. saundersiae* in cattle (Chapter 3.4) and
- *Crotalaria sparthioides* intoxication in cattle, an example of hepatic encephalopathy (Chapter 3.5).

Ingestion of the fungus *Aspergillus clavatus* may induce a tremorgenic condition in cattle. In Chapter 4.1 and 4.2, the pathology of acute and chronic *A. clavatus* poisoning in cattle are reported, respectively. Based on the nature of the lesions in this neuromycotoxicosis, the condition is described in the category of neuronopathies and axonopathies.

References

1. Anderson LAP, De Kock WT, Pachler KGR (1967) The structure of vermeerin, a sesquiterpenoid dilactone from *Geigeria africana* Gries. Tetrahedon 23:4153-4160
2. Anderson LAP, Joubert JPJ, Prozesky L, Kellerman TS, Schultz RA, Procos J, Olivier PM (1983) The experimental production of krimpsiekte in sheep with *Tylecodon grandiflorus* (Burm. F.) Toelken and some of its bufadienolides. Onderstepoort J Vet Res 50:301-307
3. Anderson LAP, Schultz RA, Joubert JPJ, Prozesky L, Kellerman TS, Erasmus GL, Procos J (1983) Krimpsiekte and acute cardiac glycoside poisoning in sheep caused by bufadienolides from the plant *Kalanchoe lanceolata* Forsk. Onderstepoort J Vet Res 50:295-300
4. Anderson LAP, Schultz RA, Kellerman TS, Kotzé SM, Prozesky L, Erasmus GL, Labuschagne L (1985) Isolation and characterization of and some observations on poisoning by bufadienolides from *Cotyledon orbiculata* L. var. *orbiculata*. Onderstepoort J Vet Res 52:21-24
5. Colegate SM, Dorling PR (1992) The isolation and identification of plant toxins. In: James LF, Keeler RF, Bailey EM, Cheeke PR, Hegarty MP (eds) Poisonous Plants: Proceedings of the Third International Symposium Iowa State University Press, Ames, Iowa, pp 273-281
6. De Kock WT, Pachler KGR, Ross WF, Wessels PL (1968) Griesenin and dihydrogriesenin, two new sesquiterpenoid lactones from *Geigeria africana* Gries-I structures. Tetrahedon 24:6037-6043
7. Enslin PR, Naudé TW, Potgieter DJJ, Van Wyk AJ (1966) 1α, 2α-Epoxyscillirosidine, the main toxic principle of *Homeria glauca* (W & E). N.E. Br. Tetrahedron 22:3213-3220
8. Fourie N (1994) Isolation of the cardiotoxin from gousiekte-inducing plants and investigation of the pathogenesis and diagnosis of the disease. PhD thesis, University of Pretoria
9. Fourie N, Erasmus GL, Schultz RA, Prozesky L (1995) Isolation of the toxin responsible for gousiekte, a plant-induced cardiomyopathy of ruminants in southern Africa. Onderstepoort J Vet Res 62:77-87
10. Kellerman TS, Coetzer JAW, Naudé TW (1988) Plant Poisonings and Mycotoxicosis of Livestock in Southern Africa, 1st edn, Oxford University Press, Cape Town
11. Kellerman TS, Coetzer JAW, Naudé TW (1992) Plant poisonings and mycotoxicosis of livestock in Southern Africa. In: James LF, Keeler RF James LF, Keeler RF, Bailey EM, Cheeke PR, Hegarty MP (eds) Poisonous Plants: Proceedings of the Third International Symposium Iowa State University Press, Ames, Iowa, pp 43-54
12. Kellerman TS, Erasmus GL, Coetzer JAW, Brown JMM, Maartens BP (1991) Photosensitivity in South Africa. VI. Experimental induction of geeldikkop in sheep

with crude steroidal saponins from *Tribulus terrestris*. Onderstepoort J Vet Res 58:47-53
13. Kellerman TS, Naudé TW, Fourie N (1996) The distribution, diagnoses and estimated economic impact of plant poisonings and mycotoxicosis in South Africa. Onderstepoort J Vet Res 63: 65-90
14. Louw PGJ (1943) Lantanin, the active principle of *Lantana camara* L. I. Isolation and prelimary results on the determination of its constitution. Onderstepoort J Vet Sci Anim Ind 18:197-202
15. Louw PGJ (1948) Lantadene A, the active principle of *Lantana camara* L. II. Isolation of Lantadene B and oxygen functions of lantadene A and lantadene B. Onderstepoort J Vet Sci Anim Ind 23:233-238
16. Louw PGJ (1949) Lantadene A, the active principle of *Lantana camara* L. III. Pyrolytic distillation of lantadene A. Onderstepoort J Vet Sci Anim Ind 22:321-327
17. Louw PGJ (1949) The cardiac glycoside from *Urginea rubella* Baker. Part I. Isolation and properties of rubellin. Onderstepoort J Vet Sci Anim Ind 22:313-314
18. Louw PGJ (1952) Transvaalin, a cardiac glycoside isolated from *Urginea burkea*, Bkr. (Transvaal slangkop). Onderstepoort J Vet Res 25:123-131
19. Louw PGJ (1953) A cardiac glycoside from *Urginea rubella* Baker. Part II. Hydrolysis of rubellin and pre-rubellin. Onderstepoort J Vet Res 26:141-148
20. Manske RHF (1931) The alkaloids of *Senecio* species. I. The necines and necic acids from *S. retrorsus and S. jacobaea*. Can J Res 5:651-659
21. Marais JSC (1944) Monofluoroacetic acid, the toxic principle of 'gifblaar' *Dichapetalum cymosus* (Hook) Engl. Onderstepoort J Vet Sci Anim Ind 20:67-73
22. Miles CO, Munday SC, Holland PT, Lancester MJ, Wilkens AL (1992) Further analysis of bile crystals from sheep grazing *Panicum schinzii* (sweet grass). Aust Vet J 69:34
23. Miles CO, Munday SC, Holland PT, Smith BL, Embling PP, Wilkens AL (1991) Identification of a sapogenin glucuronide in the bile of sheep affected by *Panicum dichotomiflorum* toxicosis. N Z Vet J 39:150-152
24. Miles CO, Wilkens AL, Erasmus GL, Kellerman TS (1994) Photosensitivity in South Africa. VIII. Ovine metabolism of *Tribulus terrestris* during experimentally induced geeldikkop. Onderstepoort J Vet Res 61:351-359
25. Miles CO, Wilkens AL, Erasmus GL, Kellerman TS, Coetzer JAW (1994) Photosensitivity in South Africa. VII. Chemical composition of biliary crystals from a sheep with experimentally induced geeldikkop. Onderstepoort J Vet Res 61:215-222
26. Naudé TW, Potgieter DJJ (1966) A preliminary note on the isolation and pharmacological actions of the toxic principles of *Homeria glauca* (W & E). N.E. Br. J S Afr Vet Med Assoc 37:73-75
27. Naudé TW, Potgieter DJJ (1971) Studies on South African cardiac glycosides. I. Isolation of toxic principles of *Homeria glauca* (W & E). N.E. Br. Onderstepoort J Vet Res 38:255-278
28. Schultz RA, Fourie N, Basson KM, Labuschagne L, Snyman LD, Prozesky L (2001) Pavetamine: an inhibitor of protein synthesis in the heart. In: Proceedings 6th International Symposium of Poisonous Plants (ISOPP6), 5-10 August, Glasgow, Scotland (in print)
29. Steyn DG (1934) The Toxicology of Plants in South Africa, Central News Agency. Johannesburg, South Africa
30. Steyn DG (1949) Vergiftiging van Mens en Dier, Van Schaik Bpk, Pretoria

31. Van Wyk AJ, Enslin PR (1968) Bufadinolides in *Moraea polystachya* Ker. And *M. graminicola* Obem. J S Afr Chem Inst 22:186-190
32. Van Wyk AJ, Enslin PR (1969) Bufadinolides from Iridaceae. J S Afr Chem Inst 22:66-70
33. Van Wyk AJ, Enslin PR (1969) A new toxic principle of *Homeria glauca* (Wood & Evans) N.E. Br. J S Afr Chem Inst 22:186-190
34. Watt HE (1909) The alkaloids of *Senecia latifolius*. Proc Chem Soc 25:68
35. Watt JM, Breyer-Brandwijk MG (1962) The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd edn, E & S Livingstone Ltd, Edinburgh and London

CHAPTER 2 : LYSOSOMAL STORAGE DISEASES

Chapter ***2.1***

CLASSIFICATION

A wide variety of lysosomal storage diseases have been described in domestic animals and humans [6,9]. The hallmark of these disorders is the accumulation of a specific substrate(s) in lysosomes due to a deficient activity of a lysosomal enzyme or enzymes. Phenotypically, these disorders are diverse but the nervous system is commonly involved. This is explained by the fact that neurons are metabolically active cells for many of the substrates and they represent a postmitotic, permanent cell population [9]. The cytoplasm of affected cells in various tissues contain enlarged lysosomes with uncatabolized substrate(s) in solution or complexed with other chemical species. Histologically, cells with lysosomes filled with storage material display a vacuolated or granular cytoplasm.

The lysosomal storage diseases are classified according to the defective enzyme (acid hydrolase) or the primary stored substrate. The diseases are reviewed elsewhere and the following classification is used in domestic animals [9]:

Sphingolipidoses
- Gangliosidoses
- Galactocerebrosidosis
- Glucocerebrosidosis
- Sphingomyelin lipidosis
- Galactosialidosis

Glycoproteinoses
- α-Mannosidosis
- β-Mannosidosis
- Fucosidosis

Mucopolysaccharidoses
Glycogen storage disease type II
Ceroid-lipofuscinosis

Most lysosomal storage diseases are genetic disorders. A few conditions however, are induced by the ingestion of toxic plants such as the locoweeds *(Astragalus and Oxytropis* spp.) from North America, South America, and China and the poison peas *(Swainsona* spp.) found in Australia [2,4,7,10]. A novel plant-induced lysosomal storage disease in goats feeding on *Ipomoea carnea* in Mozambique is reported (Chapter 2.2). The plants contain the indolizidine alkaloid swainsonine, which not only inhibits lysosomal α-mannosidose but also mannosidase II [1,6].

Recently, a plant-induced storage disease in goats caused by the ingestion of *Sida carpinifolia* was reported from Brazil [3]. Light and electron microscopical features in affected cells were consistent with a storage disease and the pattern of lectin histochemical staining in nerve and pancreatic cells indicated an α-mannosidosis. Biochemical assays were not performed but the condition probably is identical to the storage disease induced by swainsonine.

The ceroid-lipofuscinoses is a group of storage diseases characterised by the lysosomal accumulation of a fluorescent lipopigment within neurons and other cell types [9]. A specific enzyme derangement has not been elucidated. They have been described in cattle, sheep, goats dogs, cats, and human as inherited conditions. In southern Africa, cattle, sheep, horses and pigs may develop a progressive, ascending paresis and paralysis following ingestion of the plants *Trachyandra laxa* and *T. divercata* [5,8]. Affected animals have prominent and widespread neuronal lipofuscinosis consistent with ceroid-lipofuscinosis. A similar neurological condition associated with *T. divercata* has been reported from western Australia [9].

References

1. Dorling PR, Huxtable CR, Colegate SM (1980) Inhibition of lysosomal a-mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens.* Biochem J 191:649-651
2. Dorling PR, Huxtable CR, Vogel P (1978) Lysosomal storage in *Swainsona* spp. toxicosis: an induced mannosidosis. Neuropath Appl Neurobiol 4:285-295
3. Driemeier D, Colodel EM, Gimeno EJ, Barros SS (2000) Lysosomal storage disease caused by *Sida carpinifolia* in goats. Vet Pathol 37:153-159
4. Everist SL (1981) Poisonous plants of Australia, 2nd edn, Angus & Robertson, Sydney, Australia, pp 966
5. Grant CC, Basson PA, Kidd AB (1985) Paralysis and lipofuscin-like pigmentation of stock caused by the plant *Trachyandra laxa* var. *laxa*. Onderstepoort J Vet Res 52:255-259
6. Jolly RD, Walkley SU (1997) Lysosomal storage diseases: an essay in comparative pathology. Vet Pathol 34:527-548
7. Locke KB, McEwan DR, Hamdorf IJ (1980) Experimental poisoning of horses and cattle with *Swainsona canescens var. homiana.* Aust Vet Jnl 56:379-383
8. Newsholme SJ, Schneider DJ, Reid C (1985) A suspected lipofuscin storage disease of sheep associated with the ingestion of the plant *Trachyandra divercata* (Jacq.) Kunth. Onderstepoort J Vet Res 52:87-92
9. Summers BA, Cummings JF, De Lahunta A (1985) Veterinary Neuropathology. Mosby: St. Louis, pp 214-236
10. Van Kampen KR, James LF (1969) Pathology of locoweed poisoning in sheep. Pathol Vet 6:413-423

Chapter ***2.2***

A LYSOSOMAL STORAGE DISEASE INDUCED BY *IPOMOEA CARNEA* IN GOATS IN MOZAMBIQUE

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Abstract
A novel plant-induced lysosomal storage disease was observed in goats from a village in Mozambique. Affected animals were ataxic, with head tremors and nystagmus. Because of a lack of suitable feed, the animals consumed an exotic hedge plant growing in the village that was identified as *Ipomoea carnea* (shrubby morning glory, Convolvulaceae). Feeding *I. carnea* plant material to goats reproduced the toxicosis. In acute cases, histologic changes in the brain and spinal cord comprised widespread cytoplasmic vacuolation of neurons and glial cells in association with axonal spheroid formation. Ultrastructurally, cytoplasmic storage vacuoles in neurons were membrane bound and consistent with lysosomes. Cytoplasmic vacuolation was also found in neurons in the submucosal and mesenteric plexuses in the small intestine, in renal tubular epithelial cells, and in macrophage-phagocytic cells in the spleen and lymph nodes in acute cases. Residual alterations in the brain in chronic cases revealed predominantly cerebellar lesions characterised by loss of Purkinje neurons and gliosis of the Purkinje cell layer. Analysis of *I. carnea* plant material by gas chromatography-mass spectrometry established the presence of the mannosidase inhibitor swainsonine and two glycosidase inhibitors, calystegine B_2 and calystegine C_1, consistent with a plant-induced α-mannosidosis in the goats. The described storage disorder is analogous to the lysosomal storage diseases induced by ingestion of locoweeds *(Astragalus and Oxytropis)* and poison peas *(Swainsona).*

Key words: *Ipomoea carnea*, goats, lysosomal storage disease, swainsonine, glycosidase inhibitors, calystegine B_2, calystegine C_1

Introduction

Most lysosomal storage diseases are genetic disorders [32], but a few are induced by the ingestion of toxic plants such as the locoweeds *(Astragalus and Oxytropis* spp.) from North America, South America, and China [36] and the poison peas *(Swainsona* spp.) found in Australia [13,15,25]. These plants belong to the family Fabaceae (Leguminosae) and contain the indolizidine alkaloid swainsonine, an inhibitor of lysosomal α-mannosidase and mannosidase II [12,28]. The action of swainsonine results in a lysosomal storage disorder that closely mimics α-mannosidosis, characterised by the accumulation of incompletely processed oligosaccharides rich in α-mannosyl and β-*N*-acetyl glucosamine moieties [2,12]. Histologically, there are cytoplasmic vacuoles in cells of the nervous system and other tissues. Neurons, as is the case in most lysosomal storage diseases, are most consistently affected because they represent a postmitotic, permanent cell population [32].

Animals consuming these plants exhibit a variety of clinical signs, reflecting derangement of the nervous system in particular and of other tissues. Depression, rough hair coat, staggering gait, muscle tremors, ataxia, and nervousness, especially when stressed, may be seen [19,22,25]. Generally, these plants are grazed only during periods of food shortage, but some animals apparently develop a liking for them to such an extent that individuals selectively consume the plants despite the availability of other feed [15,19].

A novel plant-induced lysosomal storage disease has been observed in goats feeding on *Ipomoea carnea.* The condition was reproduced experimentally, and swainsonine and two calystegine glycosidase inhibitors from the plant were isolated and identified.

Materials and methods

Description of outbreak

During the dry season (Jun-Sep) of 1993, a nervous condition was noted in local Landin goats in a village 35 km south-west of Maputo, Mozambique (32'25'E, 26'05'S). The village, Paulo Samuel Kankhomba, was founded in 1978 after independence of Mozambique from Portugal, and most of its initial inhabitants are former soldiers. At the time of the outbreak, all villagers were involved in small-scale subsistence farming. Like most villages in Mozambique, the houses were built of local material (reed, mud, and a few stones) and separated by hedges of growing plants. Very few people in the village kept cattle, but approximately 50 of 620 families kept goats. Because theft was a major problem in the village, goat rearers kept their animals in rudimentary pens at night. During the day, young boys took out the animals to the field for grazing. Until the signing of the peace accord

Fig. 1: *Ipomoea carnea* growing as a hedge in the village in Mozambique in which goats were intoxicated.

in October 1992, the ongoing civil war and the extent of land under cultivation restricted the grazing areas of the animals from the village.

The most prominent clinical signs in affected goats consisted of ataxia, head tremors, and nystagmus. Goats continued to eat until the progressive course of the nervous symptoms finally rendered the animals unable to walk and graze normally. Goats of all ages and both sexes were affected. Conflicting information was obtained about the course of the condition, but most animals apparently died within a few weeks of showing the first clinical signs. A questionnaire survey conducted in July 1994 among 25 of the goat farmers keeping a total of 295 goats indicated that approximately 10% of the animals had died because of this condition during the previous 12 months. Cases had sporadically been observed as early as 1987, but a drastic increase in the number of affected animals occurred in 1992. Spontaneous recovery was occasionally reported.

Affected goats were observed consuming a plant grown as a hedge around the homesteads and pens (Fig. 1). In the dry season, this plant was one of the few that maintained its leaves. After other probable local plant or mycotoxicoses such as poisoning by *Solanum kwebense, Trachyandra* spp., *Cynanchum spp., Sarcostemma viminale, Aspergillus clavatus,* and Phalaris [24] had been ruled out, the plant, identified *as Ipomoea carnea* (Fig. 2) was tested for its toxicity in goats.

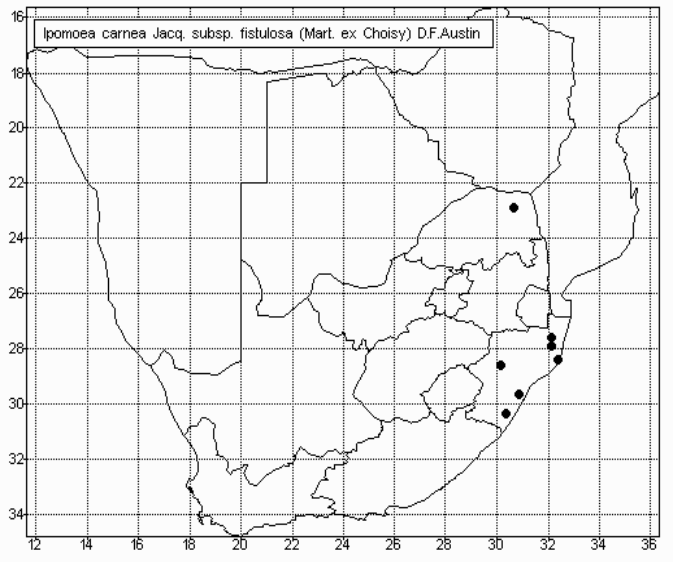


Fig 2: Distribution of *Ipomea carnea subsp. fistulosa* (courtesy Botanical Research Institute, Pretoria, South Africa).

Plant identification

Samples of the incriminated hedge plant were sent to the herbarium of the National Institute for Agricultural Research (INIA) in Maputo, Mozambique, for identification. Staff of the Botanical Research Institute, Pretoria, South Africa, confirmed identification.

Identification of toxic principles

Plant material was collected from the village in December 1995 for isolation and identification of alkaloids. Dried, milled leaves (1.05 g) were soaked in methanol for 16 hr in a Soxhlet apparatus (Kontes, Vincland, NJ), and the alkaloidal fraction was purified by ion-exchange chromatography on a 5- X 0.5-cm column of Dowex 5OW-X8 (Bio-Rad Laboratories, Richmond, CA) (NH_4^+ form) [27]. The 0.5% aqueous ammonium hydroxide eluate was evaporated to dryness to give the base fraction, containing alkaloids and basic amino acids, as a pale yellow, partially crystalline solid (18 mg; 1.7% yield). Portions of this material, which contained the alkaloidal constituents, were used for further analysis as required. The identity of alkaloids was established by gas chromatography-mass spectometric (GC-MS) analysis of the trimethylsilyl (TMSI) derivatives, prepared by treatment with *N*-methyl-*N*trimethylsilyl-trifluoroacetamide in pyridine at 60 °C for 1 hr [27,30,31]. Analyses were performed on a GCMS instrument (5890 Series 11 gas

chromatograph, 5971 mass-selective detector, Hewlett-Packard, Palo Alto, CA) operating at 70 eV, an on-column injector, and a 60-m X 0.32-mm inside diameter SE-30 fused silicon column. The column was programmed from 120 to 300 ºC at 10 ºC/min.

Field cases

Necropsies were performed on three affected goats (Goats Nos.1-3) from the village. Goat 1 was an one-year-old male, and Goats Nos. 2 and 3 were females, 1.5-2 year of age. They were kept at the Veterinary Faculty, Maputo, for 2 weeks, 6 months, and 1 year, respectively, after the onset of clinical signs before being slaughtered and necropsied.

Experimental cases

Dosing trial. Fresh leaves of the incriminated plant were collected daily and fed to four healthy male goats (Goats Nos. 4-7), 9-12 months old, which had not been previously exposed to the plant. They were housed in separate pens on cement and observed on a daily basis. Prior to the trial, the goats were dewormed with ivermectin and weighed. Weighing was continued once weekly throughout the trial. Goats Nos. 4 and 5 were given 50 g plant material/kg body mass/day, and Goats Nos. 6 and 7 received 25 g plant material/kg body mass/day. The goats also had free access to freshly cut grass and water and, only initially, to small amounts of the fodder tree *Leucaena leucocephala.*

Clinical pathology. Before dosing and once weekly during the course of the experiment, venous blood was collected and the following parameters were measured by routine methods: haemoglobin, haematocrit, total erythrocyte and leukocyte counts, differential leukocyte counts, concentrations of total serum proteins, blood urea nitrogen (BUN), creatinine, total bilirubin, and activities of alanine transaminase, alkaline phosphatase, aspartate transaminase (AST), creatine kinase, y-glutamyltransferase (GGT), and lactate dehydrogenase (LDH).

Pathology. From each goat that was necropsied, the brain, spinal cord, a portion of the sciatic nerve, and samples of liver, kidney, spleen, lung, and myocardium were fixed in 10% formalin for light rnicroscopy. In addition, samples of small intestine were collected from Goats Nos. 4-6. The brains were serially sectioned, and blocks were prepared from levels cut at the olfactory tubercle and cortex, cerebral cortex, basal nuclei, thalamus, mesencephalon, pons, and medulla oblongata. Specimens were routinely processed and stained with haematoxylin and eosin (HE). For electron microscopy, formalin-fixed specimens of cerebellum of Goats Nos. 4 and 5 were postfixed in 2,5% gluteraldehyde. Semithin sections were stained with

toluidine blue (TB) for tissue orientation, and ultrathin sections were viewed with a transmission electron microscope.

Results

Plant identification

The plant ingested by the affected goats was identified as *Ipomoea carnea* Jacq. Subsp. *Fistulosa* (Mart ex Choisy) D.F. Austin belonging to the family Convolvulaceae. Commonly the plant is known as shrubby morning glory (Texas). A specimen originating from the village with the affected goats was deposited at the Herbarium of the INIA in Maputo (voucher 51806).

The plant is erect, densely leafed, and almost unbranched, growing as shrubs to 3 m high (Fig. 1). The leaves are ovate to lanceolate, 10-25 cm long, with petioles 2-10 cm long. Flowers occur in clusters and are funnel shaped, deep pink to rose-purple, and 5-9 cm long. A full botanical description has been published [5].

Chemical investigation of *Ipomoea carnea*

Extraction of the milled leaves of *I. carnea* with methanol followed by ion-exchange chromatography gave the fraction containing alkaloids and basic amino acids. Analysis of the extract by capillary GC-MS of its TMSI derivative showed the presence of 2 major constituents at 13.90 (31%), and 15.94 (50%) minutes, a secondary component at 17.17 (7%) minutes, and 3 n-minor constituents at 14.20, 14.47, and 14.90 minutes, each of which comprised <4% of the total extract (Fig. 2). The later (15.94 minutes) major peak had a retention time (R_t) and mass spectrum identical in all respects to that of the tetra-TMSi derivative of a standard sample of calystegine B_2 (Fig. 3), with a base peak at m/z 217 characteristic of compounds having three adjacent trimethylsilylated secondary hydroxyl groups. The secondary component (17.17 minutes) had a fragmentation pattern similar to that of calystegine B_2, but showed an ion at m/z 536, corresponding to loss of 15 amu from a molecular ion at m/z 551, indicating the presence of an additional trimethylsilylated hydroxyl group. The alkaloid is therefore a pentahydroxy calystegine, and comparison with an authentic standard showed it to have an identical R_t to that of calystegine C_1 (Fig. 3). The three minor constituents had mass spectra generally consistent with alkaloids of the calystegine type, but the signals were too weak for specific identification.

The earlier (13.90 minute) major peak did not correspond in R_t to any of the TMSi derivatives of available calystegine standards. Moreover, the mass spectrum did not show fragments indicative of this class of compounds, and the molecular ion at m/z 389 and the base peak at m/z 185 were inconsistent with the nortropane structural type characteristic of all calystegines. The R_t and fragmentation pattern did, however, correspond to those of the tri-TMSi derivative of swainsonine (Fig.

3), and GC-MS analysis of an authentic standard of this alkaloid confirmed the identity of this component of the extract with swainsonine.

The relative composition was determined as area percentage of the total ion current for the chromatogram in the 12-20-minute range.

Characterisation of calystegine B_2, calystegine C_1 and swainsonine

The R_t of the major component (15.94 minutes) was identical to that of the TMSI derivative of authentic calystegine B_2. The mass spectrum of this peak also corresponded with that of tetra-TMSi calystegine B_2, eims m/z $[M]^+$ 463 (0.1), 448 (2), 373 (6), 284 (6), 259 (14), 244 (12), 229 (7), 218 (20), 217 (100), 170 (5), 156 (6), and 147 [18].

The secondary component peak at R_t 17.17 minutes corresponded in both its R_t and mass spectrum to penta-TMSi calystegine C_1, with eims mlz $[M\text{-}15]^+$ 536 (0.1), 461 (7), 347 (9), 332 (7), 317 (6), 291 (6), 258 (6), 244 (7), 218 (20), 217 (100), and 147 (24) [31].

The other major component with R_t 13.90 minutes was identical in its R_t to tri-TMSi swainsonine, and its mass spectrum also corresponded with this compound, eims m/z $[M]^+$ 389 (30), 374 (34), 299 (30), 260 (83), 185 (100), 170 (39), 157 (20), 147 (27), and 143 (39) [30].

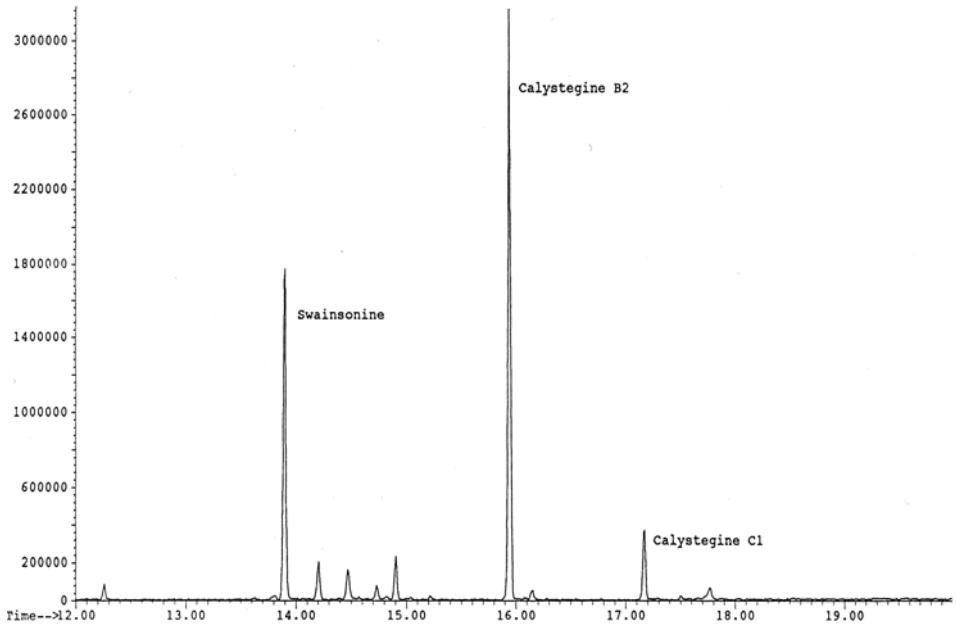
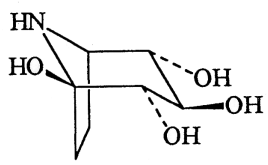
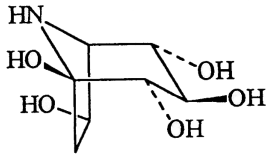


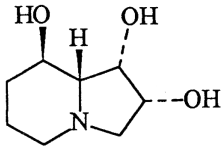
Fig. 3: Total ion chromatogram of the alkaloid extract from *Ipomea carnea,* analyzed by GC-MS of the trimethylsilyl derivative (y-axis = abandunce).



Calystegine B_2



Calystegine C_1



Swainsonine

Fig. 4: Structures of glycosidase-inhibitory alkaloids present *in Ipomoea carnea.*

Field cases

Clinical signs. The 3 field cases (Goats Nos.1-3) showed head tremors and ataxia. Goat 2 also exhibited hyperaesthesia and high stepping gait, and goat No. 3 had nystagmus.

Pathology. Oedema of the lumbar part of the spinal cord and congestion of meninges was noted in goat No. 1. Goat No. 2 had marked asymmetry and atrophy of the cerebellum. No significant gross lesions were observed in Goat No. 3.

In Goat No. 1, neurons and glial cells in the brain and spinal cord were distended with numerous small, spherical intracytoplasmic vacuoles (Fig. 5). Cytoplasmic vacuolation was present in the cerebral cortex, thalamus, brain stem, Purkinje cells in the cerebellum, and dorsal and ventral horn neurons in the spinal cord. The extent of the vacuolation in the cerebellum was best seen in semithin sections stained with TB (Fig. 6). In addition to Purkinje neurons, some stellate neurons and Golgi neurons were also affected.

Eosinophilic, round, homogenous, occasionally finely granular axonal spheroids were conspicuous in the brain and spinal cord in areas of cytoplasmic vacuolation, often in close association with larger neurons. There were some large, fusiform

torpedoes in the granular cell layer of the cerebellum, sometimes clearly originating from Purkinje cells (Fig. 5). Other lesions in the brain of Goat No. 1 comprised gliosis and capillary accentuation in the caudal colliculi and pronounced vacuolation of the white matter of the rostral caudate nucleus. Cytoplasmic vacuolation was also detected in renal tubular epithelial cells and in macrophage-phagocytic cells in the spleen and lymph nodes.

Histologic lesions in Goats Nos. 2 and 3 were similar in nature but were more widespread in Goat No. 3. In the cerebellum, there was degeneration and loss of Purkinje neurons, affecting most folia in Goat No. 3 (Fig. 7). Some of the persisting Purkinje neurons were shrunken and possessed condensed cytoplasm and hyperchromatic nuclei. In areas of Purkinje cell loss, there was vacuolation and gliosis of the Purkinje cell layer and proliferation of astrocytes in the molecular layer associated with capillary accentuation and endothelial cell hyperplasia (Fig. 7). In the most severely affected folia, the granular layer had decreased cellularity. Cytoplasmic vacuolation of neurons and glial cells was rarely observed in sections stained with HE, and few axonal spheroids were present.

Accumulations of small amounts of yellowish green to yellowish brown finely granular pigment interpreted to be lipofuscin in the cytoplasm of glial cells and apparently free-lying in the neuropil associated with mild gliosis were noticed in some brain stem nuclei in Goats Nos. 2 and 3. Macrophage-phagocytic cells in the spleen of Goat No. 2 were vacuolated.

Experimental cases

Feeding trial and clinical signs. Initially, all 4 experimental animals (Goats No. 4-7) were reluctant to eat plant material. Goat No. 7 was excluded from the trial because no plant was consumed even after periods of fasting. Goat No. 5 ate only petioles and stems; therefore, the daily dose was exclusively provided in the form of petioles and stems detached from the leaves, which were readily consumed from week 2 onwards.

After the first week of the feeding trial, weight losses were registered as animals were given reduced amounts of other food sources to encourage the intake of *I. carnea.* Goat No. 4 consumed most of the leaves provided after the first 3 days. Just prior to the initiation of central nervous system signs, the animal reduced its intake of *I. carnea* leaves. Slow movements were observed from day 39, and head tremors, a high stepping gait, and ataxia were observed from day 56 onwards. Nystagmus was evident only from day 68. Goat No. 4 was euthanised on day 72.

Goat No. 5 exhibited head shaking from day 54 and progressive weakness from day 59. After an episode of diarrhoea, the animal became bloated and died on day 74 while attempts were made to pass a stomach tube.

Abnormal behaviour characterised by hyperaesthesia, pica, and upward movements of the head was noted in Goat No. 6 from day 78. The animal only

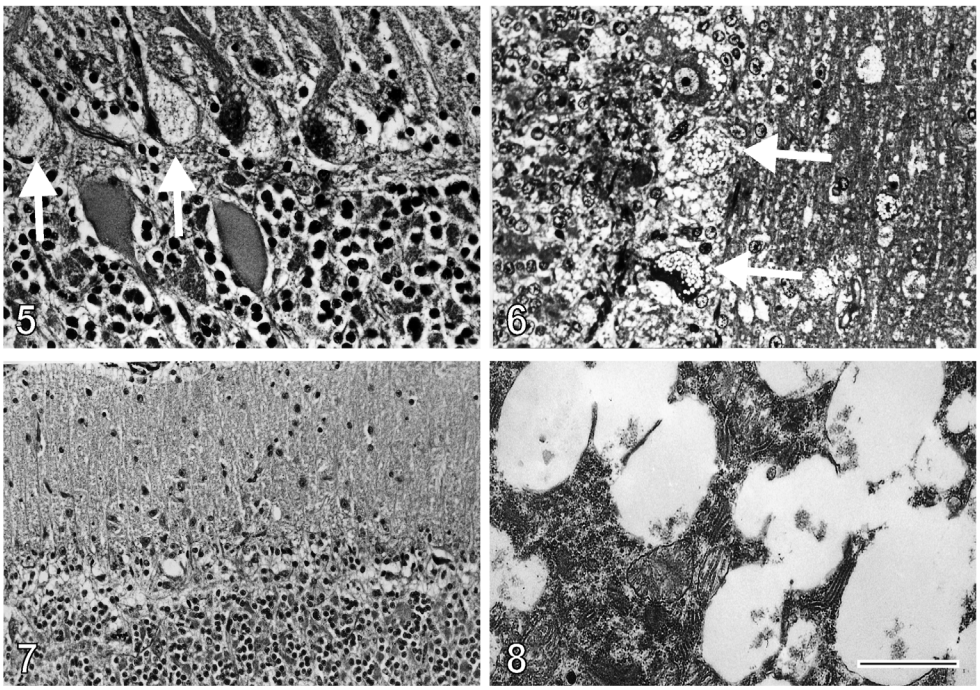


Fig. 5: Cerebellum of Goat No. 1 with naturally occurring *Ipomoea carnea* toxicosis. Purkinje neurons in the cerebellum are distended by numerous cytoplasmic vacuoles (arrows). Note torpedoes in the granular cell layer. HE.
Fig. 6: Cerebellum of Goat No. 2 with naturally occurring *Ipomoea carnea* toxicosis. Note the presence of numerous intracytoplasmic storage vacuoles in Purkinje cells. Semithin section, toluidine blue staining.
Fig. 7: Cerebellum of Goat No. 3 with chronic *Ipomoea carnea* toxicosis. Note loss of Purkinje neurons, vacuolation and gliosis of the Purkinje cell layer, and gliosis in the molecular layer. HE.
Fig. 8: Electron micrograph. Cerebellum of Goat No. 4 that had been fed *Ipomoea carnea* for 72 days. Storage vacuoles in the cytoplasm of a Purkinje neuron appear largely empty, containing small amounts of membranous or granular material. Bar = 1 μm.

started to consume a portion of the *I. carnea* leaves from day 49, but all of the leaves were consumed after day 65. The animal developed diarrhoea, became emaciated, and died on day 89 of the experiment.

Pathology. Goat No. 6 showed minor changes in haemoglobin and haematocrit values. In goats No. 4-6, there were fluctuations of AST levels during the trial. In addition, a slight increase of LDH and a 10-fold increase of CK during the last weeks of the feeding trial occurred in Goat No. 4. BUN and creatinine levels

remained normal for Goat No. 5, but levels of BUN increased for Goats Nos. 4 and 6 before considerable amounts of plant material had been consumed. The total protein content of the blood fluctuated in all the experimental goats. The lowest level was registered for Goat No. 6 with 53 g/litre (normal range, 64-78 g/litre) towards the end of the experiment. The other parameters remained within normal limits.

Gross lesions in Goat No. 4 comprised moderate hydropericardium, slight oedema of the spinal cord, and meningeal congestion. In Goats No. 5 and 6, mild brain oedema was present.

Goats No. 4-6 showed cytoplasmic vacuolation in neuronal and glial cells and axonal spheroids in the nervous system similar to those described in Goat No. 1. Pronounced status spongiosis of the optic tract was observed in Goat No. 5. Vacuolation of neurons in the submucosal and mesenteric plexuses in the small intestine and in tubular epithelial cells in the kidney was also noted in these cases.

Electron microscopy. Ultrastructurally, the vacuoles were membrane bound, containing amorphous membranous fragments or small amounts of granular material within an electron-lucent background (Fig. 8).

Discussion

Ipomoea carnea is a plant of tropical American origin; nevertheless, one subspecies is now pantropical. *Ipomoea carnea subsp. carnea* seems to be confined to its natural distribution, from Peru to Mexico, and *I. carnea subsp. fistulosa* has its natural distribution from Argentina to Florida and Texas and has been introduced to the tropics of the Eastern Hemisphere and Hawaii, where it has often escaped from cultivation [6]. In southern Africa it is found in Zimbabwe, Mozambique, and South Africa [6] and in Zambia and Malawi [17].

Ipomoea carnea flowers throughout the year. *Ipomoea carnea subsp. carnea* prefers dry habitats, whereas *I. carnea subsp. fistulosa* prefers wet habitats. In southern Africa, *I. carnea subsp. fistulosa* is cultivated as an ornamental and in hedges and windbreaks and often occurs as culture relics and escapes from cultivation. It can become established in disturbed areas, such as along roadsides, and to a limited extent in grassland, along river banks, and in other moist areas.

Earlier reports of *I. carnea* poisoning came from Sudan and India [21,35] and toxicity in livestock after the consumption of *I. crassicaulis* (= *I. carnea)* [8,16] has been diagnosed in Karawag, Indonesia [7]. Although the toxicity of the plant and its effects on the nervous system have been reported by these and other authors, a detailed histopathologic examination of the nervous system was either inconclusive or not done [10,16,21,34,35], and the toxic principle(s) and mechanism of intoxication was not determined.

In feeding trials with *I. carnea* in ruminants, elevations in levels of AST and arginase suggested that the nervous signs were a consequence of hepatic damage

rather than primary involvement of the brain and spinal cord [1,10]. In the present study, AST levels in the experimental goats could not be related to hepatic lesions, but a 10-fold increase in CK levels was demonstrated in Goat No. 4, which developed the most apparent central nervous system signs.

Cytoplasmic vacuolation in cells in the nervous system, particularly neurons, and in other tissues in animals with *I. carnea* poisoning are consistent with a lysosomal storage disorder. These histologic changes closely resemble those of the storage diseases induced in livestock by ingestion of locoweeds *(Astralagus and Oxytropis)* [36,37] and poison peas *(Swainsona)* [13,20,25]. In these disorders, storage vacuoles in routinely prepared histologic sections are empty, reflecting the solubility of the undigested metabolises in water or organic solvents used in paraffin embedment. However, granular or membranous remnants may be detected in the vacuoles with electron microscopy [38]. Spheroids, representing swollen axons, and torpedoes, which are enlargements of the proximal segments of Purkinje axons, have also been described in locoweed and *Swain*sona poisoning [18,19,23,25,36,38].

Clinical signs of acute and chronic *I. carnea poi*soning probably reflect impairment of tissues affected by vacuolar lesions, particularly nervous tissue, and range from general depression and ruminal and digestive disorders to staggering gait, reluctance to move, abduction and weakness of the hind limbs, general weakness, tremors, dullness, incoordination, posterior paresis and paralysis, diarrhoea, lacrimation, nasal discharge, and pallor of visible mucous membranes [10,16,21,33,35]. Hyperaesthesia, head tremors, and nystagmus were observed in some of the affected goats.

Clinical recovery in the plant-induced lysosomal storage diseases may occur after animals are withdrawn from the toxic plants [19] and is probably related to the disappearance of neuronal cytoplasmic vacuolation, axonal dystrophy, and meganeurites upon disease reversal [20]. Reversal of clinical signs will probably occur when exposure to the toxic plant was brief or in animals in the early stages of intoxication. In goats necropsied six months and one year after the onset of clinical signs of *I. carnea* poisoning; neuronal cytoplasmic vacuolation was uncommon, and only a few spheroids and no significant loss of neurons in the cerebrum or spinal cord were detected. Residual nervous lesions in these goats were, however, conspicuous in the cerebellum and included degeneration, atrophy, and loss of Purkinje cells and gliosis of the Purkinje cell layer. These findings are in agreement with previous observations suggesting that Purkinje cells are particularly vulnerable to injury in α-mannosidosis [35]. The cerebellar lesions combined with ectopic neurite growth and persistence of associated synapses probably account for the irreversible symptoms of toxicity in some chronic cases [38]. Pigmentation in certain brain areas was also noted in chronic cases of *Swainsona* toxicity [18, 38].

The identification of swainsonine and calystegines B_2 and C_1 as the biologically active substances in *I. carnea* provided further evidence of a lysosomal storage

disorder in the goats. Swainsonine is a potent, reversible inhibitor of α-mannosidase and is present in locoweeds *(Astragalus and Oxytropis)* and *Swainsona* species [9,28], all of which are members of the plant family Leguminosae. Swainsonine toxicosis is thus analogous to heritable α-mannosidosis, which has been described in Angus, Galloway, and Murray Grey cattle and in Persian and mixed-breed cats [32]. Calystegines B_2 and C_1 are also powerful glycosidase inhibitors, affecting β-glucosidase and α- and β-galactosidase [4,31]. Inhibition of α- and β-galactosidase would produce phenocopies of the human genetic lysosomal storage defects Gaucher's disease and Fabry's disease, respectively [11]. Significant indicators of these conditions are epileptiform seizures and vacuolation of Purkinje cells.

Calystegines have so far been identified in members of the plant families Convolvulaceae, Solanaceae, and Moraceae. Their co-occurrence with swainsonine has previously been reported only in two *Ipomoea* species of very limited distribution in Australia, *Ipomoea* sp. Q6 [aff. *calobral*] (Weir vine) and *I. polpha.* Weir vine has been associated with induction of a lysosomal storage disease in livestock [30].

Although this analysis did not permit an accurate quantitative measurement of the alkaloid content in the *I. carnea* sample, the GC-MS detector response was comparable to that observed for the Australian samples, indicating a combined level of calystegines and swainsonine of ca. 0.1%. It has been estimated that locoweeds containing at least 0.001% swainsonine are capable of producing neurologic damage if consumed regularly over a sufficient period of time [29]; the content in *I. carnea* is therefore far in excess of the level necessary to induce poisoning. The exceptional potency of swainsonine as an α-mannosidase inhibitor does not require high levels for toxicity to occur; rather, the length of the grazing period is highly significant because continuous suppression of enzyme activity will lead to the cellular vacuolation characteristic of the poisoning [29].

Except for a single outbreak of a fatal posterior paralysis in exotic goats in Tanzania [26], there have been no reports of the toxicity of *I. carnea* from eastern and southern Africa [24,39]. *Ipomoea carnea* is known to have been planted in Zambia, Zimbabwe, Malawi, and Mozambique [17], and it appears to be widely distributed in southern Africa, especially in the southern part of Mozambique. A few other toxic *Ipomoea* species are known. *Ipomoea batatas,* damaged by various fungi or chemicals, produces stress metabolises of the 3-substituted furan type, causing mouldy sweet potato poisoning, a condition similar to acute bovine pulmonary oedema and emphysema [40]. *Ipomoea violacea,* whose seeds together with certain other morning glories contain derivatives of lysergic acid diethylamide, has hallucinogenic effects in humans [14]. This species does not occur in southern Mozambique [3].

Although *I. carnea* is widely distributed in southern Mozambique, the circumstances that prevailed in the specific area at the time of the outbreak, especially the ongoing civil war and the drought, had restricted the grazing area.

Goats were most likely forced to consume plants in the village that were otherwise not browsed, including *I. carnea.* Nevertheless, after the drought was over, goats showing central nervous symptoms still continued consuming *I. carnea.* These observations suggest that once the goats were forced to eat the plant, some developed a liking for the plant, consuming it even after other food sources became available, as has been observed for *Swainsona* [15,19].

After confirming the toxicity of the plant, the villagers and extension workers were informed about the potential danger of *I. carnea.* Many hedges of *I. carnea* were then removed from the village, resulting in a drastic decrease in the number of new poisoning cases.

Acknowledgements

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References

1. Adam SEI, Tartour G, Obeid HM, Idris OF (1973) Effects of *Ipomoea carnea* on the liver and on serum enzymes in young ruminants. J Comp Path 83:531-542
2. Alroy J, Orgad U, Ucei AA, Gavris VE (1985) Swainsonine toxicosis mimics lectin histochemistry of mannosidosis. Vet Pathol 22:311-316
3. Arnold TH, de Wet BC (1993) Plants of southern Africa: names and distribution. Mem Bot Surv South Afr 62:592-594
4. Asano N, Kato A, Oseki K, Kizu H, Matsui K (1995) Calystegins of *Physalis alkekengi var. francheti* (Solanaceae). Structure determination and their glycosidase inhibitory activities. Eur J Biochem 229: 369-376
5. Austin DF (1977) *Ipomoea caryiea* Jacq. v. *Ipoinoea fistulosa* Mart. ex Choisy. Taxon 26:235-238
6. Austin DF, Huaman Z (1996) A synopsis of *Ipomoea* (Convolvulaceae) in the Americas. Taxon 45:3-38
7. Bahri S (1983) Toxicity of *Ipomoea crassicalis* for guinea pigs. Penyakil Hewan 15:103-104
8. Chaudhuri H, Ramaprabhu T, Ramachandran V (1994) *Ipomoea carnea* Jacq. A new aquatic weed problem in India. J Aquat Plant Manag 32:37-38
9. Colegate SM, Dorling PR, Huxtable CR (1979) A spectroscopic investigation of swainsonine: an α-mannosidase inhibitor isolated from *Swainsona canescens.* Aust J Chem 32:2257-2264
10. Damir HA, Adam SE, Tartour G (1987) The effects of *Ipomoea carnea* on goats and sheep. Vet Hum Toxicol 29:316-319

11. Dorling PR (1984) Lysosomal storage diseases in animals. In: Dingle JT, Dean RT, Sly W (eds), Lysosomes in biology and pathology, Elsevier, Amsterdam, The Netherlands, pp 347-379
12. Dorling PR, Huxtable CR, Colegate SM (1980) Inhibition of lysosomal a-mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens.* Biochem J 191:649-651
13. Dorling PR, Huxtable CR, Vogel P (1978) Lysosomal storage in *Swainsona* spp. toxicosis: an induced mannosidosis. Neuropathol Appl Neurobiol 4:285-295
14. Ellenhorn MJ, Bareeloux DG (1988) Medical toxicology. Diagnosis and treatment of human poisoning, Elsevier, New York, NY, pp 1512
15. Everist SL (1981) Poisonous plants of Australia, 2nd ed, Angus & Robertson, Sydney, Australia, pp 966
16. Gahlot AK, Gupta HK (1984) Toxicity of the plant "Vilayati akadi" *(Ipomoea carnea)* in live-stock. Indian J Vet Med 4:142
17. Gonçalves ML (1987) Convolvulaccae-Cuscutaccae. In: Launert E (ed) Flora Zambesiaca, Flora Zambesiaca Managing Committee, London, England, Vol 8, pp 117-118
18. Hartley WJ (1971) Some observations on the pathology of *Swainsona* spp. poisoning in farm livestock in Eastern Australia. Acta Neuropathol (Berl) 18:342-355
19. Huxtable CR, Dorling PR (1982) Poisoning of livestock by *Swainsona* spp. current status. Aust Vet J 59:50-53
20. Huxtable CR, Dorling PR, Walkley SU (1982) Onset and regression of neuroaxonal lesions in sheep with mannosidosis induced experimentally with swainsonine. Acta Neuropathol (Berl) 58:27-33
21. Idris OF, Tartour G, Adam SEI, Obeid HM (1973) Toxicity to goats of *lpomoea carnea.* Trop Anim Health Prod 5:119-123
22. James LF, Hartley WJ, Van Kampen KR (1981) Syndromes of *Astragalus* poisoning in livestock. J Am Vet Med Assoc 178: 146-150
23. James LF, van Kampen KR, Hartley WJ (1970) Comparative pathology of *Astragalus* (locoweed) and *Swainsona* poisoning in sheep. Pathol Vet 7:116-125
24. Kellerman TS, Coetzer JAW, Naudé TW (1988) Plant poisonings and mycotoxicosis of livestock in Southern Africa, Oxford University Press, Cape Town, South Africa, pp 47-82
25. Locke KB, MeEwan DR, Hamdorf IJ (1980) Experimental poisoning of horses and cattle with *Swainsona canescens var. homiana.* Aust Vet J 56:379-383
26. Maselle RM, Mbassa GK, Jiwa SFH, Ndemanisho E (1989) An epidemic of fatal posterior paralysis and sudden death in goats: a case report. Beitr Trop Landwirtsch Veterinirmed 27(H2): 211-215
27. Molyneux RJ, Benson M, Wong RJ (1988) Australine, a novel pyrrolizidine alkaloid glucosidase inhibitor from *Castanospennum australe.* J Nat Prod 51:1198-1206
28. Molyneux RJ, James LF (1982) Loco intoxication: indolizidine alkaloids of spotted locoweed *(Astragalus lentiginosus).* Science 216:190-191
29. Molyneux RJ, James LF, Ralphs MH (1994) Polyhydroxy alkaloid glycosidase inhibitors from poisonous plants of global distribution: analysis and identification. In: Colegate SM, Dorling PR (eds) Plant-associated toxins. Agricultural, phytochemical and ecological aspects, CAB International, Wallingford, England, pp 107-112

30. Molyneux RJ, McKenzie RA, O'Sullivan BM, Elbein AD (1995) Identification of glycosidase inhibitors swainsonine and calystegine B2 in Weir Vine *(Ipomoea* sp. Q6 [aff. calobral) and correlation with toxicity. J Nat Prod 58:878-886
31. Molyneux RJ, Pan YT, Goldmann A, Tepfer DA, Elbein AD (1993) Calystegins, a novel class of alkaloid glycosidase inhibitors. Arch Biochem Biophys 304:81-88
32. Summers BA, Cummings JF, de Lahunta A (1995) Veterinary neuropathology, Mosby, St. Louis, MO, pp 208-350
33. Tartour G, Adam SEI, Obeid HM, Idris OF (1974) Development of anemia in goats fed with *Ipomoea carnea.* Br Vet J 130:27I-279
34. Tartour G, Obeid HM, Adam SHI, Idris OF (1973) Haematological changes in sheep and calves following prolonged oral administration of *Ipomoea carnea.* Trop Anim Health Prod 5:284-292
35. Tirkey K, Yadava KP, Mandal TK (1987) Effect of aqueous extract of *Ipomoea carnea* on the haematological and biochemical parameters in goats. Indian J Anim Sci 57:1019-1023
36. Van Kampen KR, James LF (1969) Pathology of locoweed poisoning in sheep. Pathol Vet 6:413-423
37. Van Kampen KR, James LF(1970) Pathology of locoweed (A*stragalus lentiginosus)* poisoning in sheep: sequential development of cytoplasmic vacuolation in tissue. Pathol Vet 7:503-508
38. Walkely SU, Siegel DA (1989) Comparative studies of the CNS in swainsonine-induced and inherited feline α-mannosidosis. In: James LF, Elbein AD, Molyneux RJ, Warren CD (eds) Swainsonine and related glycosidase inhibitors, Iowa State University Press, Ames, IA, pp 57-75
39. Watt JM, Breyer-Brandwijk MG (1962) The medicinal and poisonous plants of southern and eastern Africa, 2nd ed, E.& S. Livingstone, Edinburgh, Scottland, pp 1457
40. Wilson BJ, Garst JE, Linnabary RD, Doster AR (1978) Pulmonary toxicity of naturally occurring 3-substituted furans. In: Keeler RF, Van Kampen KR, James LF (eds) The effects of poisonous plants on livestock (proceedings of a joint United States-Australian symposium on poisonous plants), Academic Press, New York, NY, pp 311-323

Chapter ***2.3***

β-MANNOSIDOSIS IN SIMMENTALER CALVES

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Abstract

β-Mannosidosis was diagnosed in two neonatal Simmentaler calves. The animals were unable to rise or stand at birth, showed intermittent hyperextension of the hind limbs when in lateral recumbency, and opisthotonus. Affected calves had doming of the frontal bone, narrowed palpebral fissures, superior brachygnathism and posteriorly orientated pinna. The calves also had a constant fine head tremor and intermittent nystagmus. Necropsy revealed hydrocephalus and myelin pallor in the cerebrum and cerebellum. Microscopically, prominent cytoplasmic vacuolation was present in neurons of the brain and spinal cord, tubular epithelial cells of the kidneys, follicular cells of the thyroid, and macrophages of lymphoid tissues. In the white matter of the cerebrum and cerebellum, a deficiency of myelin and axonal spheroids were demonstrated. Electron microscopy of the cerebellum revealed membrane-bound vacuoles containing granular material or membranous fragments within an electron-lucent background in the cytoplasm of neurons. β-Mannosidosis was biochemically confirmed in one calf by the absence of lymphocyte β-mannosidase activity. In cattle, β-mannosidosis has previously been reported in Salers calves but not in the Simmentaler breed.

Key words: β-mannosidosis, lysosomal storage disease, Simmentaler, calf, cytoplasmic vacuolation, myelin paucity

Introduction

β-Mannosidosis is an inherited, autosomal recessive disorder of glycoprotein catabolism characterised by a deficiency of tissue and plasma β-mannosidase activity and the accumulation of oligosaccharide substrates for β-mannosidase in lysosomes [20,21]. This storage disease was originally described in Nubian goats [11] and has subsequently been reported in cattle [4] and humans [6,33]. Calves with β-mannosidosis are affected at birth and vacuolation of multiple cell types in various body tissues, including the nervous system, is noticeable at light microscopy. Affected animals are born with multiple defects and do not survive the neonatal period [1,4,18]. A definitive diagnosis relies upon the demonstration of a profound deficiency or absence of lymphocyte or tissue β-mannosidase in affected calves [4].

Bovine β-mannosidosis has been described in Salers calves [1,4,18]. This report documents clinical, biochemical and pathological findings of β-mannosidosis in Simmentaler calves.

Materials and methods

Case history

In August 1995 a farmer brought a neurological disorder in new-born Simmentaler calves to our attention. Calves came from a large herd of Simmentaler cows (some were South Devon and Afrikaner crossbred) upon which five pedigree Simmentaler bulls were used (30 cows per bull in separate camps). In one group, a two-year-old bull sired 8 abnormal calves of both sexes and 18 clinically normal calves.

Two calves were obtained for examination, a two-day-old male calf (Calf No. 1) and a four-day-old female calf (Calf No. 2). The other six affected calves were not examined in detail but were identified by the owner on the presence of clinical signs and gross defects. Clinical signs in the calves were remarkably similar. They were affected at birth, were recumbent and unable to rise or stand and could maintain sternal recumbency for a short period when assisted. When in lateral recumbency, they sometimes showed rigid extension of the hind legs accompanied by opisthotonus (Fig. 1). Withdrawal reflexes to painful stimuli were present, and the calves were able to urinate and defecate. A dome-shaped skull, mild superior brachygnathism and vertical narrowing of the palpebral fissures were noted. The pinnae were directed posteriorly (Fig. 1). The calves had a constant fine head tremor and periods of swinging motions of the head. Their eyes exhibited intermittent, rapid, nystagmus that appeared more prominent when the animals were disturbed.

Genetic studies were not performed on the Simmentaler herd. The incriminated bull was culled following the diagnosis of β-mannosidosis in his progeny, while

Fig. 1: Simmentaler calf (Calf No. 2) with β-mannosidosis. Note recumbency, extension of the hind legs and posteriorly directed pinna.

the farmer requested to keep the identities of the affected calves and their parents confidential.

Biochemistry

Lymphocyte α-mannosidase and β-mannosidase activities were determined on blood samples from an affected calf (No. 2), two clinically normal one-week-old siblings (Calves Nos. 3, 4) and a 10-day-old Simmentaler calf from the same herd but from different parents (Calf No. 5; Table 1). Blood was collected in tubes containing acid citrate dextrose as anticoagulant. Samples were kept at 4ºC in an insulated container during transportation, and were received at The South African Institute for Medical Research, Johannesburg within 6 h of collection. Leukocytes were prepared using modifications of a previously decribed technique [30]. The modification was necessary because the presence of dextran did not lead to the desired flocculation of the red cells (and the continuing suspension of white cells). In order to recover the white cells and lyse the red by osmotic shock, equal volumes of the dextran containing mixture were transferred to four glass test tubes and centrifuged. The supernatants were discarded and 1,5 ml chilled water was added to each pellet. The resulting mixture was repeatedly drawn into and expelled from a 1 ml automatic pipette for a period of one minute. A 0, 5 ml volume of 3.6% NaCl was then added to each preparation to restore isotonicity. Following

centrifugation and the removal of the supernatants (containing the remains of the lysed red cells) a further one ml volume of water was added and the procedure repeated until the pelleted white cells were free of red cells. The white cells were frozen at –70 ºC until assayed. α-Mannosidase activity was assayed using the fluorogenic substrate 4-methylumbelliferone-alpha-D-mannoside and β-mannosidase using the substrate 4-methylumbelliferone-beta-D-mannoside according to the method of Masson and coworkers [27] as adapted Jones *et al* [20,21].

Pathology

The calves were killed by intravenous pentobarbital overdose. During the necropsies, a range of organs and tissues including the brain, spinal cord, peripheral nerve (sciatic nerve) were collected in 10 % buffered formalin for histological examination. Tissue samples were routinely prepared and stained with haematoxylin and eosin (HE). Selected sections of the brain and spinal cord were also stained with luxol fast blue/periodic acid-Schiff/haematoxylin (LFB/PAS/H) and luxol fast blue/Holmes (LFB/H). Sections of brain from an age-matched clinically normal calf (Calf No. 6) were used as a control.

Samples of the cerebellum from one calf (No.2) were also fixed by immersion in 2,5% gluteraldehyde in 0,1 M sodium cacodylate buffer (at pH 7,3 - 7,4) within 15 min of euthanasia. Following fixation at room temperature for 24 h, specimens were post-fixed in 2 % osmium tetroxide, dehydrated in a graded ethanol series, passed through propylene oxide, and embedded in Polarbed 812. Semithin (1-2 μm) sections were stained with toluidine blue for tissue orientation. Ultrathin sections were stained with uranyl acetate and lead citrate and studied with a transmission electron microscope.

Results

Biochemistry

Results of enzyme assays for α-mannosidase and β-mannosidase activities are given in Table 1. The absence of lymphocyte β-mannosidase was used as a biochemical confirmation of β-mannosidosis.

Table 1: Determinations of α-mannosidase and β-mannosidase activities in one clinically affected and three clinically normal calves.

Identification of calf	**α-mannosidase activity U (ηmoles/hr/mg protein)**	**β-mannosidase activity U (ηmoles/hr/mg protein)**
No. 1 (affected calf)	Not done	Not done
No. 2 (affected calf)	176,4	No discernible activity
No. 3 (normal sibling)	217,8	17,5
No. 4 (normal sibling)	159,6	13,3
No. 5 (different parents)	102,6	11,2
No. 6 (age-matched control)	Not done	Not done

Gross lesions

Both calves revealed mild internal hydrocephalus with dilation of the lateral ventricles. Poor demarcation between the grey and white matter in the cerebrum and cerebellum was observed (Figs. 2, 3). Kidneys were moderately swollen and on cut surface the cortices were slightly greenish to greyish-green.

Light microscopical lesions

Nervous tissue:
With minor exception, lesions were similar in the two affected calves. Cytoplasmic vacuolation of multiple cell types occurred in various organs. In the cerebrum, a large proportion of neurons contained roundish cytoplasmic vacuoles. The vacuoles were of variable size, mostly empty although larger vacuoles contained eosinophilic thin strands of material. A variable degree of vacuolation was observed among neurons: in some cells the vacuoles were numerous giving affected neurons a foamy appearance, while in others only few vacuoles were evident. Neuronal nuclei were either centrally or peripherally located but appeared normal.

In the cerebellum, cytoplasmic vacuolation was prominent in Purkinje cells (Fig. 4). These neurons often contained a few large peripherally located vacuoles, which coalesced and distorted the cell shape. In addition, loss of Nissl substance, eosinophilia of the cytoplasm and prominent cytoplasmic processes (axons) were present. In one calf (No. 2), loss of Purkinje cells, vacuolation of the Purkinje cell layer, proliferation of Bergmann's glia and perivascular gliosis in the molecular layer were noticeable in several gyri. Axonal spheroids, which appeared lightly eosinophilic in sections stained with HE, were occasionally present in the granular layer of the cerebellum in one calf (No. 2, Fig. 5). Cytoplasmic vacuolation was also noted in Golgi II cells and in deep nuclei.

Fig. 2: Coronal section of a cerebral hemisphere (Calf No. 2). There is mild ventricular dilation and no clear distinction between grey and white matter indicating a paucity of myelin.
Fig. 3: Cross section of the cerebellum and medulla oblongata: Calf No. 2. There is no visible white matter due to a severe deficiency of myelin.

Light microscopy confirmed deficiency of myelin in the cerebral and cerebellar white matter. This was best illustrated by a reduction in myelin staining with luxol fast blue in the cerebrum and cerebellum when compared with sections of the age-matched control calf (No. 6; Figs. 6,7). In areas of most profound myelin paucity, axons were prominent but appeared intact while roundish axonal spheroids

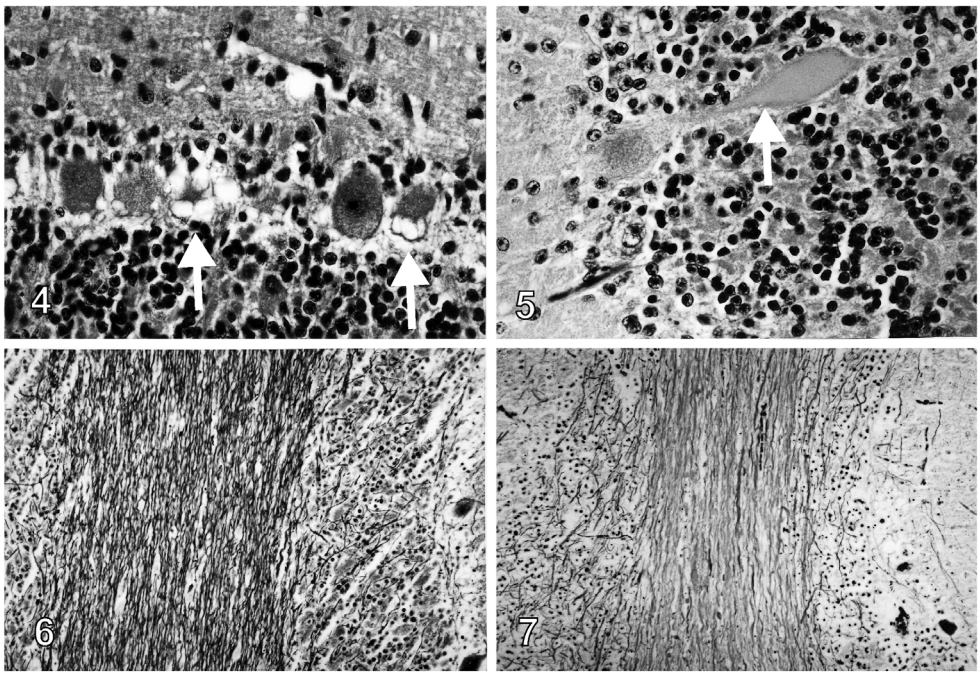

Fig. 4: Cerebellum; Calf No. 1. The cytoplasm of Purkinje cells contain vacuoles of varying size causing distortion of some cells (arrows). HE.
Fig. 5: Cerebellum; Calf No. 2. Axonal swelling of fusiform shape in a Purkinje cell (torpedo, arrow). HE.
Fig. 6: Cerebellar folium; control calf (No. 6). LFB/H.
Fig. 7: Cerebellar folium; β-mannosidosis-affected calf (No. 1). A marked reduction in myelin in the white matter is evident. Axons appear intact. LFB/H.

occasionally surrounded by a myelin sheath were seen. Oligodendrocytes often appeared finely vacuolated.

In the spinal cord, vacuolation of neurons in the dorsal and ventral horns was marked and axonal spheroids identified in the grey matter (Fig. 8). The degree of myelination in the white matter of the spinal cord of the affected calves was similar to that of the control calf as assessed by luxol fast blue staining.

Sections of the sciatic nerve revealed vacuolation of Schwann cells and of perivascular cells, and multifocal vacuolation of myelin. Fine vacuolation of ganglion cells in the plexuses of the intestine was noticeable.

Other tissues:
In the kidneys, severe vacuolation of epithelial cells in especially the proximal and distal convoluted tubules was noted (Fig. 9). The vacuoles were of different size: both large and small vacuoles were present within a cell, while other epithelial cells appeared to contain either few large vacuoles or numerous small vacuoles of simi-

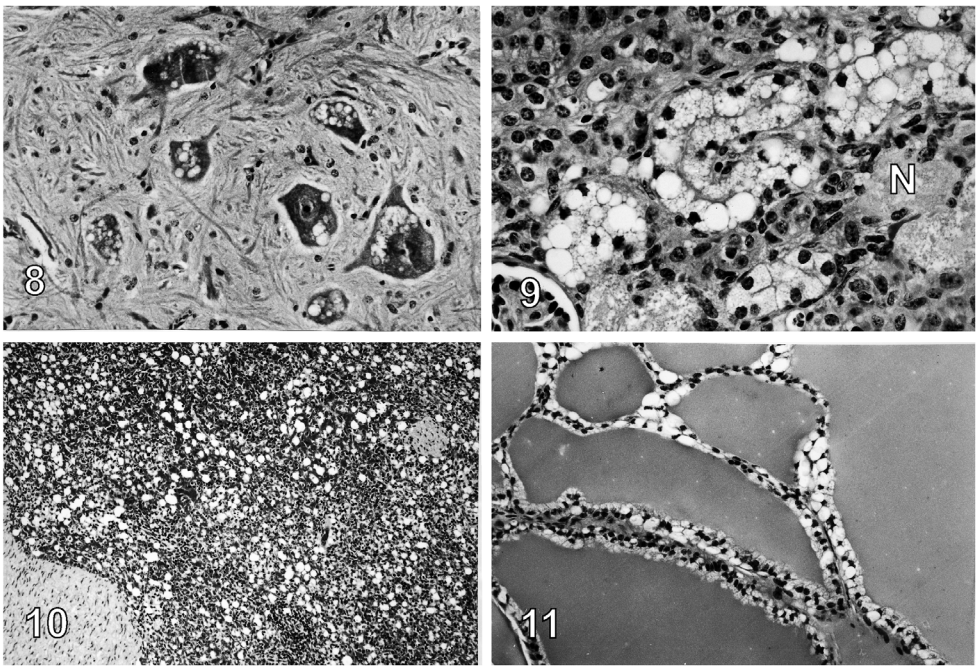

Fig. 8: Spinal cord; Calf No. 2. Ventral horn neurons reveal prominent cytoplasmic vacuolation. HE.
Fig. 9: Kidney; Calf No. 1. Several tubular epithelial cells contain multiple cytoplasmic vacuoles. Some tubules are necrotic (N). HE
Fig. 10: Spleen; Calf No. 2. The red pulp has a vacuolated appearance on low power caused by widespread cytoplasmic vacuolation. HE.
Fig. 11: Thyroid gland; Calf No. 1. Note diffuse vacuolation of follicular epithelial cells. HE.

lar size. Epithelial cells in the cortex not vacuolated were degenerative with a distended granular, lightly eosinophilic cytoplasm, or, less commonly, were necrotic. Tubular epithelial cells in the medulla and pelvic epithelial cells showed mild cytoplasmic vacuolation. Tubules especially in the medulla frequently contained protein and cellular casts.

In the spleen, the parenchyma had a vacuolated appearance due to severe cytoplasmic vacuolation of cells interpreted to be macrophages (Fig. 10). The white pulp was severely depleted and lymphoid nodules few and smaller than normal. Less severe cytoplasmic vacuolation occurred in the mesenteric lymph nodes, thymus, and Peyer's patches.

Sections of the thyroid gland revealed follicles of variable size containing colloid. Follicular epithelial cells were markedly vacuolated containing multiple small vacuoles or one to three large vacuoles (Fig. 11). In the medulla of the adre-

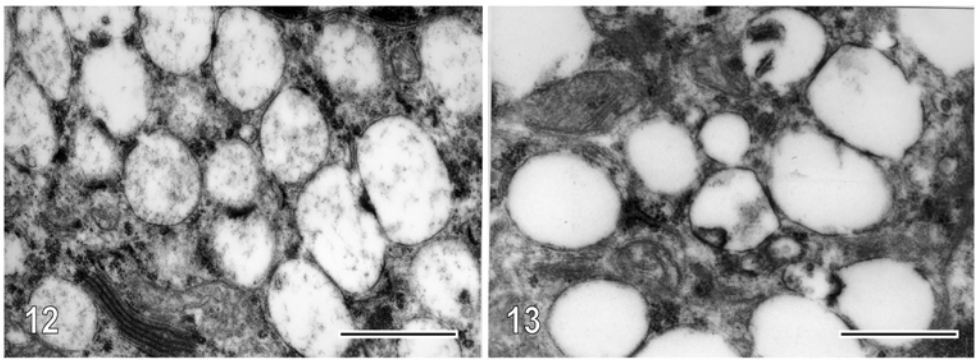

Fig. 12: Transmission electron micrograph. Cerebellum; Calf No. 2. The cytoplasm of a Purkinje cell is distended by membrane-bound vacuoles containing granular material within an electron-lucent background. Uranyl acetate and lead citrate. Bar = 1 μm.
Fig. 13: Transmission electron micrograph. Cerebellum; Calf No. 2. In this Purkinje cell, the lysosomes are generally empty or occasionally contain membranous fragments. Uranyl acetate and lead citrate. Bar = 0,5 μm.

nal gland, scattered cells had cytoplasmic vacuolation. Vacuolation was prominent in Kuppfer cells and macrophages in the liver.

Ultrastructural lesions

Purkinje cells and Golgi II cells in the cerebellar cortex revealed multiple membrane-bound cytoplasmic vacuoles 0,5-3,8 μm in diameter. The vacuoles contained finely granular or occasionally scattered membranous fragments within an electron-lucent background (Figs. 12, 13).

Discussion

In this study, one of the two affected Simmentaler calves (No. 2) had no detectable β-mannosidase activity in lymphocytes, allowing a definitive diagnosis of β-mannosidosis [4]. This storage disease has been studied in detail in Salers calves but has not previously been reported in the Simmentaler breed or, to the best of our knowledge, in any other cattle breed.

Clinically and pathologically, β-mannosidosis in Salers and Simmentaler calves are similar and closely resembles the condition in Nubian goats [13,19]. In Salers calves there is strong evidence that β-mannosidase deficiency is a genetic autosomal recessive condition [4]. Data in support of an autosomal recessive mode of inheritance in the present case include: the pregnant animals were not exposed to plants known to cause an induced storage disease; the parents of the calves were

phenotypically normal; both male and female calves were affected; and distinctive lesions in both affected calves (Nos. 1, 2) were observed.

The goat and bovine β-mannosidase cDNA sequences encoding the enzyme have been reported [5,22,23]. The molecular basis for the storage disease in the goat was found to be a single base deletion at position 1398 in the 2640-bp coding region, resulting in a deduced peptide of 481 amino acids instead of a 879 amino acid peptide [23]. More recently, a single-base deletion at position 2574 in bovine β-mannosidosis was identified, creating a premature stop codon in the protein coding region [5,22]. Diagnostic tests were developed to detect the mutations in both species [22,23].

Human β-mannosidosis is more variable in presentation and cases generally have a milder clinical expression and a later stage of onset. The phenotypes range from mild peripheral neuropathy and depression to facial dysmorphism, mental retardation, and speech and hearing defects [31]. In animals there is lysosomal storage of the trisaccaride Manβ1-4GlcNAcβ1-4GlcNAc and the disaccharide Manβ1-4GlcNAc [21]. The primary storage product in the human disease is the disaccharide, Manβ1-4GlcNAc [31]. It needs to be established however, whether differences in disease expression between ruminants and humans are primarily related to the type of mutations, species differences in developmental requirements for enzyme function, the nature of the storage products, or effects on thyroid function.

In affected Salers and Simmentaler calves, light microscopy confirmed cytoplasmic vacuolation of multiple cell types with prominent involvement of neurons in the brain and spinal cord, tubular epithelial cells in the kidneys, follicular cells in the thyroid, and macrophages in lymphoid tissues (thymus, lymph nodes, spleen and Peyer's patches) [1,4,17,28]. Ultrastructurally, affected cells had membrane-bound lysosomes sometimes filled with electron-lucent material. The nature, severity, and regional distribution of cytoplasmic vacuolation in the nervous system was studied in great detail in Salers calves [28]. The authors used semithin histologic sections allowing a more accurate assessment of affected cells than routinely prepared tissue sections stained with HE. In the calves studied by them, cytoplasmic vacuolation was demonstrated in most neurons and glial cells in the cerebral cortex but was more variable and less extensive in the brain stem. In the cerebellum, Purkinje cells and Golgi II cells were affected. Cytoplasmic vacuolation was noticeable in ventral horn and occasional dorsal horn neurons and glia in the spinal cord. Other cells affected in the nervous system were perivascular cells, endothelial cells and choroid plexus and ependymal epithelial cells [28]. In contrast to goats, Salers calves with β-mannosidosis are not deaf although there is cytoplasmic vacuolation in a range of cells in the ears of affected animals [29].

Myelin deficiency was prominent in Salers calves, in the Simmentaler calves in this study, and in goats with β-mannosidosis [4,19,24,28]. The regional distribution of hypomyelination in the central nervous system in affected Salers calves was similar to that described in caprine β-mannosidosis. The most severe reduction of

myelin was demonstrated in regions, which become myelinated late in gestation such as the corpus callosum, while early myelinating areas such as the spinal cord showed mild or no hypomyelination. The reason for the hypomyelination is unknown. Calves and goats with β-mannosidase deficiency however, suffer from congenital hypothyroidism and it has been proposed that thyroid hormone deficiency may play a role in the pathogenesis of myelin deficiency in β-mannosidosis [3,26,28].

Axonal spheroids have been reported in Salers calves [28] and goats [19,25] with β-mannosidosis. Electron microscopy showed that the spheroids in goats contained variable proportions of dense bodies, vesicles, membranous whorls, mitochondria, and neurofilaments [19,25]. Axonal spheroids have also been described in bovine [2,9,17] and feline α-mannosidosis [30], the lysosomal storage diseases induced by the ingestion of locoweeds (*Astralagus* and *Oxytropis* spp.) and poison peas (*Swainsona* spp.) in sheep [8,10,15,16] and the plant *Ipomea carnea* in goats [7]. The pathogenesis and significance of axonal spheroid formation is not clear. They may occur independent of myelin abnormalities [28], and may be reversible and without observable neurological sequellae in the ovine foetus in locoweeed poisoning [10,25]. In adult sheep with α-mannosidosis, the onset of neurological signs coincided with the appearance of axonal lesions rather than cytoplasmic vacuolation [8,19], while in swainsonine-treated rats axonal dystrophy developed in parallel to the accumulation of storage material and vacuolation in neurons [15]. Proposed mechanisms for the development of axonal lesions may relate to the accumulation of storage material in nerve cells bodies or interference with axonal transport [19,28].

α-Mannosidosis has been described in Aberdeen Angus [2,17], Galloway [9] and Murray Grey breeds [12]. In this storage disease, clinical signs and lesions are less prominent than those reported in β-mannosidosis and a variation in the phenotypic expression within and between breeds have been reported. Calves may be born prematurely or die soon after death, while in others clinical signs become apparent within the first year of life or even up to 15 months of age. Affected calves are often in poor condition and show general enlargement of lymph nodes and mild hydrocephalus.

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References

1. Abbitt B, Jones MZ, Kasari TR, Storts RW, Templeton JW, Holland PS, Castenson PE (1991) β-Mannosidosis in twelve salers calves. J Am Vet Med Assoc 198:109-113
2. Barlow RM, MacKellar A, Newlands G, Wiseman A, Berrett S (1981) Mannosidosis in Aberdeen Angus cattle. Vet Rec 109:441-445

3. Boyer PJ, Jones MZ, Nachreiner RF, Refsal KR, Common RS, Kelley J, Lovell KL (1990) Caprine β-mannosidosis. Abnormal thyroid structure and function in a lysosomal storage disease. Lab Invest 63:100-106
4. Bryan L, Schmutz S, Hodges SD, Snyder FF(1993) Bovine β-mannosidosis: pathologic and genetic findings in Salers calves. Vet Pathol 30:130-139
5. Chen H, Leipprandt R, Traviss CE, Sopher BL, Jones MZ, Cavanagh KT, Friderici KH (1995) Molecular cloning and characterization of bovine β-mannosidase. J Biol Chem 270:3841-3848
6. Cooper A, Sardharwalla IB, Roberts MM (1986) Human β-mannosidase deficiency. N Engl J Med 315:1231
7. De Balogh KKIM, Dimande AP, Van der Lugt JJ, Molyneux RJ, Naudé TW, Welman WG (1999) A lysosomal storage disease induced by *Ipomoea carnea* in goats in Mozambique. J Vet Diagn Invest 11:266-273
8. Dorling PR, Huxtable CR, Vogel P (1978) Lysosomal storage in *Swainsona* spp. toxicosis: an induced mannosidosis. J Neuropathol Appl Neurobiol 4:285-295
9. Embury DH, Jerrett IV (1985) Mannosidosis in Galloway calves. Vet Pathol 22:548-551
10. Hartley WJ (1971) Some observations on the pathology of *Swainsona* spp. poisoning in farm livestock in Eastern Australia. Acta Neuropathol (Berl) 18:342-355
11. Hartley WJ, Blakemore WF (1973) Neurovisceral storage and dysmyelinogenesis in neonatal goats. Acta Neuropathol (Berl) 25:325-333
12. Healy PJ, Cole AE (1976) Heterozygotes for mannosidosis in Angus and Murray Grey cattle. Aust Vet J 52:385-386
13. Healy PJ, Seaman JT, Gardner IA, Sewell CA (1981) β-Mannosidase deficiency in Anglo Nubian goats. Aust Vet J 57:504-507
14. Huxtable CR, Dorling PR, (1982) Poisoning of livestock by *Swainsona* spp. current status. Aust Vet J 59:50-53
15. Huxtable CR, Dorling PR (1985) Mannoside storage and axonal dystrophy in sensory neurons of Swainsonine-treated rats: morphogenesis of lesions. Acta Neuropathol (Berl) 68:65-73
16. James LF, van Kampen KR, Hartley WJ (1970) Comparative pathology of *Astragalus* (locoweed) and *Swainsona* poisoning in sheep. Pathol Vet 7:116-125
17. Jolly RD, Thompson KG (1978) The pathology of bovine mannosidosis. Vet Pathol 15:141-152
18. Jolly RD, Thompson KG, Bayliss SL, Vidler BM, Orr MB, Healy PJ (1990) *β*-Mannosidosis in a Salers calf: a new storage disease of cattle. N Z Vet J 38:102-105
19. Jones MZ, Cunningham JG, Dade AW, Alessi DM, Mostosky UV, Vorro JR, Benetiz JT, Lovell KL (1983) Caprine β-mannosidosis: clinical and pathological features. J Neuropathol Exp Neurol 42:268-285
20. Jones MZ, Dawson G (1981) Caprine β-mannosidosis: inherited deficiency of β-D-mannosidose. J Biol Chem 256:5185-5188
21. Jones MZ, Laine RA (1981) Caprine oligosaccharide storage disease: accumulation of β-mannosyl(1-4)β-N-acetylglucosaminyl(1-4)β-N-acetylglucosamine in brain. J Biol Chem 256:5181-5184
22. Leipprandt JR, Chen H, Horvath JE, Qiao XT, Jones MZ, Friderici KH (1999) Identification of a bovine β-mannosidosis mutation and detection of two β-mannosidase pseudogenes. Mamm Genome 10:1137-1141

23. Leipprandt JR, Kraemer SA, Haithcock BE, Chen H, Dyme JL, Cavanagh KT, Friderici KH, Jones MZ (1996) Caprine β-mannosidase: sequencing and characterization of the cDNA and identification of the molecular defect of caprine β-mannosidosis. Genomics 37:51-56
24. Lovell KL, Jones MZ (1983) Distribution of central nervous system lesions in β-mannosidosis. Acta Neuropathol (Berl) 62:121-126
25. Lovell KL, Jones MZ (1985) Axonal and myelin lesions in β-mannosidosis: ultrastructural characteristics. Acta Neuropathol (Berl) 65:293-299
26. Lovell KL, Jones MZ, Patterson J, Abbitt B, Castenson P (1991) Thyroid structure and function in bovine β-mannosidosis. J Inherit Metab Dis 14: 228-230
27. Masson PK, Lundblad A, Autio S (1974) Mannosidosis: detection of the disease and of heterozygotes using serum and leucocytes. Biochem Biophys Res Commun 56:296-303
28. Patterson JS, Jones MZ, Lovell KL, Abbitt B (1991) Neuropathology of bovine β-mannosidosis. J Neuropathol Exp Neurol 50:538-546
29. Render JA, Lovell KL, Keller CB, Storts RW, Abbitt B, Jones MZ, Castenson PE, Hunter JF (1992) The ocular and otic pathology of bovine β-mannosidosis. J Vet Diag Invest 4:96-98
30. Skoog WA, Beck WS (1956) Studies on the fibrinogen, dextran and phytohaemagglutinin methods of isolating leukocytes. Blood 11:436-454
31. Thomas GH, Beaudet AL (1995) Disorders of glycoprotein degradation and structure: α-mannosidosis, β-mannosidosis, fucosidosis, sialidosis, aspartylglucosaminuria, and carbohydrate-deficient glycoprotein syndrome. In: Scriver AR, Beaudet AL, Sly WS, Valle D, Stanbury JB, Wyngaarden JB, Fredrickson DS (eds) The Metabolic and Molecular Basis of Inherited Disease, pp 2529-2562
32. Vandevelde M, Frankhauser R, Bichsel P, Wiesman U, Herschkowitz N (1982) Hereditary neurovisceral mannosidosis associated with α-mannosidase deficiency in a family of Persian cats. Acta Neuropathol (Berl) 58:64-68
33. Wenger DA, Sujansky E, Fennessey PV, Thompson JN (1986) Human β-mannosidase deficiency. N Engl J Med 315:1201-1205

Chapter ***2.4***

CEREBELLAR CORTICAL DEGENERATION IN CATTLE CAUSED BY *SOLANUM KWEBENSE*

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Abstract

'Maldronksiekte' is an irreversible cerebellar disorder of cattle due to ingestion of the plant *Solanum kwebense* in certain parts of South Africa. The toxicosis is characterised by degeneration, necrosis and loss of cerebellar Purkinje cells and ultimately in cerebellar atrophy. Intoxication of cattle by *S. fastigiatum* in Brazil manifests with similar clinical signs and pathology and, based on the accumulation of membranous cytoplasmic bodies in affected Purkinje cells, the condition is believed to be an induced lysosomal storage disease. The pathology of 'maldronksiekte' was studied in three typical field cases and the ultrastructural changes in the cerebellar cortex are reported. Affected Purkinje cells displayed two types of degeneration and necrosis: they were either swollen, pale and had fine vacuolation of the perikaryon or were shrunken, intensely eosinophilic and less prominently vacuolated. Loss of Purkinje neurons was evidenced by empty baskets, Bergmann's cell gliosis in areas of Purkinje cell loss, and the presence of spheroids and torpedoes in the granular layer. Ultrastructurally, neurons with a swollen perikaryon showed depletion of granular endoplasmic reticulum, empty dilated cisternae of endoplasmic reticulum and swollen mitochondria with partial loss of cristae. In a few Purkinje cells, the cytoplasm contained small numbers of lamellar bodies and membranous bodies. In shrunken neurons, the highly condensed cytoplasm contained distended Golgi saccules, dense clusters of granular endoplasmic reticulum and swollen mitochondria. Vacuolated neurons and axonal spheroids were present in the fastigial, interposital and lateral nuclei as well as in vestibular nuclei. The folia and central cerebellar white matter displayed mild multifocal Wallerian degeneration. Lectin histochemistry was applied to formalin-fixed paraffin-embedded sections of cerebellum. The vacuoles in the cytoplasm of some distended Purkinje cells and of neurons in cerebellar nuclei stained strongly with *Canavalia ensiformis* (ConA) agglutinin. Weak positive staining with *Triticum vulgaris* (WGA) and succinyl-WGA (S-WGA) agglutinin in swollen and shrunken Purkinje cells were noted. The cerebellum of the affected cattle were examined for evidence of apoptosis by means of commercial deoxyuridine triphosphate nick-end labeling (TUNEL) method. Apoptosis was not detected in Purkinje cells and neurons in the cerebellum of cattle with 'maldronksiekte'. This study showed that cerebellar Purkinje neurons are consistently and particularly severely affected in *S. kwebense* intoxication and that the condition is morphologically reminiscent of a cerebellar cortical degeneration. Small numbers of membranous bodies in affected Purkinje cells and the neuronal degeneration and necrosis may result from the inability of these neurons to metabolise a plant toxin or cellular substrate.

Key words: *Solanum kwebense*, cerebellar cortical degeneration, Purkinje cells, cattle

Introduction

Ingestion of the plant *Solanum kwebense* N.E. Br. (Solanaceae) may induce a neurological condition known in South Africa as 'maldronksiekte' (which means, literally, mad-drunk-disease) [23]. The plant is a branched shrub up to 2 m in height and bears characteristic orange-red to scarlet berries (Figs. 1,2).

Fig. 1: *Solanum kwebense* (arrow) grows up to two metres high often in the shade of *Acacia erubescense* (geelhaak) trees.
Fig. 2: Characteristic berries of *Solanum kwebense* (courtesy Oxford University Press, Cape Town) .

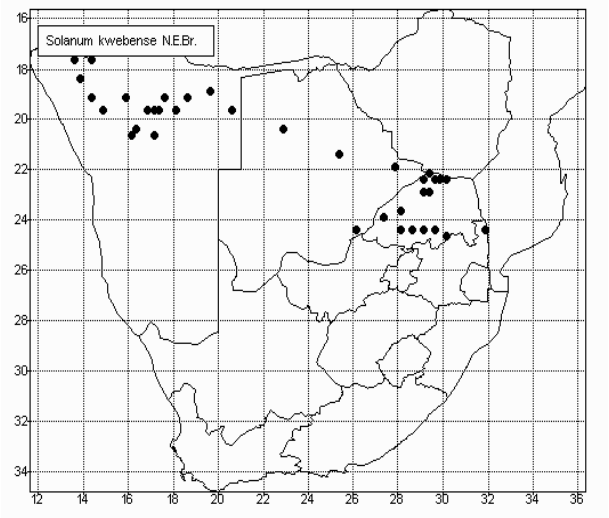


Fig. 3: Distribution of the plant *Solanum kwebense* (courtesy Botanical Research Institute, Pretoria).

Although the plant occurs in the Northern Province of South Africa, Zimbabwe, Botswana and Namibia (Fig. 3), 'maldronksiekte' has only been reported in the northern and north-western parts of the Northern Province [15]. On these farms, overgrazing may causes replacement of the palatable *Panicum maximum* grass, growing predominantly in the shade of *Acacia erubescense* (geelhaak) trees, by *S. kwebense* (Fig. 1). Once *S. kwebense* becomes established, other grasses will not grow in their vicinity and cattle may be forced to feed on it [15]. The toxic principle of *S. kwebense* has not been characterised.

Clinical signs relate to cerebellar dysfunction and are precipitated by various stimuli such as exercise, handling and fright [15,23]. Signs may vary from lateral head tilt, ataxia or muscle tremors to a temporary loss of balance or epileptiform seizures. In mildly affected animals, hypermetria and dysmetria often accompany a tilted head or rigid neck. Some animals show stargazing, extension of the neck, a wide-based crouching stance followed by stumbling. Severely affected cattle often fall down, exhibit temporarily muscle tremors and after regaining their feet recover rapidly from the attack. Pienaar and co-workers described clinical signs and gross and light microscopical pathology in experimentally produced and field cases of 'maldronksiekte' [23]. Some cattle had gross evidence of cerebellar atrophy with thin folia. Light microscopical changes in the cerebellum revealed cytoplasmic vacuolation and swelling, necrosis and eventual loss of Purkinje cells.

In this publications the macro- and microscopical pathology of 'maldronksiekte' in 3 cattle are described. This is also the first report on the ultrastructural changes in the cerebellar cortex of this intoxication. In addition, lectin histochemistry was applied to sections of the cerebellum and evidence for DNA fragmentation in

degenerating neurons in the cerebellum was sought by means of a modified terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) method.

Materials and methods

Animals

Three affected cattle (Bovine Nos. 1-3) ranging in age from 15 to 36 months were obtained from farms were *Solanum kwebense* poisoning was previously diagnosed and sent to the Onderstepoort Veterinary Institute for study. The animals exhibited clinical signs consistent with ''maldronksiekte' [15,23].

Pathology

The animals were killed by intravenous injection of pentobarbitone sodium and necropsied. Specimens of brain, spinal cord including spinal ganglia and sciatic nerve were fixed by immersion in 10% buffered formalin, processed routinely and embedded in paraffin wax. Sections were cut at 4-6 µm thickness and stained with haematoxylin and eosin (HE). Selected sections were stained with luxol fast blue/Holmes (LFB/H) and luxol fast blue/periodic acid-Schiff/haematoxylin (LFB/PAS/H) [16].

Specimens of the cerebellum of the three animals were collected (within 10-15 min of euthanasia), diced into 1 mm cubes and fixed by immersion in 2,5% gluteraldehyde in 0,1 M sodium cacodylate buffer (at pH 7,3 - 7,4) within 15 min of euthanasia. Following fixation at room temperature for 24 h, specimens were post-fixed in 2 % osmium tetroxide, dehydrated in a graded ethanol series, passed through propylene oxide, and embedded in Polarbed 812. Semithin (1-2 µm) sections were stained with toluidine blue for tissue orientation. Ultrathin sections were stained with uranyl acetate and lead citrate and studied with a transmission electron microscope (Jeol JEM Ex Mk I).

Lectin histochemistry

Sections of the cerebellum from the affected cattle and a normal bovine were used for lectin histochemical examination. The histochemical technique has been described previously [1]. Paraffin sections (5 µm) of formalin-fixed tissue were mounted on slides coated with poly L-lysine and dried overnight at 55 ºC. The sections were then deparaffinized and rehydrated in series of xylene, alcohol (100%, 96% and 70%) and Milli Q (every step two times for five min). The slides were treated with 0.1% pronase (Boehringer Mannheim, GmbH, Germany) in Milli Q for 5 min, rinsed in Milli Q and endogenous peroxidase was blocked by immersing the slides in 0.3% hydrogen peroxide in methanol for 30 min. After

rinsing in phosphate-buffered saline (PBS) with 0.1% Tween 20, the slides were incubated with each of nine biotinylated lectins (Vector Laboratories, Burlingame, CA, USA;Table 1) overnight. The biotinylated lectins were used at the following concentrations: jack bean (*Canavalia ensiformis* (*ConA*)) agglutinin 2 µg/ml, horse gram (*Dolichos biflorus* (DBA)) agglutinin 10 µg/ml, peanut (*Arachis hypogea* (PNA)) agglutinin 10 µg/ml, castor bean (*Ricinus communis* (RCA-I)) agglutinin 2 µg/ml, soybean (*Glycine max* (SBA)) agglutinin 20 µg/ml, gorse (*Ulex europaeus* UEA-I)) agglutinin 100 µg/ml, wheat germ (*Triticum vulgaris* (WGA)) agglutinin 2 µg/ml, succinyl wheat germ (*succinyl*-WGA (S-WGA)) agglutinin 12,5 µg/ml, and griffonia (*Bandeira simplicifolia* BSL-I)) agglutinin 10 µg/ml. After rinsing in PBS/Tween, the sections were incubated with avidin-biotin-peroxidase complex (Vectastain ABC Kit, Vector Laboratories, Burlingame, CA, USA) for 45 minutes, washed in PBS, incubated in 3,3 diaminobenzidine (DAB), counterstained with Mayer's haematoxylin, rehydrated and coverslipped. Incubation of each lectin with its corresponding sugar (Table 1) served as a control for binding specificity [26]. The sugars were diluted in PBS and incubated for 30 min at a concentration of 0,2 M except for L-fucose which was used at a dilution of 0,1 M and D-glucosamine at a dilution of 0,5 M. Incubation of tissue sections with PBS alone served as non-specific negative controls.

Table 1: Lectins used on sections of cerebellum of cattle poisoned with *Solanum kwebense*

Lectin	**Major sugar specificity***	**Binding inhibitor***
Canavalia ensiformis	α-D-Man, α-D-Glc	α-D-methyl-Man
Triticum vulgaris	β-D-GlcNAc, NeuNAc	NeuNAC
Dolichos biflorus	α-D-GalNAc	α-D-GalNAc
Arachis hypogea	β-D-Gal(1-3)GalNAc	Lactose
Glycine max	α-D-GalNAc, α-D-Gal	α-D-GalNAc
Succinyl-WGA	$(\beta\text{-}(1\text{-}4)\text{-D-GlcNac})^2$	β-D-GlcNAc
Ricinus communis	β-D-Gal	Lactose
Bandeira simplicifolia	β-D-Gal	Lactose
Ulex europaeus	α-L-fucose	α-L-fucose

* Gal = Galactose; GalNAc = N-acetyl-galactosamine; GlcNac = N-acetyl-glucosamine; NeuNAc = N-acetyl-glucosamine; Man = Mannose

In situ detection of apoptosis

To detect apoptotic cells in the cerebellum of the affected cattle, a modified terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) method (NeuroTACS™ II *in situ* apoptosis detection kit,

R&D Systems, Minneapolis, USA) was used according to the manufacturer's instructions. Briefly, 5-µm-thick paraffin-embedded sections of formalin-fixed cerebellum were mounted on slides coated with poly L-lysine, dried and heated at 58°C for 5 min. The sections were then deparaffined, treated with NeuroPore™ for 30 min, washed, treated with H_2O_2, washed with terminal deoxynucleotidyl transferase (TdT) labeling buffer and incubated with labeling reaction mix for one hour at 37°C. After washing, the sections were incubated with streptavidine/horseradish peroxidase solution, stained with diaminobenzidine and counterstained. Nuclease-generated slides of cerebellum were used as a positive control. As a negative control, TdT was excluded in the protocol.

Results

Gross pathology

Gross inspection of the brains revealed a slightly reduced size of the cerebellum in two of the three animals.

Light microscopy

The cerebellum in the three animals revealed widespread degeneration and loss of Purkinje cells (Figs. 4-6). The degree and extent of neuronal changes varied among folia and there was no distinct pattern of distribution of the lesions in the cerebellum. Purkinje cells showed two types of degeneration and necrosis. A large proportion of neurons were swollen and pale and exhibited fine vacuolation of the perikaryon while a few contained large, irregular vacuoles (Figs. 4,5). The vacuoles did not stain with PAS. Other Purkinje cells were shrunken and deeply eosinophilic and less severely vacuolated. Nuclei in both swollen and shrunken cells were often eccentric and pyknotic or fragmented. Dendrites of affected Purkinje cells were occasionally swollen and more prominent than usual. Empty basket formation as a result of loss of Purkinje cells was noticed in sections stained with LFB/H.

Isolated axonal spheroids, presumed to originate from Purkinje neurons, and very few torpedoes in the granular layer were seen. There was also vacuolation of the molecular layer and proliferation of glial cells (Bergmann's glia) in several folia (Fig. 6). In two animals, a reduction in thickness of the molecular layer and depletion of the granular cell layer in folia exhibiting extensive loss of Purkinje cells were apparent (Fig. 7).

A small number of vacuolated neurons and axonal spheroids were present in fastigial, interposital, lateral nuclei and in vestibular nuclei. The folia and central cerebellar white matter displayed mild multifocal Wallerian degeneration and gliosis (Fig. 8). Small numbers of perivascular macrophages in the white matter contained yellowish-brown, granular pigment resembling lipofuscin.

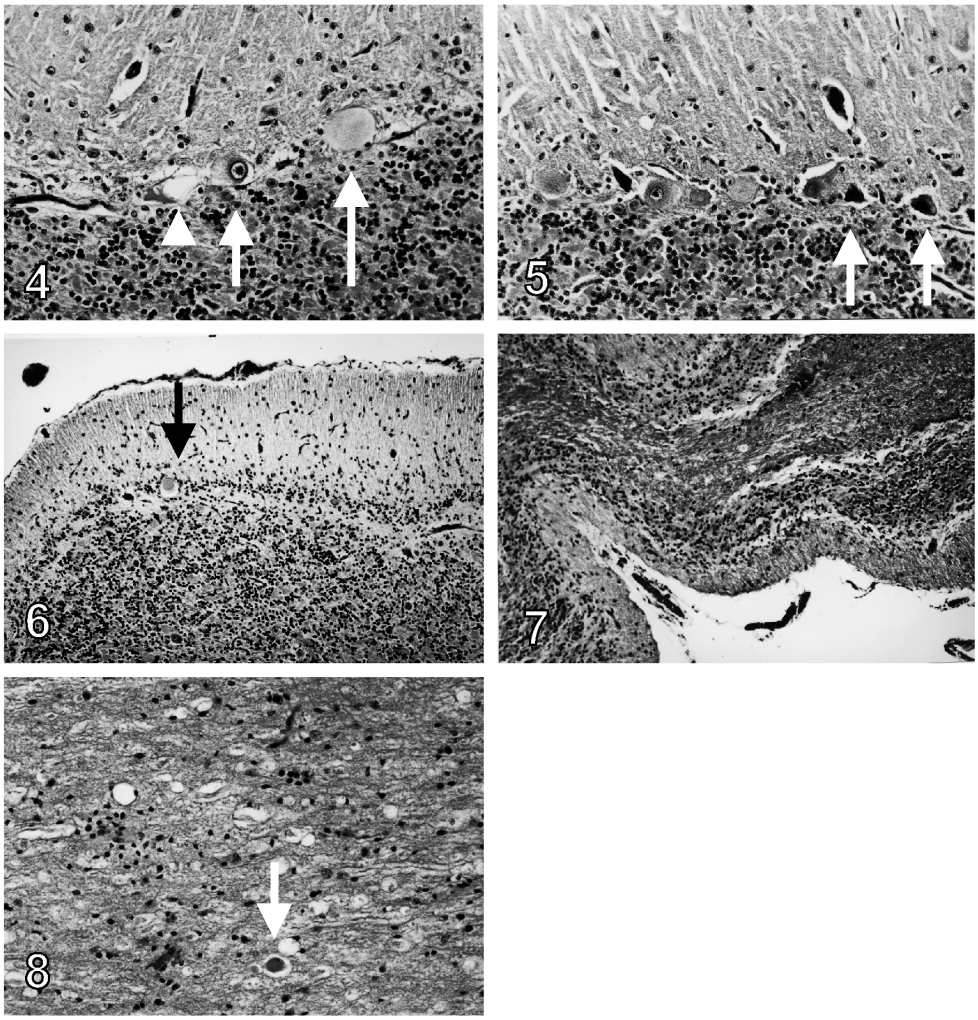


Fig. 4: Cerebellum: Bovine No. 1. Three Purkinje cells with degenerative changes. Note pale staining of cell and a fine vacuolar appearance of the perikaryon (long arrow) and nuclear margination in another cell (short arrow). The third Purkinje neuron contains several large cytoplasmic vacuoles (arrowhead). HE

Fig. 5: Cerebellum: Bovine No. 1. Purkinje cells are pale-staining or shrunken and hyperchromatic (arrows). HE

Fig. 6: Cerebellum: Bovine No. 2. Low power view illustrating loss of Purkinje cells with a single preserved but degenerative Purkinje cell remaining (arrow). Note an increase in Bergmann's glia. HE.

Fig. 7: Cerebellum: Bovine No. 3. Severely affected folium showing total loss of Purkinje cells, thinning of the molecular layer and depletion of the granular layer. HE.

Fig. 8: Cerebellum: Bovine No. 3. Vacuolation of neuropil, spheroid formation (arrow) and gliosis in folial white matter. HE.

Transmission electron microscopy

Purkinje neurons with a distended perikaryon showed depletion of the granular endoplasmic reticulum especially in the perinuclear cytoplasm. These areas contained irregular, empty dilated cisternae of endoplasmic reticulum as well as few membranous bodies sometimes within a vacuole or within a lysosomal configuration, and swollen mitochondria with partial loss of cristae (Figs. 9-11). Occasional bundles of normal neurofilaments were detectable. A few Purkinje cells contained increased numbers of lamellar bodies (Fig. 12). Nuclei of most of the affected Purkinje cells were shrunken, had irregular outlines, and showed marginal clumping of chromatin or fragmentation.

In Purkinje cells that were shrunken, the cytoplasm was markedly increased in density and showed clusters of granular endoplasmic reticulum, aggregates of distended Golgi saccules, and few membranous bodies (Figs. 13, 14). Roundish membrane-bound bodies, interpreted to be lysosomes, were filled with fine granular material and were abundant in several shrunken neurons (Fig. 14). Mitochondria in several affected neurons were increased in number, swollen and frequently contained membranous vesicles. The nuclear outline was often irregular, the nuclei more electron dense with clumping of chromatin and pyknosis. No storage material was detected in Purkinje cells and synapses were normal.

Degenerated Purkinje cell axons were encountered in the granule cell layer. These axons contained lamellated or membranous bodies, vesicular elements, electron-dense bodies and mitochondria (Fig. 15). Myelin sheaths were intact but disproportionately thin in several axons.

Lectin histochemistry

In the cerebellum of affected cattle, the cytoplasm of a moderate number of Purkinje neurons, cells in the granular layer (including Golgi cells) and neurons in cerebellar nuclei stained strongly with ConA. Neurons that were positive displayed either diffuse or localised cytoplasmic staining in areas of vacuolation (Fig. 16). Only a few shrunken, degenerated Purkinje cells stained slightly. Positive staining with ConA was also seen in astrocytes and in macrophages in the white matter mainly in perivascular locations in areas with Wallerian degeneration (Fig. 17). Incubation of each lectin with its corresponding binding inhibitor consistently blocked binding of the lectin to tissue sections. Neurons were also not stained in the negative control sections.

The cytoplasm of a small number of Purkinje cells and neurons in cerebellar

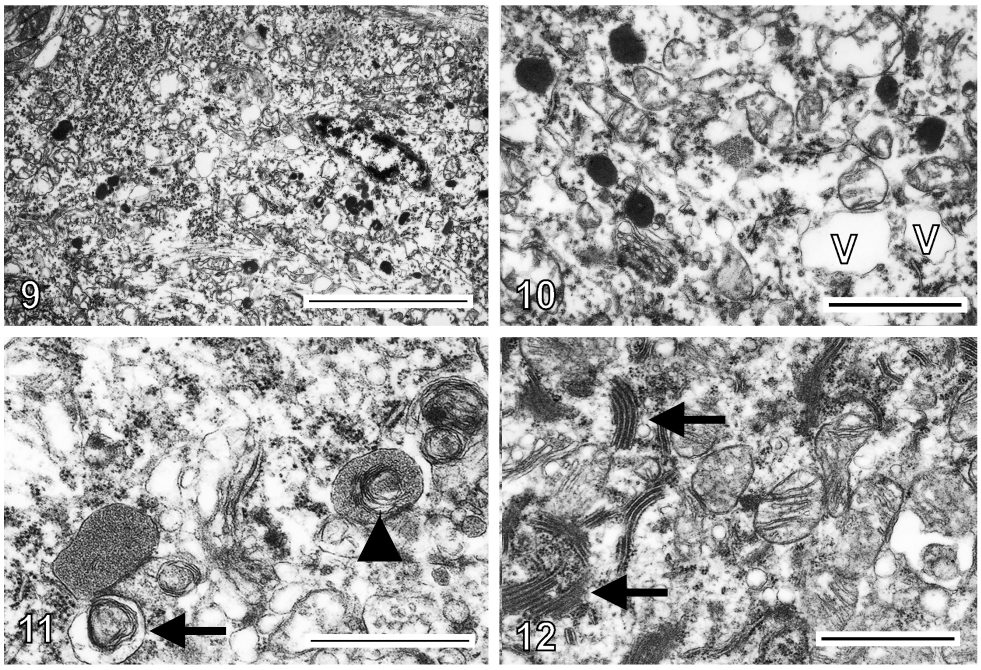


Fig. 9: Transmission electron micrograph. Cerebellum: Bovine No 1. In this Purkinje cell there is perinuclear (central) depletion of endoplasmic reticulum. The cytoplasm contains vacuoles, dense bodies, swollen mitochondria, some neurofilaments and a pycnotic nucleus. Bar = 4 μm.
Fig. 10: Transmission electron micrograph. Cerebellum: Bovine No. 1. Higher magnification of Purkinje cell cytoplasm illustrating membrane-bound vacuoles (V), dispersion of endoplasmic reticulum, dense bodies and intact Golgi apparatus. Bar = 1 μm.
Fig. 11: Transmission electron micrograph. Cerebellum: Bovine No. 2. This Purkinje neuron contains several membranous bodies within a vacuole (arrow) or within a lysosomal configuration (arrowhead). Bar = 1 μm.
Fig. 12: Transmission electron micrograph. Cerebellum: Bovine No. 2. Increased numbers of lamellar bodies (arrows) within a Purkinje cell neuron. Bar = 1 μm.

nuclei showed weakly positive granular staining with WGA and S-WGA. Both swollen and shrunken Purkinje cells were stained by the two lectins. Positive staining with WGA and S-WGA was also noted in macrophages and astrocytes, whereas sections from the control bovine and the negative control sections did not stain. There was no staining in the cerebellum with the lectins DBA, PNA, SBA, RCA-I, BSL-I and UEA-I.

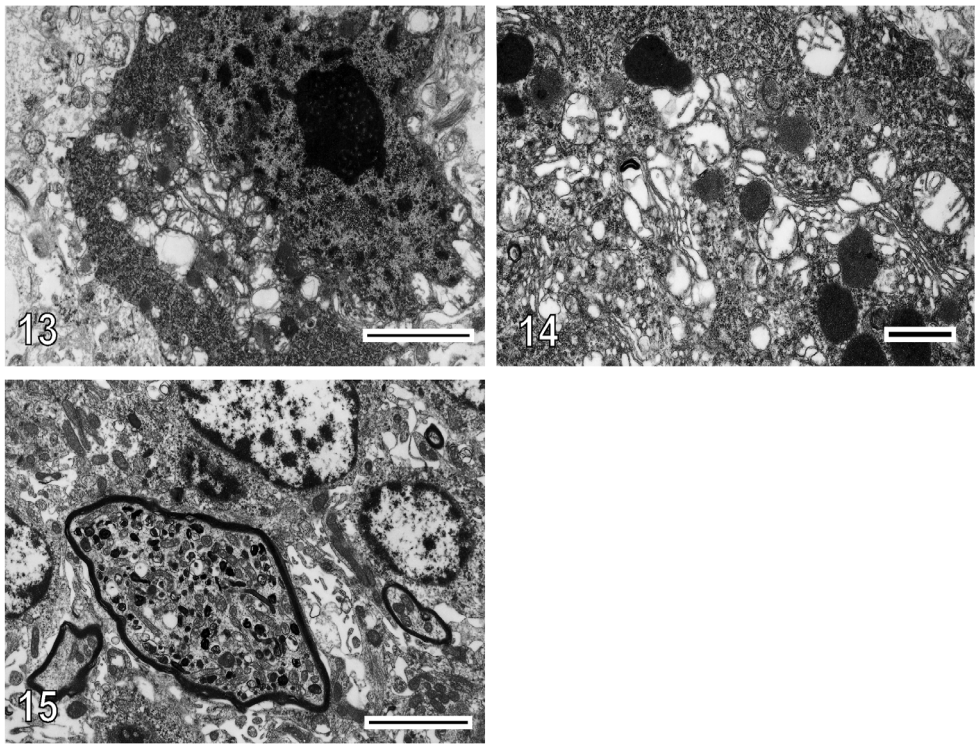
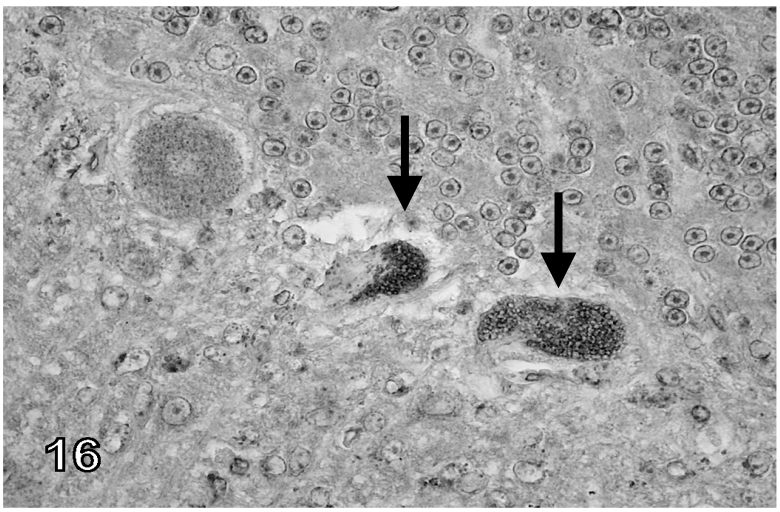


Fig. 13: Transmission electron micrograph. Cerebellum: Bovine No. 3. Shrunken Purkinje cell; the cytoplasm is markedly increased in density and contains predominantly clusters of granular endoplasmic reticulum at the periphery of the cell body, distended Golgi saccules and swollen mitochondria. Bar = 4 μm.
Fig. 14: Transmission electron micrograph. Cerebellum: Bovine No. 3. Higher magnification of a shrunken Purkinje cell with highly condensed cytoplasm. Note swollen mitochondria, distended Golgi complexes, dense bodies and lysosomes. Bar = 1 μm.
Fig. 15: Transmission electron micrograph. Cerebellum: Bovine No. 3. Axonal spheroid within the granule cell layer. The axon is surrounded by a relatively thin myelin sheath and contains membranous bodies, vesicular elements, electron-dense bodies and organelles including mitochondria. Bar = 4 μm.

In situ detection of apoptosis

Only an occasional cell in the cerebellum of two of the affected animals and the control bovine showed positive, nuclear staining. It was not possible to identify the cell type(s) affected based on morphology. Positive control slides treated with nuclease were strongly positive while there was no positive staining in the negative control slides.



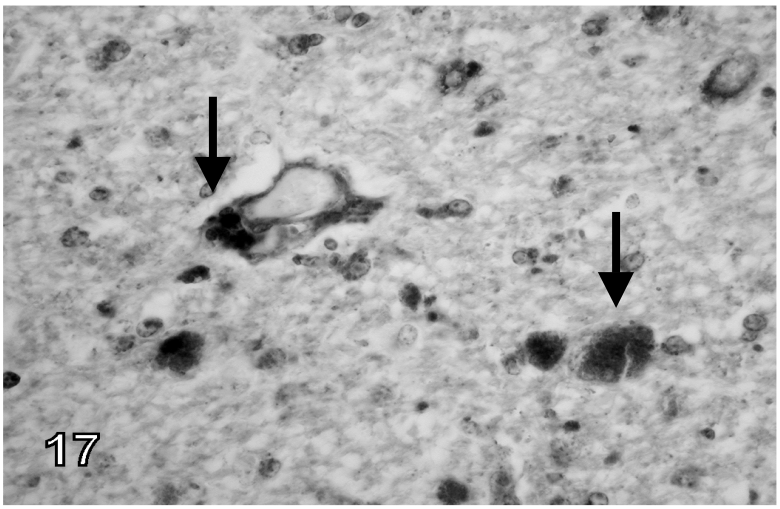


Fig. 16: Cerebellum: Bovine No. 2. Positive staining of the cytoplasm of two vacuolated Purkinje neurons (arrows). Purkinje cell at left with no cytoplasmic staining. Cerebellum incubated with ConA and counterstained with Mayer's haematoxylin.
Fig. 17: Cerebellum: Bovine No. 2. Lectin binding of macrophages (arrows) in perivascular areas and in the white matter in an area of Wallerian degeneration. Tissue incubated with ConA and counterstained with Mayer's haematoxylin.

Discussion

'Maldronksiekte' is an irreversible cerebellar disorder of cattle due to ingestion of *S. kwebense*. It is clinically and pathologically similar to a syndrome of cattle in

Brazil, Uruguay and the United States following ingestion of *Solanum fastigiatum* (and another as yet unidentified *Solanum* sp.) [7,24], *S. bonariensis* [24] and *S. dimidiatum* [18], respectively. In Australia, a cerebellar disorder characterised by degeneration and loss of Purkinje cells has been described in goats grazing *S. cinereum* [4]. Different bodies (lamellar, membranous, vesiculo-membranous and dense) in degenerated Purkinje cells, axons and dendrites have been demonstrated in cattle poisoned by *S. fastigiatum* [7,24]. Lamellar bodies, representing stacked derivatives of endoplasmic reticulum, have been described in normal Purkinje neurons and axons, and in a number of diverse pathological processes [3, 11]. They are regarded as non-specific and their presence may merely reflect degenerative processes in injured Purkinje cells [3,11]. The presence of membranous cytoplasmic bodies gave rise to the hypothesis by Riet-Correa and co-workers that *S. fastigiatum* is an induced lysosomal storage disease, probably a gangliosidosis [24]. A subsequent publication on the pathology of *S. fastigiatum* and a *Solanum* sp. in cattle in Brazil described osmiophilic inclusions, mainly composed of vesiculo-membranous bodies, in affected Purkinje cells [7]. The authors speculated that the active principle of *S. fastigiatum* forms a complex with lipid material that cannot readily be metabolised by Purkinje cells, and that the intoxication does not result from an enzymatic lysosomal defect as has been confirmed in storage diseases [14].

In the present study of 'maldronksiekte', small numbers of cytoplasmic membranous bodies similar to those reported in *S. fastigiatum* toxicosis [24] were demonstrated with electron microscopy in swollen Purkinje cells. These membranous bodies may represent storage material and most likely accounted for the vacuolated appearance of swollen Purkinje cells and the positive staining with the lectins ConA, WGA and S-WGA. Lectins are carbohydrate binding proteins of non-immune origin and, when bound to visulants, they allow localisation and identification of complimentary carbohydrates in cells [6,10]. The application of lectin histochemistry in the study and diagnosis of some lysosomal storage diseases has been accomplished [1,9,21]. ConA binds to α-mannosyl residues and is specific for α-D-Mannose and α-D-Glucose while the positive staining with WGA and S-WGA suggest the presence of terminal moieties consisting of β-D-N-acetyl-glucosamine and acetyl-neuraminic acid [6,10]. The pattern of lectin staining in 'maldronksiekte' partially agrees with those observed in neurons in intoxication with *Sida carpinifolia* [9], locoweed (*Astragalus lentiginosus*), swainsonine (*Swainsona galegifolia*) and α-mannosidosis in human, calves and cats [1]. In these storage diseases, the catabolism of α-mannosyl residues, which are constituents of N-linked glycoproteins, is altered because of decreased α-mannosidase activity. This results in strong positive staining of affected neurons with ConA, WGA and S-WGA as opposed to weak staining with the latter two lectins in *S. kwebense* intoxication. The indolizidine alkaloid swainsonine also inhibits Golgi mannosidase II resulting in the formation of hybrid glycoproteins [29].

Small numbers of dense and lamellar bodies were found but are interpreted as non-specific inclusions. Mitochondrial lesions comprising swelling, loss of cristae and the formation of membranous vesicles occurred in swollen and/or shrunken Purkinje cells. These changes may in part be related to fixation.

In ‘maldronksiekte’, the Purkinje cell is most consistently and severely affected cell type evidenced by widespread degeneration, necrosis and eventual loss of cells. Extensive necrosis of cell populations is unusual in the lysosomal storage diseases [25]. Cerebellar Purkinje cells are also consistently affected in most cases of cerebellar cortical degeneration or abiotrophy different domestic animal species [8, 25]. Cerebellar cortical abiotrophic diseases have been reported in several cattle breeds, but detailed electron microscopical studies in cattle are limited [8]. In bovine familial convulsions and ataxia in Aberdeen Angus cattle, increased numbers of non-specific tubulovesicular profiles of agranular endoplasmic reticulum, neurofilaments and dense-cored vesicles have been documented in swollen Purkinje cell axons [2].

In dogs, cerebellar degeneration has been studied in many different breeds and an autosomal recessive mode of inheritance was demonstrated in those breeds where genetic studies were possible [25]. Degenerated Purkinje cells that were swollen and vacuolated or shrunken and more eosinophilic, as in this study, occur concurrently in several conditions [5]. Multiple vacuoles continuous with the endoplasmic reticulum, loss of ribosomes, increased prominence of lamellar bodies derived from endoplasmic reticulum [5], stacked cisternae of presumed ER-origin [20], progressive loss of cytoplasmic volume and organelles [27] and spaces containing degenerated organellar material of undetermined origin [22] have been reported in cerebellar degenerations in dogs. Purkinje cells also showed the most profound changes in a late-onset cerebellar degeneration in Brittany Spaniels [12]. In this disorder, massive neurofilament accumulation occurred in degenerating cells. Excessive neurofilament accumulation may cause slow axonal transport leading to neuronal degeneration but may also represent a non-specific neuronal response to a wide variety of diseases [12].

The precise mechanism of neuronal degeneration in cerebellar cortical degenerations in dogs, cats and cattle has not been defined. It was postulated that the primary neuronal degeneration in hereditary striatonigral and cerebello-olivary degeneration in Kerry Blue Terriers reflects an excitotoxic degeneration involving neurons that have glutamate receptors and receive axon terminals that elaborate glutamic acid as the excitatory neurotransmitter [8]. In man, Purkinje cells are most severely affected in spinocerebellar ataxia type 6 (SCA6), one of the eight neurodegenerative disorders caused by a tri-nucleotide (CAG) repeat expansion within the affected gene [13,17,30]. In SCA6, the CAG repeat has been identified in the gene encoding the alpha1A-calcium channel protein. Aggregations of this channel protein in Purkinje cells, in which this protein is most intensively expressed, in SCA6 is most likely the mechanism of neurodegeneration following interference of normal function of this channel.

In some affected Purkinje cells, degenerative changes resembled those reported in apoptosis and included cell shrinkage, chromatic clumping and dilation of endoplasmic reticulum [28]. DNA fragmentation indicating apoptosis was, however, not demonstrated in a significant number of degenerated Purkinje cells and neurons in the cerebellum by a modified TUNEL method. Apoptosis has been described in the normal developing brain, in traumatic brain injury and in a number of chronic neurodegenerative disorders including motor neuron disease and Alzheimer's disease in humans [31]. Whether or not apoptotic neuronal cell death plays a role in cerebellar cortical degeneration in domestic animals needs further investigation.

Leaves and stems of *S. kwebense* were collected from the source farm and analysed. Low levels of calystegines were detected in one sample. There were no detectable levels of swainsonine and other known glycosidase inhibitory alkaloids in the plant material. Calystegin B_2 and related alkaloids have been detected in leaves and fruit from *S. dimidiatum* from Texas [19]. Differences in toxin levels between different *Solanum* spp. may be explained by the fact that plants produce alkaloids only at certain growth stages or in specific locations, and that alkaloids are more readily extracted from seeds rather than leaf material (RM Molyneux, Western Regional Research Centre, Agricultural Research Service, US Department of Agriculture, Albany, CA 94710, personal communication, 2001). There have also been postulates that the alkaloids are produced by endophytic fungi, rather than the plant, in which case the presence of the toxic principle(s) could be highly variable (RM Molyneux, personal communication, 2001).

In conclusion, 'maldronksiekte' has several morphological features reminiscent of an acquired (plant-induced) cerebellar cortical degeneration. The selective vulnerability of Purkinje cells in this plant toxicosis is unusual for a lysosomal storage disease in which different populations of neurons in the brain are generally affected [25]. The presence of carbohydrate moieties in affected Purkinje cells, however, suggests that there may be an inability of cerebellar neurons to metabolise a plant toxin or cellular substrate.

References

1. Alroy J, Orgad U, Ucci AA, Gavris VE (1985) Swainsonine toxicosis mimics lectin histochemistry of mannosidosis. Vet Pathol 22:311-316
2. Barlow RM (1981) Morphogenesis of cerebellar lesions in bovine familial convulsions and ataxia. Vet Pathol 18:151-162
3. Bestetti G, Rossi GL (1980) The occurrence of cytoplasmic lamellar bodies in normal and pathologic conditions. Acta Neuropathol (Berl) 49:75-78
4. Bourke CA (1997) Cerebellar degeneration in goats grazing *Solanum cinereum* (Narrawa burr). Aust Vet J 75:363-365
5. Cummings JF, deLahunta A (1988) A study of cerebellar and cerebral cortical degeneration in Miniature Poodle pups with emphasis on the ultrastruture of Purkinje cells. Acta Neuropathol (Berl) 75:261-271

6. Damjanov I (1987) Lectin cytochemistry and histochemistry. Lab Invest 57:5-20
7. De Barros SS, Riet-Correa F, Andujar MB, Lombardo de Barros CS, Méndez MC & Schild AL (1987) *Solanum fastigiatum* var. *fastigiatum* and *Solanum sp*. poisoning in cattle: ultrastructural changes in the cerebellum. Pesquisa Veterinária Brasileira 7, 1-5
8. De Lahunta A (1990) Abiotrophy in domestic animals: a review. Can J Vet Res 54:65-76
9. Driemeier D, Colodel EM, Gimeno EJ, Barros SS (2000) Lysosomal storage disease caused by *Sida carpinifolia* in goats. Vet Pathol 37:153-159,
10. Goldstein IJ, Hayes CE (1978) The lectins: carbohydrate-binding proteins of plants and animals. Adv Carbohydrate Chem Biochem 35:127-340
11. Herndon RM (1964) Lamellar bodies, an unusual arrangement of the granular endoplasmic reticulum. J Cell Biol 20:338-342
12. Higgens RJ, LeCouteur RA, Kornegay JN, Coates JR (1998) Late-onset progerssive spinocerebellar degeneration in Brittany Spaniel dogs. Acta Neuropathol (Berl) 96:97-101
13. Ishikawa K, Owada K, Ishida K, Fujigasaki H, Shun Li M, Tsunemi T, Ohkosi N, Toru S, Mizutani T et al (2001) Cytoplasmic and nucelar polyglutamine aggregates in SCA6 Purkinje cells. Neurology 56:1753-1756
14. Jolly RD, Walkley SU (1997) Lysosomal storage diseases: an essay in comparative pathology. Vet Pathol 34:527-548
15. Kellerman TS, Coetzer JAW, Naudé TW (1988) Plant Poisonings and Mycotoxicosis of Livestock in Southern Africa. Oxford University Press, Cape Town, pp 64-82
16. Margolis G & Pickett JP (1956) New applications of the Luxol fast blue myelin stain. Lab Invest 5:459-474
17. Martin JB (1999) Molecular basis of neurodegeneraive disorders. New Engl J Med 340:1970-1980
18. Menzies JS, Bridges CH & Bailey EM (1979) A neurological disease of cattle associated with *Solanum dimidiatum*. South West Vet 32:45-49
19. Molyneux RJ, Lynn JF, Ralphs MH, Pfister JA, Panter KE, Nash JR (1994) Polyhydroxy alkaloid glycosidase inhibitors from poisonous plants of global distribution. In: Colegate SM, Dorling PR, Allen JG, Huxtable CR, Mead RJ, Panter KE (eds) Plant-associated Toxins: Agricultural, Phytochemical and Ecological Aspects, CAB International, pp 107-112
20. Montgomery DL, Storts RW (1984) Hereditary striatonigral and cerebello-olivary degeneration of the Kerry Blue Terrier. II. Ultrastructural lesions in the caudate nucleus and cerebellar cortex. J Neuropathol Exp Neurol 43:263-275
21. Murnane RD, Ahern-Rindell AJ, Prieur DJ (1989) Lectin histochemistry of an ovine lysosomal storage disease with deficiencies of β-galactosidase and α-neuraminidase. Am J Pathol 135:623-630
22. Nesbit JW, Ueckermann JF (1981) Cerebellar cortical atrophy in a puppy. J S Afr Vet Assoc 52:247-250
23. Pienaar JG, Kellerman TS, Basson PA, Jenkins WL, Vahrmeijer J (1976) Maldronksiekte in cattle: a neuronopathy caused by *Solanum kwebense* N.E. Br. Onderstepoort J Vet Res 43:67-74
24. Riet-Correa F, Méndez MC, Schild AL, Summers BA & Oliveira JA (1983) Intoxication by *Solanum fastigiatum* var. *fastigiatum* as a cause of cerebellar degeneration in cattle. Cornell Vet 73, 240-256

25. Summers BA, Cummings JF, De Lahunta A (1995) Veterinary Neuropathology, Mosby, St. Louis, pp 214-236,
26. Szumanska G, Vorbrodt AW, Mandybur TI, Wisniewski HM (1987) Lectin histochemistry of plaques and tangles in Alzheimer's disease. Acta Neuropathol (Berl) 73:1-11
27. Troncoso JC, Cork LC, Price DL (1985) Canine inherited ataxia: ultrastructural observation. J Neuropathol Exp Neurol 44:165-175
28. Trump BJ, Berezesky IK, Chang SH, Phelps PC (1997) The pathways of cell death: oncosis, apoptosis, and necrosis. Tox Pathol 25:82-88
29. Tulsiani DRP, Touster O (1983) Swainsonine causes the production of hybrid glycoproteins by human skin fibroblasts and rat liver Golgi preparations. J Biol Chem 258:7578-7585
30. Yang Q, Hashizume Y, Yoshida M, Wang Y, Goto Y, Mitsuma N, Ishikawa K, Mizusawa H (2000) Morphological Purkinje cell changes in spinocerebellar ataxia type 6. Acta Neuropathol (Berl) 100:371-376
31. Yuan J, Yankner B (2000) Apoptosis in the nervous system. Nature 407:802-809

CHAPTER 3 : MYELINOPATHIES

Chapter ***3.1***

CLASSIFICATION

As an encompassing term, myelinopathies denote non-inflammatory conditions of the central or peripheral nervous system in which the primary event relates to some disorder of myelin formation, maintenance, or stability [2]. This clearly is a morphological classification irrespective of cause or mechanism of disease. The myelinopathies are subclassified into four categories [2]:

- hypomyelinations/dysmyelinations (e.g. Border disease),
- leukodystrophic and myelinotic diseases (e.g. globoid cell leukodystrophy),
- spongiform myelinopathies (idiopathic e.g. congenital brain oedema in Hereford calves and toxic or metabolic e.g. maple syrup urine disease), and
- non-myelinic spongiform encephalomyelopathies (e.g. bovine spongiform encephalopathy and scrapie).

Conditions reported in this chapter fall into the category of spongiform myelinopathies in which the dominant pathological feature is vacuolation of myelin in the absence of a significant degree of myelin breakdown or phagocytosis.

Prominent vacuolation of neural tissue, as seen by light microscopy, is generally referred to as status spongiosis and may result from vacuolation within processes of the neuropil, vesiculation of myelin sheaths, or swelling of astrocytes or oligodendrocytes [1]. To determine the structural basis for such spongy vacuolation requires ultrastructural examination. Most commonly, intramyelinic vacuolation is caused by splitting of myelin lamellae along the intraperiod line [3]. This splitting may produce variably sized, empty vacuoles lined by myelin lamellae at multiple levels and multiple loci along an internode. Separation of lamellae at the intraperiod line reopens the extracellular space originally obliterated within the spiralling processes of the myelinating cell. Myelinic vacuolation may also arise by separation at the major dense line thereby reopening the intracellular compartment of the myelinating cell, or by ballooning of the periaxonal space [2].

References

1. Adornato B, Lampert P (1971) Status spongiosis of nervous tissue. Acta Neuropathol (Berl) 19:271-289
2. Jubb KVF, Huxtable CR (1993) The Nervous System. In: Jubb KVF, Kennedy PC, Palmer N (eds) Pathology of Domestic Animals 4th edn Academic Press, Inc, San Diego, pp 267-439
3. Kreutzberg GW, Blakemore WF, Graeber MB (1997) Cellular pathology of the central nervous system. In: Graham DI, Lantos PL (eds) Greenfield's Neuropathology, 6th edn, Arnold, London, pp 85-140

Chapter ***3.2***

STATUS SPONGIOSIS, OPTIC NEUROPATHY, AND RETINAL DEGENERATION IN *HELICRYSUM ARGYROSPHAERUM* POISONING IN SHEEP AND A GOAT

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Abstract

Lesions of natural *Helichrysum argyrosphaerum* poisoning were studied in eight sheep and one goat. Light microscopic examination revealed widespread, bilaterally symmetrical status spongiosis of the white matter of the brain consistently present in the subependymal area adjacent to the lateral ventricles, cerebellar peduncles, and brain stem in all animals. In three animals, the ultrastructural finding of intramyelinic vacuolation due to splitting of the myelin lamellae at the intraperiod lines indicated myelin oedema. There was also mild distension of perivascular and extracellular spaces in the severely affected areas. Significant changes were absent in neurons, glial cells, axons, or blood vessel walls. Myelin oedema associated with degeneration and loss of axons and myelin and astrocytic gliosis was present in the intraorbital and intracranial portions of the optic nerves. In the intracanalicular portions of the nerves in three animals that were studied, more chronic lesions consisting of fibrosis and atrophy of the nerve suggested that the optic neuropathy follow compression of the nerve in the optic canal as a result of myelin oedema. The toxic principle of the plant also caused a degenerative retinopathy in five animals. The essential histopathologic change was degeneration and loss of the photoreceptor outer segments predominantly in the nontapetal retina. These retinal lesions were associated with hyperplasia and hypertrophy and with migration of the pigmented epithelium, focal retinal separation, and depletion and loss of the nuclear layers.

Key words: Central nervous system, goats, *Helichrysum argyrosphaerum*, myelin oedema, optic neuropathy, sheep, status spongiosis, toxic retinopathy

Introduction

Blindness, paresis, and paralysis in sheep and cattle grazing on pastures consisting mainly of the plant *Helichrysum argyrosphaerum* DC. (Asteraceae, wild everlasting, 'sewejaartjie') has been reported from Namibia [2]. Its toxicity was confirmed by feeding plant material to sheep [2]. Light microscopic evaluation of affected animals revealed bilaterally symmetrical status spongiosis of the white matter of the brain and spinal cord. Lesions were most striking in the periventricular white matter, middle cerebellar peduncles, and pyramidal and optic tracts. Myelinic vacuolation and necrosis of the optic nerves and necrosis of the retina primarily involving the photoreceptor layer were also described.

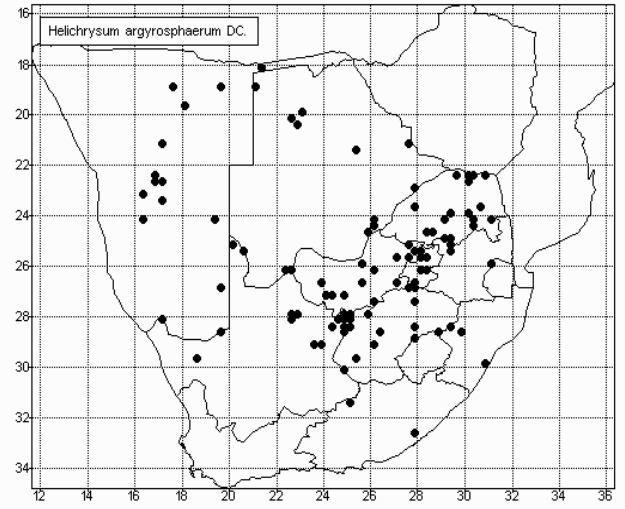

Fig. 1: Distribution map of the plant *Helichrysum argyrosphaerum* (courtesy Botanical Research Institute, Pretoria).

Notwithstanding the wide distribution of *H. argyrosphaerum* in southern Africa (Fig. 1), outbreaks of the toxicosis have been restricted to Namibia [11]. The plant is commonly found on exposed, disturbed ground and may become abundant whenever the natural pasture is heavily grazed. The toxic principle of the plant is unknown, and no investigative work has been performed on the toxicosis since its original description in 1978 [2]. In 1988, outbreaks of *Helichrysum* poisoning were diagnosed in sheep and goats from two farms in the northwestern Cape Province, South Africa. Abnormally high rainfall in the late summer and early spring (February-May) in the area ended a 9-year drought. During August, outbreaks of blindness, paresis, and paralysis were observed in weaner lambs 45 months of age and a few goat kids and ewes on two farms after the animals had

Fig. 2: More than 60% of the surface area in a camp is covered by *Helicrysum* plants.
Fig. 3: Higher magnification of the plant *Helichrysum argyrosphaerum*.

been in camps for two months. Animals were seen grazing *H. argyrosphae*rum, which covered more than 60% of the surface area of the camps (Figs. 2,3). *Helichrysum argyrosphaerum* is a small prostate plant with silvery-white to rosy-purple flowerheads. These outbreaks of *Helichrysum* poisoning in sheep and goats provided an opportunity for detailed study of the lesions in the optic nerve and retina and to describe the ultrastructural changes in the brain and optic nerve.

Materials and Methods

Case history

Formalin-fixed tissues specimens from six sheep, five 5-7-month-old lambs (Nos. 1-5) and one adult sheep (No. 6), and three live animals (Nos. 7-9) with *H. argyrosphaerum* poisoning were studied. Live animals comprised two sheep (an adult ewe [No. 7] and a 6-month-old lamb [No. 8]) and a 7-month-old Boer goat (No. 9), which were obtained 4-8 weeks after the onset of clinical signs in the flocks. Animals were blind and had dilated and unresponsive pupils and nystagmus. One lamb (No. 8) was paretic, and one ewe (No. 7) exhibited unilateral circling and frequent head shaking. The nervous signs became accentuated when the animals were disturbed or handled. One goat (No. 9) and an age-matched control were examined by direct and indirect ophthalmoscopy. In the blind goat, there was slightly increased reflectivity of the tapetal fundus and a few irregular, darkbrown pigmented areas on the nontapetal fundus.

Pathology

Formalin-fixed portions of brain and spinal cord and the entire eyes were received from six animals (Nos. 1-6). Live animals (Nos. 7-9) were killed by an intravenous overdose of pentobarbitone sodium, and a complete necropsy was performed. At necropsy, the entire brain and spinal cord, eyes, portion of the ischiatic nerve, and a range of tissue specimens including the liver and kidney were collected for light microscopy. The intracanalicular portion of the optic nerves were removed by cutting a block of sphenoid bone (optic block) approximately 50 x 30 x 30 mm in size [6]. The eyes and the intraorbital portion of the optic nerves were immersed in Zenker's fixative within 10 minutes of euthanasia. The other specimens were fixed for five days in 10% neutral buffered formalin. After fixation, the optic blocks were decalcified in 10% formic acid and trimmed to expose the transverse surfaces of the optic nerve in the bony optic canal. The brains of animal Nos. 7-9 were serially sectioned, and blocks were prepared from levels cut at the olfactory tubercle and cortex, cerebral cortex (frontal, parietal, temporal, and occipital lobes), basal nuclei, thalamus, mesencephalon, pons, two levels of medulla oblongata, and the spinal cord in the cervical, thoracic, and lumbosacral portions. Midsagittal slabs containing the optic nerve were trimmed from each globe. All tissues were routinely processed for paraffin embedment, and 5-6 μm sections were stained with haematoxylin and eosin (HE). Selected sections of brain, spinal cord, and optic nerves were stained with luxol fast blue/periodic acid-Schiff/ haematoxylin (LFB/PAS/H), luxol fast blue/Holmes (LFB/H), and Masson's trichrome (MT). Ocular sections were stained with PAS. Mounted unstained sections of the eyes from animal Nos. 7-9 were studied by fluorescence microscopy.

For electron microscopic examination, specimens of the cerebral subependymal white matter adjacent to the lateral ventricles and the intraorbital optic nerve were collected from three animals (Nos. 7-9). Tissues were diced into 1-mm cubes and fixed by immersion in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer within 15 minutes of euthanasia. Specimens were postfixed in osmium tetroxide, dehydrated in a graded ethanol series, cleared in propylene oxide, and embedded in Polarbed 812. Semithin sections were stained with toluidine blue for tissue orientation. Ultrathin sections were stained with uranyl acetate and lead citrate and studied with a transmission electron microscope.

Results

Gross lesions

In two animals (Nos. 7, 9) the intraorbital optic nerves were of normal thickness but were slightly yellowish-brown on cut surface. The brains and other tissues and organs appeared normal.

Histologic lesions

Light microscopic examination of the central nervous system in all animals corresponded to that of the earlier description [2] and revealed widespread status spongiosis of the white matter of the brain and spinal cord and, less frequently, of the spinal nerve roots (Figs. 4,5). Lesions were essentially bilaterally symmetrical in the brains of animal Nos. 7-9, which were studied for symmetry. Vacuoles were round to ovoid or elongate and approximately 5-100 μm in diameter; most were 5-30 μm. In severely affected areas, a weblike appearance of the white matter produced a rarified appearance of the myelin, and in these areas sections stained with LFB revealed a paucity of myelin staining. The vacuoles were empty, often multilocular, and partitioned by thin LFB-positive material interpreted as myelin and occurred independent of vascular elements. Toluidine blue-stained sections confirmed that the vacuoles were bounded by myelin lamellae. The spongy change varied in degree and extent among animals, but the most severe and consistently affected areas included the cerebral white matter adjacent to the lateral ventricles, cerebellar peduncles, and the brain stem, particularly the pons (Table 1). The spongy change was not accompanied by gliosis, inflammation, or myelin degeneration except in the white matter of the lumbosacral spinal cord and spinal nerve roots in one animal (No. 9). In this goat, vacuolation was particularly prominent as compared with the other animals; some dilated myelin sheaths contained foamy macrophages with pycnotic nuclei and phagocytosed LFB-positive and PAS-positive material. There was minor involvement of the gray matter, indicated by perivascular and pericellular oedema.

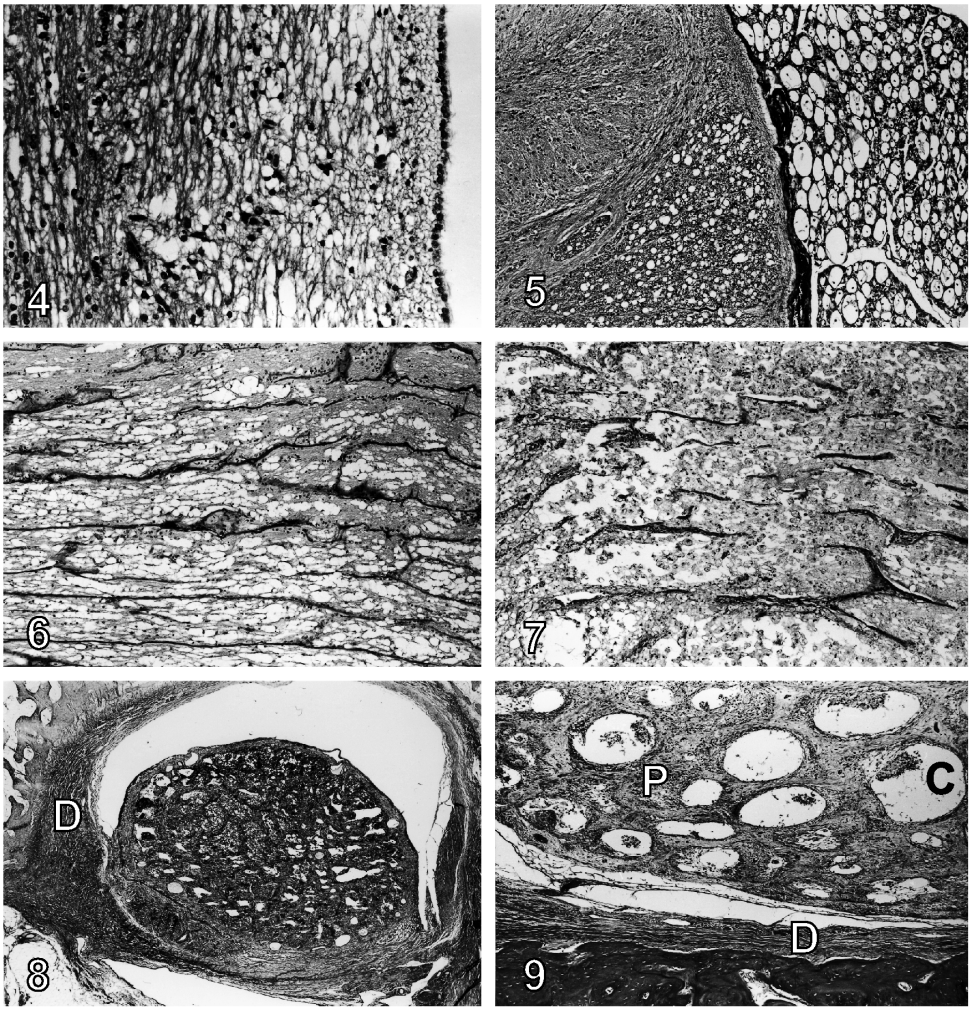

Fig. 4: Cerebrum; Sheep No. 2. Spongy degeneration of the periventricular white matter. HE.
Fig. 5: Spinal cord; Goat No. 9. Extensive myelinic vacuolation of the white matter and spinal nerve roots. Luxol fast blue/periodic acid-Schiff/hematoxylin.
Fig. 6: Optic nerve; Sheep No. 6. Longitudinal section of the intraorbital portion of the nerve showing widespread myelin vacuolation of nerve fibers. HE.
Fig. 7: Optic nerve; Sheep No. 7. Longitudinal section of the intraorbital portion of the nerve with more advanced lesions exhibited by Wallerian degeneration with marked loss of nerve fibers and thickening of pial septa. HE.
Fig. 8: Optic nerve; Sheep No. 7. Intracanalicular nerve in the bony canal is fibrotic and contracted, and the meninges, including the dura mater (D), are markedly thickened. Masson's trichrome.

Fig. 9: Optic nerve; Sheep No. 7. Higher magnification of cross section through intracanalicular optic nerve. There is fibrosis of pial septa (P), complete loss of nerve fibers with cavity formation (C), and widening of the subarachnoid space. D = dura mater. Masson's trichrome.

In the intraorbital and, to a lesser extent, the intracranial portion of the optic nerves in six animals (Nos. 1, 3, 4, 6, 8, 9), extensive myelin vacuolation was evident (Fig. 6). In the other three animals (Nos. 2, 5, 7), more advanced lesions in these portions of the nerve were characterised by destruction and marked loss of myelinated axons and accumulations of foamy macrophages consistent of Wallerian degeneration and prominent pial septa (Fig. 7). Some axons within dilated myelin sheaths had ovoid to elliptical swellings. Lesions in the intracanalicular portion of the nerves in the three animals studied (Nos. 7-9) were more chronic than those in the intraorbital and intracranial portions. In two animals (Nos. 8, 9), there was marked fibrous thickening of pial septa and loss of axons and myelin with large numbers of reactive astrocytes. In one animal (No. 7), the nerve was largely replaced by collagen, leaving a constricted fibrotic segment of intracanalicular nerve within the bony optic canal (Figs. 8,9). In the shrunken nerve, there was a complete loss of bundles of nerve fibres, leaving cavities that contained aggregates of foamy macrophages and cellular debris, thickening of the meninges, and widening of the subarachnoid space.

Table 1. Summary of distribution and severity of spongy change of the white matter in the brain and spinal cord of three animals (two sheep and one goat) with *Helichrysum argyrosphaerum* poisoning.

Severely and consistently affected	**Moderately and frequently affected**	**Mildly or inconsistently affected**
Fomix	Corona radiata	Olfactory tract
Corpus callosum	Optic radiation	Pallidum
Subcallosal fasciculus	Internal capsule	Putamen
Septum pellucidum	External capsule	Lateral geniculate body
Cerebellar peduncles	Thalamic nuclei	Cerebellar folial white matter
Spinal tract of trigeminal nerve	Hippocampus and alveus	Outer white matter of spinal cord
Pontine nuclei	Substantia nigra	
Transverse fibres of pons	Optic chiasma	
	Optic tract	
	Reticular formation	

In five animals (Nos. 1, 3, 7, 8, 9), bilateral degenerative changes were evident in the peripapillary and midzonal retina in nontapetal areas, with less extensive change in the tapetal retina. The mildest change was multifocal and coalescing areas of disruption, shortening, and loss of photoreceptor outer segments (Fig. 10).

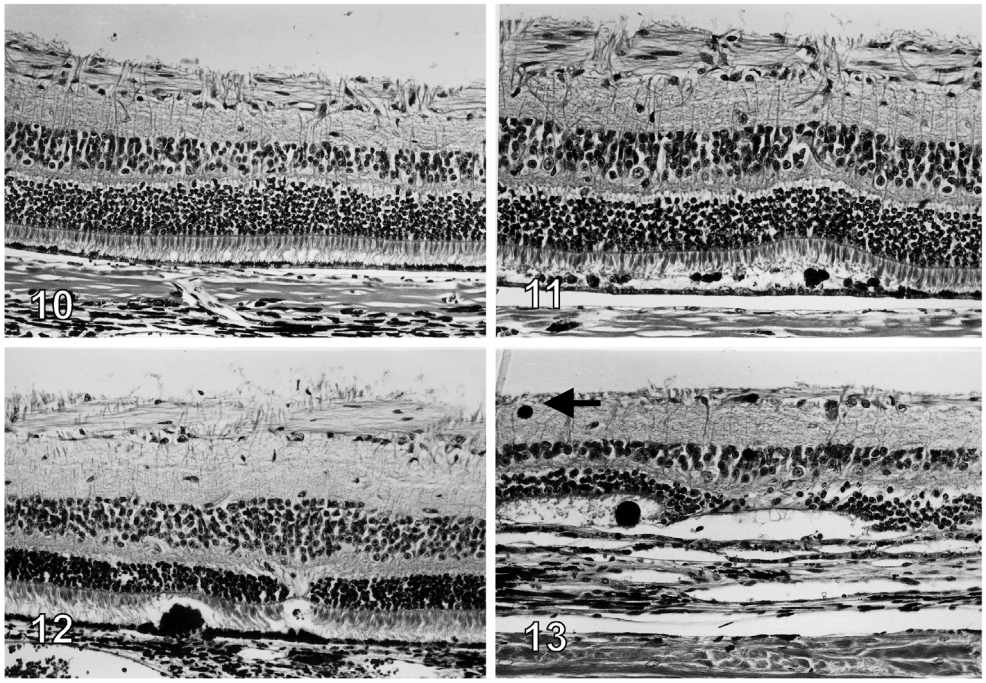

Fig. 10: Retina; Sheep No. 8. Shortening and disruption of the photoreceptor outer segments in the midzonal tapetal retina. HE.
Fig. 11: Retina; Goat No. 9. Slightly more advanced lesions indicated by small clumps of pigment-laden cells in a subretinal space associated with mild degeneration of the overlying photoreceptor layer in the peripapillary tapetal retina. HE.
Fig. 12: Retina; Goat No. 9. Large clumps of pigment-laden cells, photoreceptor degeneration and loss, and patchy loss of the outer nuclear layer in the midzonal nontapetal area. Note paucity of ganglion cells. HE.
Fig. 13: Retina; Sheep No. 7. In the peripapillary tapetal part, there is loss of the photoreceptor and outer nuclear layers, focal separation, thinning of the inner nuclear layer, and migrating pigmented cells (arrow). HE.

Slightly more advanced lesions comprised fragmentation of the outer photoreceptor segments accompanied by multifocal hyperplasia and hypertrophy of the retinal pigmented epithelium with the accumulation of small clumps of pigmented cells of various sizes within a small subretinal space, resulting in focal retinal separation (Fig. 11). The pigmented cells contained abundant melanin granules that completely obscured the nuclei. In some foci, there was attenuation or patchy loss of the outer nuclear layer overlying the pigmented cell clumps (Fig. 12). In the severely affected retina, the photoreceptor and outer nuclear layers were mostly absent, and there was thinning or focal disappearance of the inner nuclear

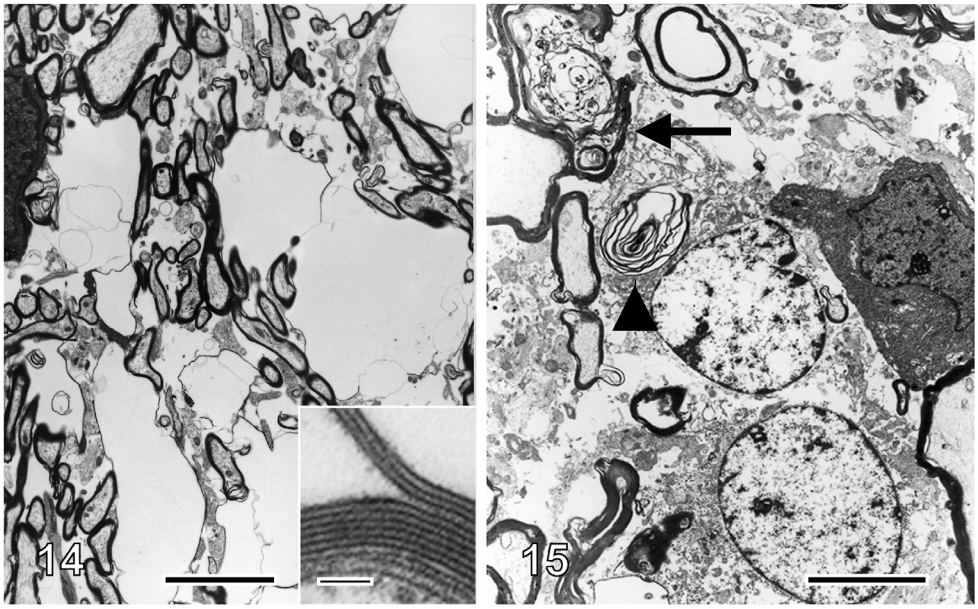

Fig. 14: Transmission electron micrograph. Cerebrum; Sheep No. 8. Multiple intramyelinic vacuoles and slightly distended extracellular spaces. Bar = 2 μm. *Inset:* Higher magnification showing separation of myelin lamellae at the intraperiod line. Bar = 0.1 μm .
Fig. 15: Transmission electron micrograph. Optic nerve; Sheep No. 7. Vacuolation, Wallerian-like degeneration of a myelinated axon (arrow), and a myelin figure in an astrocyte (arrowhead). Bar = 2 μm.

layer, multifocal retinal separation, and migration of pigment-laden cells into the inner retinal layers, including the ganglion cell layer (Fig. 13). Some extracellular oval and elongated melanin granules in the inner retina were also present. In these areas of severe retinal degeneration, portions of the pigmented epithelium were discontinuous or atrophic and devoid of pigment. No autofluorescent or PAS-positive pigment was detected in the retinal pigmented epithelium. Chronic lesions in one animal (No. 7) comprised depletion and loss of all retinal layers, resulting in a poorly cellular glial scar. A variable reduction or absence of ganglion cells and gliosis of the ganglion cell and nerve fibre layers and of the optic papilla occurred in four animals (Nos. 1, 7, 8, 9). No significant lesions were present in the other organs and tissues.

Ultrastructural lesions

Electron microscopic evaluation revealed that the status spongiosis of the white matter was due to numerous intramyelinic vacuolar spaces lined by myelin

lamellae (Fig. 14). The vacuoles were of various sizes and occurred as a result of splitting of myelin lamellae at the intraperiod lines, most commonly at the external third of the myelin sheath (Fig. 14, inset). The degree of myelin splitting differed from one myelin sheath to another, and multiple vacuoles were often noticed within the same sheath. Vacuoles were either empty or contained fragmented membranes. Distension of perivascular and extracellular spaces and of perivascular astrocytic processes was evident in some areas of pronounced myelin vacuolation. No other structural alterations were seen in neurons, axons, or other tissue elements. There was no evidence of myelin breakdown.

In the intraorbital optic nerves, the lesions consisted of myelin vacuolation, degenerating myelin and axons, reactive changes in glial cells, and the presence of macrophages. Several axons were swollen and contained vesicular structures, granular material, and remnants of organelles. Others were shrunken and had collapsed myelin sheaths. The cytoplasm of many astrocytes and oligodendrocytes was vacuolated and contained lipid droplets and myelin debris (Fig. 15). Lamellar, membranous, and dense inclusions and breakdown products of myelin were noted in phagocytic cells in areas most severely affected.

Discussion

Histologic lesions in the central nervous system and optic nerves in this study of *H. argyrosphaerum poi*soning were similar to those in previously reported cases and comprised widespread status spongiosis, with the subependymal area adjacent to the lateral ventricles, cerebellar peduncles, and brain stem consistently and most severely affected. Status spongiosis is a term applied to the sieve-like appearance of nervous tissue associated with intracellular, extracellular, and intramyelinic accumulation of fluid and artifactitious dissolution of myelin [1]. The most common morphologic alteration associated with myelin vacuolation is separation of lamellae along the intraperiod line [10], considered to indicate myelin oedema [5,17]. Electron microscopic examination of these animals confirmed intramyelinic vacuolation due to splitting of myelin lamellae at the intraperiod lines. Similar lesions of the white matter in spongiform myelinopathies have been described in a variety of disorders in humans and animals, including experimental intoxications by hexachlorophene [12,21], triethyl tin [9], cuprizone [20], and isonicotinic acid hydrazide [13] and by the plant *Stypandra imbricata* in rats [8]. In domestic animals, these lesions have been confirmed in ammonia intoxication in calves [4], bovine maple syrup urine disease [5], chronic copper toxicity of sheep [16], and closantel toxicosis in sheep (JJ van der Lugt, personal observations). Several functional disturbances may play a role in myelin splitting. In bovine maple syrup urine disease, a direct toxic effect on the myelin sheath or one mediated via the oligodendrocyte may contribute to the myelin splitting [5].

This study did not resolve the pathogenesis of the lesions in the optic nerves. Poisoning with *S. imbricata* and *S. glauca* in sheep and goats causes histologic

lesions in the optic nerves similar to those described in the animals in the present study [14,26]. Three possible explanations for the optic nerve neuropathy in *Stypandra* poisoning have been proposed: a direct toxic effect on axons, compression of the swollen optic nerve within the optic canal, or both [8,26]. Lesions in the optic nerves in *S. glauca* poisoning were most severe in the intracanalicular portion of the nerve [26]. Thus, swelling due to myelin oedema of the nerve probably results in compression within the bony canal and eventually in Wallerian degeneration of myelinated axons. A similar pathomechanism of axonal degeneration in the optic nerve has been suggested in rats dosed with stypandrol (the toxic principle isolated from *S. imbricata)* [7] in rafoxanide toxicity in sheep [18], closantel poisoning in sheep (JJ van der Lugt, unpublished observations) and goats [3] and in hexachlorophene toxicity in rats [22]. We propose a similar sequence of events in *H. argyrosphaerum* poisoning.

The unidentified toxic principle of *Helichrysum blandowskianum* causes periacinar liver necrosis, widespread haemorrhages, and presumptive hepatic encephalopathy, with spongiform change in the central nervous system, particularly in the cerebellum and pons in sheep and cattle in Australia [15]. Hepatic necrosis is not a feature of *H. argyrosphaerum* poisoning.

The toxic principle of *H. argyrosphaerum* also causes retinal degeneration. The primary retinal lesion was multifocal degeneration and loss of the photoreceptor outer segments, predominantly in the nontapetal retina and progressing to depletion and loss of all retinal layers. The observed changes in the outer layers of the retina and in the pigmented epithelium cannot be explained by the optic neuropathy because little if any histologic evidence of transneuronal degeneration is seen in retinas with optic nerve damage, even with complete transaction of the nerve [19].

A few differences in the retinal lesions were apparent between the present cases and those previously reported [2]. Hyperplasia of the pigmented epithelium and focal retinal separation with the accumulation of small clumps of pigmented cells within a small subretinal space were not described but were illustrated in one of the figures in the original report. Evaluation of eye sections of these earlier cases from the files of the Section of Pathology, Onderstepoort Veterinary Institute, confirmed the presence of these lesions. Congestion, oedema, and haemorrhages in the choroid and retina in the earlier cases were not seen in the present animals and may be related to toxin dose or plant factors. Atrophy and loss of the ganglion cells and gliosis of the ganglion cell and nerve fibre layers may be explained by the marked axonal degeneration of axons in the optic nerves [19].

The essential change in other toxic retinopathies, including poisoning by *S. glauca* [26], bracken fern (*Pteridium aquilinum*) [24,25], closantel (JJ van der Lugt, personal observations), and probably *S. imbricata* [14] is also located in the photoreceptor layer. Primary involvement of the photoreceptor outer segments in hexachlorophene retinopathy has been confirmed in rats [23].

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References

1. Adomato B, Lampert P (1971) Status spongiosis of nervous tissue. Acta Neuropathol (Berl) 19:271-289
2. Basson PA, Kellerman TS, Albl P, Von Malitz LJF, Miller ES, Welman WG (1975) Blindness and encephalopathy caused by *Helichrysum argj rosphaerum* DC. (Compositae) in sheep and cattle. Onderstepoort J Vet Res 42: 135-148
3. Button C, Jerrett I, Alexander P, Mizon W (1987) Blindness in kids associated with overdose of closantel. Aust Vet J 64:226
4. Cho DY, Leipold HW (1977) Experimental spongy degeneration in calves. Acta Neuropathol (Berl) 39:115-127
5. Harper PAW, Healy PJ, Dennis JA (1986) Ultrastructural findings in maple syrup urine disease in Poll Hereford calves. Acta Neuropathol (Berl) 71:316-320
6. Hayes KC, Nielsen SW, Eaton HD (1968) Pathogenesis of the optic nerve lesions in vitamin A-deficient calves. Arch Ophthalmol 80:777-787
7. Huxtable CR, Dorling PR, Colegate SM (1992) *Stypandra* poisoning: the role of stypandrol and proposed pathomechanisms. In: James LF, Keeler RF, Bailey EM, Cheeke PR, Hegarty MP (eds) Poisonous Plants: proceedings of the Third International Symposium, Iowa State University Press, Ames, IA, pp 464-468
8. Huxtable CR, Dorling PR, Slatter DH (1980) Myelin oedema, optic neuropathy and retinopathy in experimental Sty*pandra* toxicosis. Neuropathol Appl Neurobiol 6:221-232
9. Jacobs JM, Crertier JE, Cavanagh JB (1977) Acute effects of triethyl tin on the rat myelin sheath. Neuropathol Appl Neurobiol 3:169-181
10. Jubb KVF, Huxtable CR (1993) The nervous system. In: Jubb KVF, Kennedy PC, Palmer N (eds) Pathology of Domestic Animals, 4th ed, Academic Press, San Diego, CA, pp 267-439
11. Kellerman TS, Coetzer JAW, Naudé TW (1988) Plant Poisonings and Mycotoxicosis of Livestock in Southern Africa, Oxford University Press, Cape Town, South Africa, pp 72-74
12. Lampert P, O'Brien J, Garrett R (1973) Hexachlorophene encephalopathy. Acta Neuropathol (Berl) 23:326-333
13. Lampert PW, Schochet SS (1986) Electron microscopic observations on experimental spongy degeneration of the cerebellar white matter. J Neuropathol Exp Neurol 27:210-220
14. Main DC, Slatter DH, Huxtable CR, Constable IC, Dorling PR (1981) *Stypandra imbricala* ("blindgrass") toxicosis in goats and sheep-clinical and pathological findings in 4 field cases. Aust Vet J 57:132-135
15. McAuliffe PR, White WE (1976) "Woolly everlasting daisy" *(Helichrysum blandoskianum)* toxicity in cattle and sheep. Aust Vet J 52:366-368

16. Morgan KT (1973) Chronic copper toxicity of sheep: an ultrastructural study of spongiform leucoencephalopathy. Res Vet Sci 15:88-95
17. Powell HC, Myers RR, Lampert PW (1980) Oedema in neurotoxic injury. In: Spencer PS, Schaumburg HH (eds) Experimental and Clinical Neurotoxicology, Williams & Wilkins, Baltimore, MD, pp 118-138
18. Prozesky L, Pienaar JG (1977) Amaurosis in sheep resulting from treatment with rafoxanide. Onderstepoort J Vet Res 44:257-260
19. Spencer WH (1986) Optic nerve. In: Spencer WH (ed) Ophthalmic Pathology: An Atlas and Textbook, 3rd ed, WB Saunders, Philadelphia, PA, pp 2337-2458
20. Suzuki K, Kikkana Y (1969) Status spongiosis of CNS and hepatic damage induced by cuprizone (biscyclohexanone oxalyldihydrazone). Am J Pathol 54:307-325
21. Towfighi J (1980) Hexachlorophene. In: Spencer PS, Schaumburg HH (eds) Experimental and Clinical Neurotoxicology, Williams & Wilkins, Baltimore, MD, pp 440-455
22. Towfighi J, Gonatas NK, McCree L (1974) Hexachlorophene induced changes in central and peripheral myelinated axons of developing and adult rats. Lab Invest 31:712-721
23. Towfighi J, Gonatas NK, McCree L (1975) Hexachlorophene retinopathy in rats. Lab Invest 32:330-338
24. Watson WA, Barnett KC, Terlicki S (1972) Progressive retinal degenration (bright blindness) in sheep: a review. Vet Rec 91: 665-670
25. Watson WA, Terlicki S, Patterson DSP, Herbert CN, Done JT (1972) Experimentally produced progressive retinal degeneration (bright blindness) in sheep. Br Vet J 128:457-469
26. Whittington RJ, Searson JE, Whittaker SJ, Glastonbury JRW (1988) Blindness in goats following ingestion of *Stypandra glauca.* Aust Vet J 65:176-181

Chapter ***3.3***

MYELIN VACUOLATION, OPTIC NEUROPATHY AND RETINAL DEGENERATION FOLLOWING CLOSANTEL OVERDOSAGE IN SHEEP AND A GOAT

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Abstract

Toxicity of closantel following accidental overdosage in eleven sheep and one goat are reported. Animals were blind and were euthanised 4-70 days following treatment. In the cerebrum and cerebellum, status spongiosis of the white matter, especially involving areas adjacent to the lateral ventricles, optic radiation, thalamic nuclei, brain stem, and cerebellar peduncles, was noted. The severity of the myelin vacuolation decreased with time after treatment. Ultrastructurally, vacuoles in the cerebral white matter were intramyelinic and resulted from splitting of myelin lamellae at the intraperiod lines, which is consistent with myelin oedema. In the optic nerves, Wallerian degeneration and eventual fibrosis and atrophy of the nerves followed myelin vacuolation. Lesions in the optic nerves were more chronic in the intracanalicular portion than those in the intraorbital and intracranial portions indicating a compressive neuropathy within the optic canal. Retinal lesions were seen in all animals. Acute lesions comprised papilloedema, multifocal necrosis of the outer retinal layers especially the photoreceptor layer, and retinal separation in tapetal and nontapetal areas. In more chronic cases, the outer nuclear layer was diffusely attenuated and generally reduced to a single row of cells and remnants of photoreceptor cells were noticeable. In some of the animals, retinal scars were formed.

Key words: status spongiosis, myelin oedema, optic neuropathy, retinal degeneration, closantel, halogenated salicylanilides, sheep, goat

Introduction

The halogenated salicylanilides are a group of compounds developed mainly for their antiparasitic activity in animals [33]. Closantel and rafoxanide, which represent the most important drugs in this group, are used extensively for the control of *Haemochus* spp. and *Fasciola* spp. infestations in sheep and cattle and *Oestrus ovis* in sheep in many parts of the world, including South Africa [33].

Clinical signs in closantel and rafoxanide toxicity in small stock includes inappetence, ataxia, paresis, recumbence, and blindness with mydriasis and papilloedema [4,7,23,26,33]. There are no reported gross lesions in the central nervous system, while narrowing of the intracanalicular portion of the optic nerves has been reported in sheep with closantel intoxication [7]. Histologically, symmetrical status spongiosis of the white matter of the cerebrum, cerebellum and spinal cord, and oedema, demyelination and lytic necrosis of the optic nerves have been described. The pathogenesis of the myelin vacuolation in the nervous system has not been elucidated. Retinopathy has been reported in blind animals [3,4,7,26,28]. There is however, disagreement on the primary site of retinal damage in salicylanilide poisoning in small stock [7]. Selective involvement including necrosis of the ganglion cells [3,4,26] and degeneration of the outer retinal layers [7,28] have been described.

In this publication histological lesions in the central nervous system, optic nerves and retina as well as ultrastructural changes in the cerebrum of closantel toxicity in sheep and a goat are documented.

Materials and methods

Case history

Eleven sheep, five 2-months-old lambs from a single farm (Nos. 6, 9-12) and six 3-9 months-old lambs from different properties (Nos. 1-5, 8) and one 6-months-old Angora goat kid (No. 7) were obtained for study. The animals originated from farms where blindness following the administration of closantel was observed by the owners. In each case the animals received *c*. 1-4 times the recommended dose of 10mg/kg closantel (Flukiver, Janssens) orally. It was however, not possible to obtain accurate dosages from all farmers although most owners confirmed that the dosage given to animals in the flock was calculated on the average weight of the heaviest animals in the group. The owners reported blindness within 2-5 days following treatment. Fixed dilated pupils and ataxia were seen in four sheep (Nos. 1, 2, 4, 5) and two animals (Nos. 3, 7) were temporary recumbent.

Table 1: Summary of 11 sheep and one goat with closantel poisoning

Animal no.	**Species**	**Age (months) at time of treatment**	**Interval between treatment and necropsy (days)**
1	Sheep	3	4
2	Sheep	6	5
3	Sheep	3	10
4	Sheep	9	15
5	Sheep	8	15
6	Sheep	2	21
7	Goat	5	29
8	Sheep	4	36
9	Sheep	2	42
10	Sheep	2	56
11	Sheep	2	70
12	Sheep	2	70

Ophthalmoscopic investigations

Direct and indirect ophthalmoscopy and slitlamp biomicroscopic examination on five lambs (Nos. 6, 9-12) were performed one and two weeks following treatment and subsequently at two-weekly intervals up to the time of euthanasia. An electroretinogram (ERG) was recorded 2 weeks after overdosing in two lambs (Nos. 9, 11) and in an age-matched control lamb. The pupils were dilated with tropicamide (Mydriacyl, Alcon). The ERG's were recorded without general anaesthesia following 25 min of dark adaptation. After topical anaesthesia with proparcaine HCL (Ophthetic, Nicolet), a corneal ERG contact lens electrode was positioned. The reference electrode (25-gauge hypodermic needle) was positioned on the midline over the frontalis muscle, and the ground electrode was placed at the tip of the right ear. The retina was stimulated with a photo stimulater (Grass model PS22) placed 20 cm from the eye. A blue filter was used to obtain a mixed rod and cone response. The ERG's were recorded with an ossiloscope (Nicolet model 310). The amplification sensitivity was 100 μV/Div and the sweep speed 40 ms/DIV.

Pathology

All animals were euthanised at different intervals after closantel treatment (Table 1) by means of an intravenous overdose of pentobarbitone sodium, and a complete post-mortem examination was performed. The method of collection, fixation and preparation of tissue specimens for histology have been described [35]. Briefly, the brain and spinal cord, sciatic nerve, and a range of other tissue specimens were fixed in 10% neutral buffered formalin. The eyes and the intraorbital portion of the

optic nerves were immersed in Zenker's fixative within 10 min of euthanasia. Optic blocks containing the intracanalicular portion of the optic nerves were decalcified following fixation in formalin in seven animals (Nos. 1, 2, 6, 9-12) and trimmed to expose either the transverse or longitudinal surfaces of the optic nerves within the bony canals.

The brains of eight lambs (Nos. 1-3, 6, 9-12) were serially sectioned, and blocks were prepared from levels cut at the olfactory tubercle and cortex, cerebral cortex (frontal, parietal, temporal, and occipital), basal nuclei, thalamus, mesencephalon, pons, two levels of the medulla oblongata, and the spinal cord in the cervical, thoracic, and lumbosacral portions. From the other animals (Nos. 4,5,7,8), sections of parietal cerebral cortex, thalamus and spinal cord were cut. Midsaggital slabs containing the optic nerve were trimmed from each globe. All tissues were routinely processed for paraffin embedment, and 5-6 μm sections were cut and stained with haematoxylin and eosin (HE). Selected sections of brain, spinal cord, and optic nerves were also stained with luxol fast blue/periodic acid-Schiff/haematoxylin (LFB/PAS/H), luxol fast blue/Holmes (LFB/H) and Masson's trichrome (MT). Ocular sections were also stained with PAS. Mounted unstained sections of the eyes of two lambs (Nos. 6, 9) were studied by fluorescence microscopy.

Specimens of the cerebral subependymal white matter adjacent to the lateral ventricles were collected of two animals (Nos. 1, 3) and prepared for transmission electron microscopy as previously described [35].

Results

Ophthalmoscopic investigations

One week after treatment, the pupils of five lambs (Nos. 6, 9-12) were dilated and the menace reflex as well as direct and consensual pupillary light reflexes were absent in two lambs (Nos. 9, 11). On direct and indirect ophthalmoscopy both these lambs had papilloedema but no evidence of retinal degeneration.

One week later, the five lambs (Nos. 6, 9-12) were again examined. All were blind and the menace reflex and the direct and consensual pupillary light reflexes were absent. On direct and indirect ophthalmoscopy, retinal degeneration characterised by small pale areas in the non-tapetal fundus was seen. At this time there was no papilloedema. Slitlamp biomicroscopic examination confirmed a focal posterior capsular cataract and remnants of the hyaloid artery in one lamb (No. 11). Two weeks later, retinal degeneration in three lambs (Nos. 9-11) had worsened and increased tapetal reflectivity was present in one lamb (No. 10). One animal (No. 11) developed an increased tapetal reflectivity and bilateral horizontal nystagmus two weeks later.

Sheep Nos. 9 and 11 had non-recordable ERG's. The ERG in the control animal consisted of an early negative deflection (a-wave) and a bigger positive deflection

(b-wave). In this animal, the ERG amplitudes obtained while using the blue filter were 221 μV for the a-wave and 620 μV for the b-wave, and without a filter 243 μV for the a-wave and 701 μV for the b-wave.

Gross lesions

Apart from slight narrowing of the intracanalicular portion of the optic nerves in three animals (Nos. 8, 10,11), no lesions were noticed in other tissues and organs at necropsy.

Histological lesions

Brain and spinal cord
Lesions in the central nervous system were of similar nature and in the eight animals (Nos. 1-3, 6, 9-12) which were studied for symmetry, lesions were of similar distribution but of variable severity. There was bilateral symmetrical status spongiosis of the white matter of the brain and spinal cord. Areas consistently affected by myelin vacuolation included the cerebral white matter adjacent to the lateral ventricles (Fig. 1), optic radiation, thalamic nuclei, brain stem particularly the pons, and the cerebellar peduncles. The myelin vacuoles were round to ovoid or elongate, generally 5-30 μm in diameter, and empty. Confluence of vacuoles resulted in large loculated areas sometimes traversed by thin myelin strands as demonstrated in sections stained with LFB. The vacuolation was most extensive in animal Nos. 1-3 and there was a paucity of myelin staining in the most severely affected areas. Myelin vacuolation in the white matter of the spinal cord was mild and inconsistent, and the spinal nerve roots and peripheral nerve were spared. In animals Nos. 9-12, myelin vacuolation was mild and restricted to the periventricular white matter. No evidence of myelin degeneration and no Gitter cells were seen with special stains.

The optic chiasma in three animals (Nos. 9,10,12) revealed mild myelin vacuolation, astrocytic gliosis and multifocal Wallerian degeneration (Fig. 2).

Optic nerves
Lesions in the optic nerves of seven animals (Nos. 1, 2, 6, 9-12) were studied. In the two sheep (Nos. 1, 2) euthanised 4-5 days after treatment, widespread myelinic vacuolation in the intracanalicular portions of the nerves was evident (Fig. 3). In the other five sheep, lesions in the intracanalicular portion of the nerves were more chronic than those in the intraorbital and intracranial portions (Figs. 4 & 5). An irregular line of demarcation separated lesions of different chronicity in the intraorbital portions of the affected nerves (Fig. 4). There was swelling, fragmentation and loss of myelinated axons, multifocal accumulations of macrophages, gliosis, and thickened pial septa. In more chronic cases, the

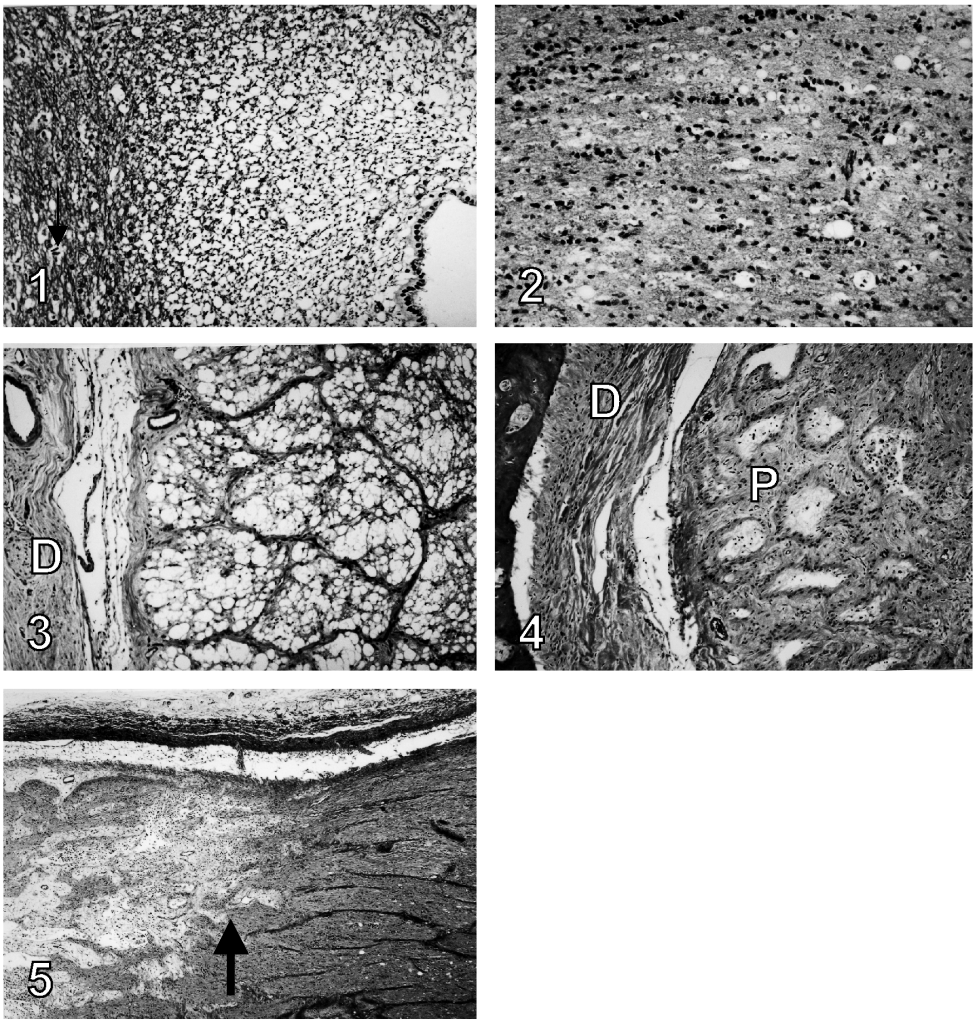

Fig. 1: Cerebrum; Sheep No. 3. Status spongiosis of the periventricular white matter. HE.
Fig. 2: Optic chiasma; Sheep No. 9. Gliosis and multifocal myelin vacuolation. HE.
Fig. 3: Optic nerve; Sheep No. 2. Myelin vacuolation in the intracanalicular portion of the nerve. D = dura mater. HE.
Fig. 4: Optic nerve; Sheep No. 5. Fibrosis of pial septa (P) with associated loss of nerve fibres and multifocal cellular infiltrates in the intracananicular portion of the nerve. Note fibrous thickening of meninges including the dura mater (D) within the bony canal. HE.
Fig. 5: Optic nerve; Sheep No. 10. There is a clear line of demarcation in a cross section of the intraorbital portion of the optic nerve (arrow). Towards the optic canal (to the left of arrow) lesions comprise necrosis and Wallerian degeneration

while towards the optic disc (to the right of arrow) less severe lesions are noted. HE.

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intracanalicular portion of the nerve was contracted and showed diffuse fibrosis with complete loss of axons and multifocal accumulations of neutrophils and macrophages (Fig. 5). There was severe fibrosis of the meninges.

Retina

In two animals (Nos. 1, 2) examined 4-5 days after overdosage, the optic disc was markedly swollen due to oedema, while the sensory retina was folded and displaced away from the edges of the disc (Fig. 6). The peripapillary and midzonal retina, involving the tapetal and non-tapetal areas, showed multifocal to coalescing necrosis, disappearance of the photoreceptor layer, outer and inner nuclear layers and outer plexiform layer, and focal haemorrhage (Fig. 7). There was occasional haemorrhage and focal neutrophil infiltration in the underlying choroid. In areas of severe necrosis, large subretinal spaces contained eosinophilic granular debris and remnants of photoreceptor segments, fibrinous material, free-lying pyknotic nuclei (likely representing cone and rod nuclei), pigmented epithelial cells, macrophages sometimes with grayish granular pigment, and red blood cells (Fig. 7). In the remainder of the retina, thinning of the outer nuclear layer together with stubby remnants of the photoreceptor inner segments or translucent membranous profiles possibly representing degenerative photoreceptor outer segments were present (Fig. 8). The inner nuclear layer was focally reduced in thickness. A small number of ganglion neurons, usually within or adjacent to foci of necrosis, were swollen and chromatolytic and revealed dispersion of Nissl substance and nuclear margination or fragmentation (Fig. 9).

In the other ten animals (Nos. 3-12) euthanised between 10-70 days after treatment, the optic disc was of normal size and the retinal changes more advanced. A consistent lesion was diffuse attenuation of the outer nuclear layer which was generally reduced to a single row of cells with scattered nuclear pyknosis noticeable (Fig. 10). The photoreceptor layer was absent in most areas. The inner retinal layers and occasionally the ganglion cell layer contained small groups of heavily pigmented cells and extracellular oval and elongated melanin granules. There was variable reduction of ganglion cells (Fig. 10). In three animals (Nos. 7, 11, 12) there were additional chronic lesions characterised by marked atrophy of the retina, loss of all layers, extensive pigment cell migration, and glial scars (Fig. 11). There was no autofluorescent or PAS-positive pigment in the retinal pigmented epithelium.

Other organs and tissues

No significant lesions were evident.

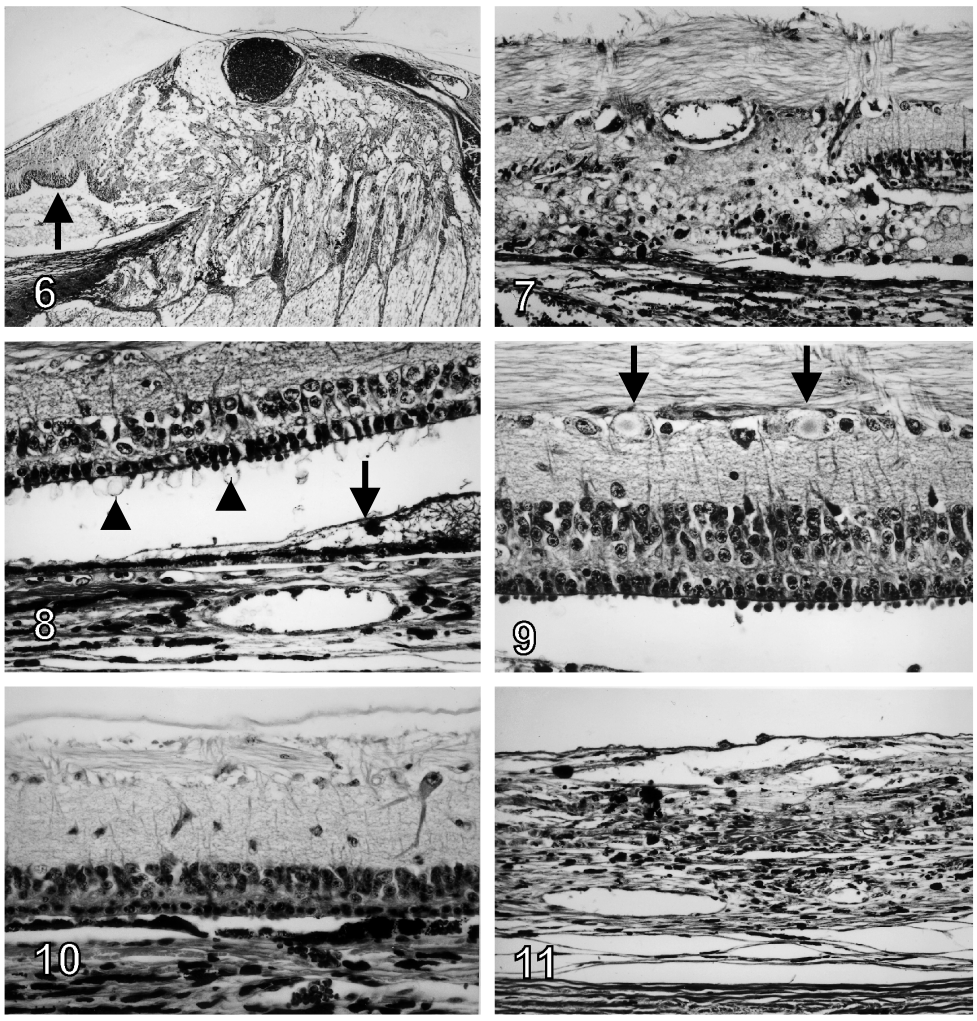

Fig. 6: Eye; Sheep No. 1. Oedema of the optic papilla and nerve, congestion of retina blood vessels, and displacement of sensory retina. Also note retinal necrosis, folding (arrow) and detachment. HE.
Fig. 7: Eye; Sheep No. 1. Necrosis and loss of outer retinal layers and formation of a subretinal space containing cellular exudate and fibrinous material in the midzonal nontapetal area. HE.
Fig. 8: Eye; Sheep No. 2. Attenuation of the outer and inner nuclear layers and a subretinal space partially filled with fibrinous material (arrow) in the peripapillary nontapetal retina. Note stubby remnants of photoreceptor cells and irregular membranous profiles (arrowheads). HE.

Fig. 9: Eye; Sheep No. 2. Necrosis of ganglion cells (arrows) in the midzonal tapetal retina. The nuclear layers are reduced in thickness and the photoreceptor layer is represented by remnants of inner segments. HE.
Fig. 10: Eye; Sheep No. 6. More advanced retinal lesions in the midzonal tapetal retina. Lesions are characterised by disappearance of the photoreceptor layer, hyperplasia and hypertrophy of pigmented epithelium, a single row of cells in the outer nuclear layer, atrophy of the outer plexiform layer and reduction in the number of ganglion cells. HE.
Fig. 11: Eye; Animal No. 10 (goat). Chronic retinal lesions comprise loss of normal architecture with atrophy, pigment cell migration, and scar tissue formation. HE.

Transmission electron microscopical lesions

The vacuoles in the cerebral white matter corresponded to distension of myelin sheaths due to splitting of myelin lamellae at the intraperiod lines (Fig. 12, inset). Vacuoles of variable size were especially noticeable at the outer portions of the myelin sheath of larger axons. The vacuoles were mostly empty although few contained small membranous fragments. There was marked distension of extracellular and perivascular spaces. Axons were well preserved in the affected nerve fibres. Glial cells, endothelial cells and neurons did not show abnormalities and myelin breakdown was not confirmed.

Discussion

In this study of accidental salicylanilide poisoning in sheep and a goat, status spongiosis of the cerebral and cerebellar white matter was a consistent lesion. This finding is in agreement with previous publications [3,22,26]. There were only mild spongy changes in the spinal cord and no involvement of peripheral myelin. The intensity of the myelin vacuolation decreased with time after treatment and would probably have resolved after a single-dose treatment without leaving residual lesions in the nervous system. We demonstrated intramyelinic vacuolation due to separation of lamellae along the intraperiod line as the structural basis for the status spongiosis in toxicity by salicylanilide anthelmintics.

Status spongiosis denotes a spongy vacuolation of white matter evident at light microscopy [1]. Electron microscopy is often required to visualise the morphological basis for the vacuolation [31]. It may be associated with intracellular, extracellular and intramyelinic accumulation of fluid or may represent an artefact [1]. The most common location of such vacuolation is intramyelinic where the accumulation of fluid results in splitting of the intraperiod line [15].

A number of conditions designated as spongiform myelinopathies are characterised by myelin splitting at the intraperiod line and the subsequent formation of intramyelinic vacuoles. Myelin splitting may be caused by the toxic

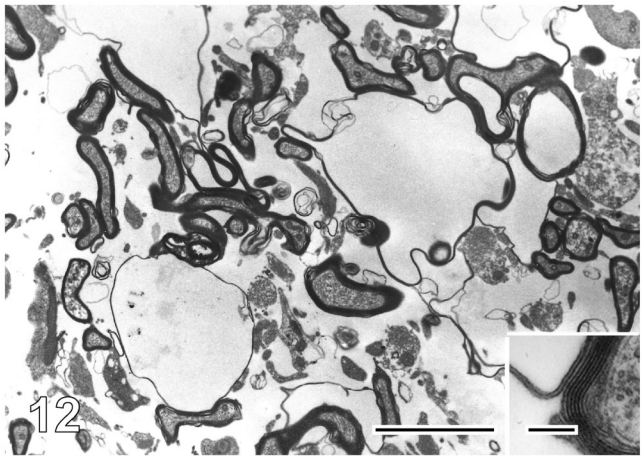

Fig. 12: Transmission electron micrograph. Cerebrum; Sheep No. 3. Multiple intramyelinic vacuolar spaces and distended extracellular spaces. Bar = 5 µm. Inset: Higher magnification to illustrate splitting of myelin lamellae at the intraperiod line. Bar = 0,1 µm.

effects of substances of exogenous origin such as in experimental poisoning by hexachlorophene [16,34], triethyl tin [12,37], cuprizone [32], isonicotinic acid hydrazide [17], nitrobenzene [21] and aniline [24] or by substances of endogenous origin such as in disorders of intermediary metabolism. In domestic animals, intoxications characterised by separation of myelin lamellae at the intraperiod line include overdosage by ammonia [5] and copper [20], ingestion of the plants *Stypandra imbricata* [10,11,18] and *Helicrysum argyrosphaerum* [35] and in diplodiosis associated with the fungus *Stenocarpella maydis* (= *Diplodia maydis*) [13,14,25]. Maple syrup urine disease, a disorder of intermediary metabolism characterised by intramyelinic vacuole formation in the brain, has been described in calves [8,9].

The pathogenesis of myelin splitting at the intraperiod line is not known. The encephalopathy in bovine maple syrup urine disease appears to be associated with a diminution of GABA-mediated neurotransmission [6]. In toxicity with nitrobenzene and aniline, uncoupling of mitochondrial oxidative phosphorylation in oligodendrocytes was suggested as the cause for the myelinic vacuolation [21,24]. Inhibition of oxidative phosphorylation interferes with ATP production and cause disturbances in the transmembrane energy-bound electrolyte transport [36]. In studies with aniline neurotoxicity in rats, a small amount of membranous debris was sporadically found in the cytoplasm of oligodendrocytes possibly indicating impairment of these cells [24]. The anthelmintic spectrum of closantel has been linked to the compound's ability to uncouple oxidative phosphorylation, but it is not known if this mechanism can explain the toxic effects in sheep and goats [2,33]. A primary myelinotoxic effect of salicylanilides on the myelin sheath,

especially since the vacuolation with overdosage of these anthelmintics is widely distributed throughout the nervous system, cannot be excluded [15].

Pathology of the optic nerves was confirmed in all cases studied. The nature and progression of the lesions showed initial myelin vacuolation leading to Wallerian degeneration and eventual irreversible fibrosis and contraction of the nerve. Optic nerve lesions in all animals, except for the two acute cases (Nos. 1, 2), were generally more chronic in the intracananicular portion of the nerve than in the intraorbital and intracranial portions. Similar optic nerve lesions were reported previously in closantel poisoning [3,7], *H. argyrosphaerum* poisoning [35] and *S. imbricata* [18] and *S. glauca* intoxication [40]. A common pathogenesis for the optic neuropathy, namely initial myelin oedema followed by swelling and compression of the nerve within the bony canal is proposed in these intoxications.

The first ophthalmological signs were absence of pupillary light reflexes and papilloedema. A loss of light reflexes is an indication of optic nerve and/or retinal damage [27]. Depigmentation of the non-tapetum and increased reflectivity of the tapetum indicate retinal degeneration. The a wave of the electroretinogram is generated by the photoreceptors and the b wave by the Müller's cells and bipolar neurons [29]. A normal ERG can be obtained despite ganglion cell degeneration. An ERG was only performed after retinal changes were ophthalmoscopically visible, making it impossible to speculate on the presence of retinal damage during the first week after treatment. Two weeks after treatment non-recordable ERG's were obtained, which was consistent with the retinal degeneration seen ophthalmoscopically [29]. The posterior capsular cataract and remnants of the hyaloid artery in Sheep No. 11 were regarded as coincidental findings.

It would seem that closantel has a direct retinotoxic effect in small stock. The observed lesions in the outer retina are not secondary to optic neuropathy since retrograde degeneration of the photoreceptor layer is not seen in optic nerve damage, even when the nerve is completely transected [30]. The photoreceptor outer segments are composed largely of compacted plasma membranes, somewhat analogous to myelin, and it was suggested that a similar toxic mechanism underlies the injury to the outer retina and myelin [7]. Acute retinal lesions were characterised by multifocal necrosis and loss of the photoreceptor layer and outer nuclear layer, and retinal separation. In more chronic lesions, the outer nuclear layer was generally reduced to a single row of neuronal nuclei with multifocal loss of the outer nuclear layer and photoreceptor layer. The rods and cones were mere vestiges or mostly absent. There was preservation of the inner retinal layers until later in the course of the intoxication when a reduction in ganglion cells was noted. Eventually, chorioretinal scars were formed. A similar type of retinopathy has been described in closantel toxicosis [7,28]. Acute lesions (papilloedema, retinal haemorrhage and exudation within a subretinal space) in two animals (Nos. 1, 2) in our study have not been reported previously but may correlate with the dosage level of closantel and the short interval between treatment and necropsy.

The retinal lesions in closantel poisoning are very similar to those described in sheep and a goat with *H. argyrosphaerum* poisoning [35]. In this plant intoxication,

degeneration and loss of the photoreceptor outer segments was considered the primary event, which progressed to loss of all retinal layers. In chronic cases of closantel poisoning, the outer nuclear layer remained as a single row of large neuronal nuclei in most parts of the retina. This change may be interpreted as selective survival of cone nuclei especially since tiny blunt processes, likely representing persistent cones, were evident in the photoreceptor layer. It would therefore seem that the photoreceptor layer, possibly the rods, is primarily involved in acute halogenated salicylanilide poisoning. Electron microscopical studies may clarify the pathogenesis of the retinopathy in both conditions. The difference in distribution of the retinopathy between the two intoxications may be dose dependent. The photoreceptor outer segments also seem to be primarily targeted in other toxic retinopathies such as poisoning by *S. glauca* [40], *S. imbricata* [18] and bracken fern *(Pteridium aquilinum)* [38,39]. Closantel intoxication may also cause blindness in dogs [19]. A dog that received six times the recommended dose showed papilloedema, retinal haemorrhages, and optic neuritis [19].

Retinal ganglion cells were affected in two ways depending on the stage of the intoxication. In acutely affected animals (Nos. 1, 2), chromatolysis of cell bodies was noted. Prozesky & Pienaar reported similar involvement of ganglion cells in rafoxanide toxicity in sheep [26]. The association of ganglion cell degeneration with diffuse myelin swelling in the optic nerves suggested that the ganglion cell lesions may either reflect a direct toxic effect on the neurons or follow axonal injury within the optic nerve. In more chronic cases, a reduction in the number of ganglion cells is most likely attributed to degeneration and loss of axons in the optic nerves.

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References

1. Adornato B, Lampert P (1971) Status spongiosis of nervous tissue. Acta Neuropathol (Berl) 19:271-289
2. Bacon JA, Ulrich RG, Davis JP, Thomas EM, Johnson SS, Condor GA, Sangster NC, Rothwell JT, McCracken RO, Lee BH, Clothier MF, Geary TG, Thompson DP (1998) Comparative *in vitro* effects of closantel and selected β-ketoamide anthelmintics on a gastrointestinal nematode and vertebrate liver cells. J Vet Pharmacol Ther 21:190-196
3. Borges AS, Mendes LCN, De Andrade AL, Machado GF, Peiro JR (1999) Optic neuropathy in sheep associated with overdosage of closantel. Vet Human Toxicol 41:378-380
4. Button C, Jerrett I, Alexander P, Mizon W (1987) Blindness in kids associated with overdose of closantel. Aust Vet J 64:226

5. Cho DY, Leipold HW (1977) Experimental spongy degeneration in calves. Acta Neuropathol (Berl) 39:115-127
6. Dodd PR, Williams SH, Gundlach AL, Harper PAW, Healy PJ, Dennis JA, Johnston AR (1992) Glutamate and γ-aminobutyric acid neurotransmitter systems in the acute phase of maple syrup urine disease and citrullinemia encephalopathies in newborn calves. J Neuroch 59:582-590
7. Gill PA, Cook RW, Boulton JG, Kelly WR, Vanselow B, Reddacliff LA (1999) Optic neuropathy and retinopathy in closantel toxicosis of sheep and goats. Austr Vet J 77:259-261
8. Harper PAW, Dennis JA, Healy PJ, Brown GK (1989) Maple syrup urine disease in calves: a clinical, pathological and biochemical study. Aust Vet J 66:46-49
9. Harper PAW, Healy PJ, Dennis JA (1986) Ultrastructural findings in maple syrup urine disease in poll Hereford calves. Acta Neuropathol (Berl) 71:316-320
10. Huxtable CR, Dorling PR, Colegate SM (1992) Stypandra poisonimg: the role of stypandrol and proposed pathomechanisms. In: James LF, Keeler RF, Bailey EM, Cheeke PR, Hegarty MP (eds). Poisonous Plants: Proceedings of the Third International Symposium, Ames: Iowa State University Press, pp 464-468
11. Huxtable CR, Dorling PR, Slatter DH (1980) Myelin oedema, optic neuropathy and retinopathy in experimental Stypandra toxicosis. Neuropathol Appl Neurobiol 6:221-232
12. Jacobs JM, Crertier JE, Cavanagh JB (1977) Acute effects of triethyl tin on the rat myelin sheath. Neuropathol Appl Neurobiol 3:169-181
13. Kellerman TS, Prozesky L, Schultz RA, Rabie CJ, Van Ark H, Maartens BP, Lübben A (1991) Perinatal mortality in lambs of ewes exposed to cultures of *Diplodia maydis* (= *Stenocarpella maydis*) during gestation. Onderstepoort J Vet Res 58: 297-308
14. Kellerman TS, Rabie CJ, Van der Westhuizen GCA, Kriek NPJ, Prozesky L (1985) Induction of diplodiosis, a neuromycotoxicosis, in domestic ruminants with cultures of indeginous and exotic isolates of *Diplodia maydis*. Onderstepoort J Vet Res 52: 35-42
15. Kreutzberg GW, Blakemore WF, Graeber MB (1997) Cellular pathology of the central nervous system. In: Graham DI, Lantos PL (eds) Greenfield's Neuropathology 6th edn Arnold: London, pp 85-140
16. Lampert P, O'Brien J, Garrett R (1973) Hexachlorophene encephalopathy. Acta Neuropathol (Berl) 23:326-333
17. Lampert PW, Schochet SS (1986) Electron microscopic observations on experimental spongy degeneration of the cerebellar white matter. J Neuropathol Exp Neurol 27:210-220
18. Main DC, Slatter DH, Huxtable CR, Constable IC, Dorling PR (1981) Stypandra imbricata ("blindgrass") toxicosis in goats and sheep - clinical and pathological findings in 4 field cases. Aust Vet J 57:132-135
19. McEntee K, Grauwels M, Clercx C, Henroteaux M (1995) Closantel intoxication in a dog. Vet Hum Toxicol 37: 234-236
20. Morgan KT (1973) Chronic copper toxicity of sheep: an ultrastructural study of spongiform leucoencephalopathy. Res Vet Sci 15:88-95
21. Morgan KT, Gross EA, Lyght O, Bond JA (1985) Morphologic and biochemical studies of a nitrobenzene-induced encephalopathy in rats. Neurotoxicology 6:105-116
22. Obwolo MJ, Odiawo GO, Ogaa JS (1989) Toxicityof a closantel-albendazole mixture in a flock of sheep and goats. Aust Vet J 66: 229-230

23. Odiawo GO, Ogaa JS, Ndikuwera J, Moulton JE (1991) Acute amaurosis in sheep due to rafoxanide neurotoxicity. Zimbabwe Vet J 22: 64-69
24. Okazaki Y, Yamashita K, Sudo M, Tsuchitani M, Narama I, Yamaguchi R, Tateyama S (2001) Neurotoxicity induced by a single oral dose of aniline in rats. J Vet Med Sci 63:539-546
25. Prozesky L, Kellerman TS, Swart DP, Maartens BP, Schultz RA (1994) Perinatal mortality in lambs exposed to cultures of *Diplodia maydis* (= *Stenocarpella maydis*) during gestation. A study of the central nervous system lesions. Onderstepoort J Vet Res 61: 247-253
26. Prozesky L, Pienaar JG (1977) Amourosis in sheep resulting from treatment with rafoxanide. Onderstepoort J Vet Res 44: 257-260
27. Scagliotti RH (1999) Comparative Neuro-ophthalmology. In: Gelatt KN (ed) Veterinary Ophthalmology 3rd ed Lippincott Williams & Wilkens, pp 1307-1400
28. Seawright AA (1989) Animal Health in Australia, Volume 2, 2nd edn, Chemical and Plant Poisonings, Australian Government Publishing Service, Canberra, pp 331-332
29. Sims MH (1999) Electrodiagnostic evaluation of vision. In: Gelatt KN (ed) Veterinary Ophthalmology 3rd ed Lippincott Williams & Wilkens, pp 483-507
30. Spencer WH (1986) Optic nerve. In: Spencer WH (ed) Ophthalmic Pathology: An Atlas and Textbook, 3rd ed, WB Saunders, Philadelphia, pp 2337-2458
31. Summers BA, Cummings JF, De Lahunta A (1995) Veterinary Neuropathology. Mosby: St. Louis, pp 1-67, 214-236
32. Suzuki K, Kikkana Y (1969) Status spongiosis of CNS and hepatic damage induced by cuprizone (biscyclohexanone oxalyldihydrazone). Am J Pathol 54:307-325
33. Swan GE (1999) The parmacology of halogenated salicylanilides and their anthelmintic use in animals. Journal of the South African Veterinary Association 70: 61-70
34. Towfighi J (1980) Hexachlorophene In: Spencer PS, Schaumburg HH (eds) Experimental and Clinical Neurotoxicology, Baltimore/London: Williams & Wilkens, pp 440-455
35. Van der Lugt JJ, Olivier J, Jordaan P (1996) Status spongiosis, optic neuropathy, and retinal degeneration in *Helichrysum argyrosphaerum* poisoning in sheep and a goat. Vet Pathol 33:495-502
36. Verity MA (1997) Toxic disorders. In: Graham DI, Lantos PL (eds) Greenfield's Neuropathology 6th edn Arnold: London, pp 755-811
37. Watanae I (1980) Organotins (triethyltin). In: Spencer PS, Schaumburg HH (eds) Experimental and Clinical Neurotoxicology, Baltimore/London: Williams & Wilkens, pp 545-557
38. Watson WA, Barnett KC, Terlicki S (1972) Progressive retinal degenration (bright blindness) in sheep: a review. Vet Rec 91: 665-670
39. Watson WA, Terlicki S, Patterson DSP, Herbert CN, Done JT (1972) Experimentally produced progressive retinal degeneration (bright blindness) in sheep. Br Vet J 128:457-469
40. Whittington RJ, Searson JE, Whittaker SJ, Glastonbury JRW (1988) Blindness in goats following ingestion of Stypandra glauca. Aust Vet J 65:176-181

Chapter ***3.4***

DIARRHOEA, BLINDNESS AND CEREBRAL SPONGY CHANGES IN CATTLE CAUSED BY *ORNITHOGALUM SAUNDERSIAE* AND *O. PRASINUM* POISONING

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Abstract

Poisoning of cattle by *Ornithogalum saundersiae* and *O. prasinum* in South Africa is described. In two subsequent years, cattle were exposed to garden waste containing toxic *O. saundersiae*. A total of 17 animals became sick of which 13 died. All affected animals showed diarrhoea and four became blind within six days of showing clinical signs. Following naturally *O. prasinum* poisoning, three cattle were dosed with toxic plant material. They developed diarrhoea followed by blindness 6-10 days after the onset of clinical signs. The three cattle were euthanised on Days 7, 18 and 74, respectively. Histology of the brain in three blind cattle following ingestion of *O. saundersiae* and of the three blind cattle after being dosed with *O. prasinum* revealed symmetrical spongiform changes in the cerebral white matter. Lesions were of variable severity and were most conspicuous in the white matter of the cerebral hemispheres and in the optic radiation and internal capsule. Ultrastructurally, intramyelinic vacuolation and distention of perivascular and extracellular spaces in the cerebral white matter were noticeable in cattle dosed with *O. prasinum*. No lesions were detected in the eyes. Toxic *O. prasinum* plant material was also given to five sheep. The sheep either died or were euthanised between Days 2-21. All sheep developed diarrhoea, while the two sheep that were euthanised 8 and 21 days after the start of the dosing trial maintained good vision and had no significant histological lesions in the nervous system and eyes at the time of necropsy.

Key words: *Ornithogalum saundersiae*, *Ornithogalum prasinum*, cattle, diarrhoea, blindness, spongy change, status spongiosis

Introduction

Several *Ornithogalum* spp. are highly toxic and may occasional cause poisoning and death in cattle, small stock and horses in South Africa [7]. Some species of this bulbous plant grow beautiful flowerheads (Fig. 1) and are often planted in gardens throughout the country. The two best known examples are *O. thyrsoides* (chinkerinchee, Star-of-Bethlehem) and *O. saundersiae* (Transvaal chinkerinchee). *Ornithogalum thyrsoides* is endemic in the Winter Rainfall area of the Cape Province and prefers moist areas such as vleis or river banks while *O.saundersiae* occurs in Mpumalanga and northern Kwazulu-Natal in South Africa, and Swaziland (Fig. 2) and has a more varied habitat [8].

Fig. 1: Flowerheads of *Ornithogalum saundersiae* (courtesy Botanical Research Institute, Pretoria).

Outbreaks of poisoning in ruminants due to *O. thyrsoides* and *O.saundersiae* often result from animals having access to garden waste containing toxic plant material [7]. Reports on the toxicity of *O. thyrsoides* dates back to 1904 when carthorses died after eating contaminated oat hay. In 1927 the toxicity of *O.saundersiae* was confirmed in several animal species. During subsequent outbreaks and dosing trials with Transvaal chinkerinchee it became apparent that affected cattle consistently develop diarrhoea and later may become temporarily or permanently blind [7].

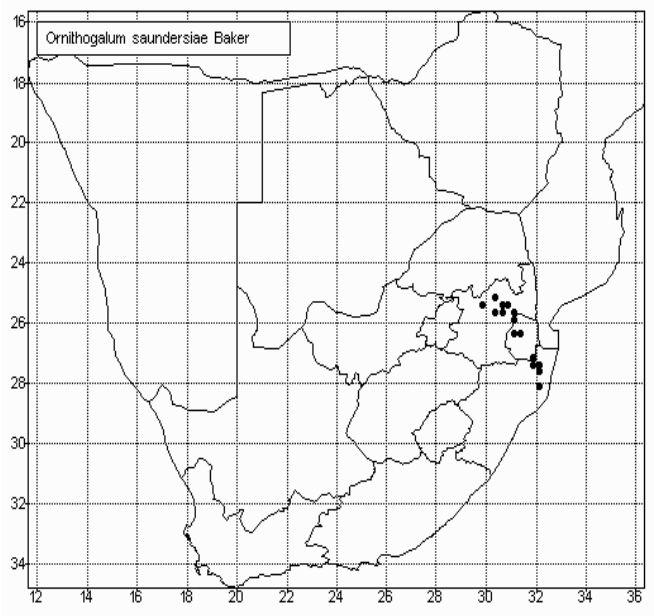

Fig. 2: Distribution of *Ornithogalum saundersiae* (courtesy Botanical Research Institute, Pretoria, South Africa).

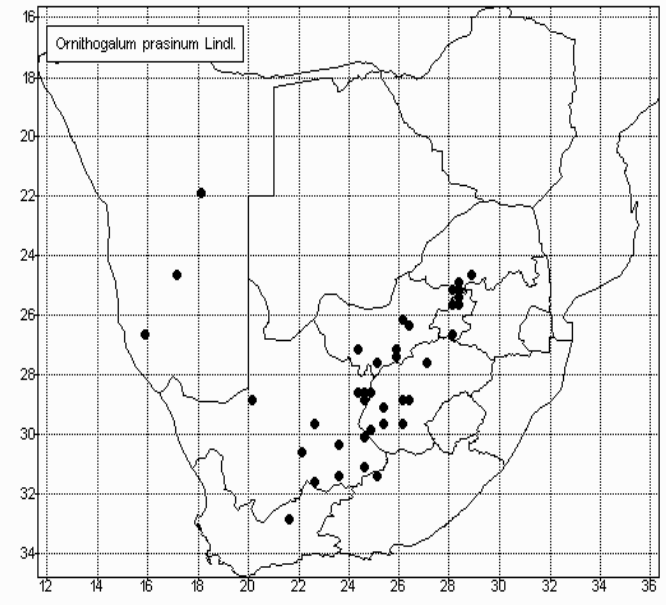

Fig 3: Distribution of *Ornithogalum prasinum* (courtesy Botanical Research Institute, Pretoria, South Africa).

Fig. 4: *Ornithogalum prasinum* growing in veld in the Northern Province.

Fig. 5: *Ornithogalum prasinum* is a bulbous plant (courtesy Oxford University Press, Cape Town).

Other *Ornithogalum* spp. (e.g. *O. conicum*, *O. ornithogaloides* and *O. prasinum*, Fig. 3) may cause poisoning in ruminants grazing infested natural veld (Fig. 4, 5). Toxicity of *O. prasinum* was confirmed in 1986 following outbreaks of diarrhoea and blindness in cattle in the Northern Province of South Africa [7].

Poisoning by *Ornithogalum* plants in cattle and sheep may cause acute death without diarrhoea or death may be preceded by severe watery or mucoid to haemorrhagic diarrhoea. In addition to the gastrointestinal signs, cattle but not sheep may develop temporary or permanent blindness. The pathogenesis of blindness in cattle has not been thoroughly investigated. Unpublished observations of a dosing trial with *O. prasinum*, included in this report, have been briefly reported [7]. Here we describe a field outbreak of *O. saundersiae* in cattle and a dosing trial with *O. prasinum* in cattle and sheep with emphasis on the nature and distribution of the nervous lesions associated with blindness in cattle.

History of field outbreaks

Ornithogalum saundersiae

A farmer near Vryheid, KwaZulu-Natal kept a group of 19 mixed breed cattle in a small camp during the winter months. In summer they grazed natural veld. The animals were bought from various sources and most of them were 14-24 months of age. In the camp they were fed hay and had access to lick blocks and water. Adjacent to the camp the farmer had a vegetable garden. At the end of May 1993 he cleaned the garden in order to start planting winter crops. The garden waste was thrown over the fence into the cattle camp. Two weeks later 11 animals developed severe watery diarrhoea within a period of 3 days, became weak and dehydrated and often walked with a staggering gait. Seven cattle died 4-7 days of the onset of clinical signs. The farmer also noticed that four cattle that developed diarrhoea and maintained a good appetite, became blind within six days of showing clinical signs. The farm was visited and it was found that the garden waste, to which the cattle had free access, contained several plant bulbs, some of which were partially eaten. In the camp an unknown plant had extensively been grazed, but no other toxic source or plants were detected in the garden waste or in the camp.

The farmer mentioned that a similar outbreak of clinical disease and mortalities in his cattle occurred the previous year. He recalled that six out of 19 cattle were affected and all died. A post mortem examination on two carcasses revealed dehydration, congestion of the mucosa of the abomasum and small intestines, and a watery diarrhoea. Bovine viral diarrhoea virus infection and lead and arsenic poisoning were initially suspected but were ruled out, and a specific diagnosis was not established at that time.

Bulbs collected from the garden waste were planted in the garden of the investigating veterinarian. At the time of flowering, whole plants were sent to the Botanical Research Institute (BRI), Pretoria, South Africa for identification. The unkown plant that was heavily grazed by the cattle in the camp was also sampled

for identification. The bulbous plant was identified as *Ornithogalum saundersiae* Bak. (Liliaceae) and the other plant collected from the camp was identified as *Nothoscordium gracile* (Ait.) Steam, an introduced plant species not known to be toxic to cattle.

Ornithogalum prasinum

Sporadic outbreaks of diarrhoea, blindness and death in cattle in the Rustenburg district, Northern Province, South Africa were investigated. The cattle had extensively grazed a bulbous plant, subsequently identified as *O. prasinum* Lindl. by staff of the BRI, Pretoria. After other possible causes of blindness and diarrhoea in cattle had been ruled out, three *Ornithogalum* spp. occurring in the Rustenburg district were evaluated for toxicity. In a pilot trail, plant material of *O. seineri*, *O. tenuifolium* and *O. prasinum* were dosed to sheep: only *O. prasinum* proved to be toxic (HE van de Pypekamp and TS Kellerman, Onderstepoort Veterinary Institute, unpublished data, 1986). It was subsequently decided to study the toxicity of *O. prasinum* in cattle and sheep in more detail, the results of which are published here.

Materials and methods

Ornithogalum saundersiae

Pathology. Necropsies were performed on three affected cattle with blindness that were killed by intravenous injection of an overdose of pentobarbitone sodium 2-10 days after becoming blind. The entire brains, spinal cords, eyes and tissue specimens from a range of organs were collected in 10% buffered formalin and submitted for histology. Paraffin sections of all tissues were prepared and stained with haematoxylin and eosin (HE) according to standard procedures. Selected sections of the brains were stained with luxol fast blue/periodic acid-Schiff/ haematoxylin (LFB/PAS/H) and luxol fast blue/Holmes (LFB/H).

Ornithogalum prasinum

Dosing trial. Plant bulbs of *O. prasinum* were collected from one of the farms in the Rustenburg district and planted in a glass house at the Section of Toxicology, Onderstepoort Veterinary Institute. At the time of the dosing trail, fresh leaves and bulbs were milled and given per stomach tube to three cattle (Bovine Nos. 1-3) and five Merino ewes (Sheep Nos. 1-5) which had not been exposed previously to the plant (Table 1). To prolong the course of the intoxication in order to try and induce blindness, one sheep and two cattle were treated with activated charcoal following the onset of clinical signs (Table 1).

Ophthalmoscopy. Two bovines were examined with direct and indirect ophthalmoscopy: Bovine No. 1 three days (on Day 17) after the onset of blindness

and Bovine No. 2 four and 14 days after the diagnosis of impaired vision (on Days 14 and 24, respectively).

Table 1: The toxicity of *Ornithogalum prasinum* in cattle and sheep

Case	**Initial live mass (kg)**	**Dosage regimen (and treatment with AC*)**	**Duration of trial (days)**	**Clinical signs and fate of animal**
Bov No. 1 (bull)	268	1 g/kg x 1 (AC: 5 g/kg on Days 3, 4)	7	Listlessness, diarrhoea, weakness, blindness, and recumbency. Euthanised on Day 7
Bov No. 2 (heifer)	260	1 g/kg x 1	18	Listlessness, anorexia, diarrhoea, weight loss, blindness and weakness. Euthanised on Day 18
Bov No. 3 (steer)	173	1 g/kg x 1 (AC: 2 g/kg on Days 2, 3, 4)	74	Listlessness, anorexia, temporary diarrhoea and blindness. Euthanised on Day 74
Sheep No. 1 (ewe)	40	5 g/kg x 1	2	Diarrhoea, anorexia and listlessness. Died on Day 2
Sheep No. 2 (ewe)	36	2,5 g/kg x 1	2	Diarrhoea, anorexia and dyspnoea. Died on Day 2
Sheep No. 3 (ewe)	24	1,25 g/kg x 1	3	Diarrhoea and anorexia. Died on Day 3
Sheep No. 4 (ewe)	38,5	1 g/kg x 1 (AC: 5 g/kg on Days 3, 4)	8	Depressed and diarrhoea. Euthanised on Day 8
Sheep No. 5 (ewe)	37,5	1 g/kg x 1	21	Depressed and diarrhoea. Euthanised on Day 21

*AC = activated charcoal; Bov = bovine

Pathology. A detailed necropsy was performed on animals that died or that were euthanised and a range of organs and tissues were fixed 10% buffered formalin for histology. These samples included representative portions of the optic nerve (intraorbital, intracananicular and intracranial parts) of two cattle (Nos. 2, 3). Specimens were embedded and sections were prepared routinely.

Portions of the midbrain of two cattle (Nos. 2, 3) were fixed in 2,5% gluteraldehyde in 0,1 M sodium cacodylate buffer (at pH 7,3 - 7,4) within 15 min of euthanasia for transmission electron microscopy. Following fixation at room temperature for 24 h, specimens were post-fixed in 2 % osmium tetroxide, dehydrated in a graded ethanol series, passed through propylene oxide, and embedded in Polarbed 812. Semithin (1-2 μm) sections were stained with toluidine

blue for tissue orientation. Ultrathin sections were stained with uranyl acetate and lead citrate and studied with a transmission electron microscope.

Results

Clinical signs

Clinical signs of animals dosed with *O. saundersiae* are given in Table 1. Bovine No. 1 developed a watery diarrhoea on Day 1 which became haemorrhagic on Day 3. The animal had difficulty in rising, walked with an unsteady gait and swaying hind quarters, and appeared depressed. On Day 6 the animal became blind and walked into fences and other objects. On Day 7 the bull was found in lateral recumbency and was euthanised.

Bovine No 2: The heifer became listless and anorexic on Day 1 and lost 34 kg of weight until Day 11. The animal showed diarrhoea throughout the course of the disease, until euthanised on Day 18. The contents of the faeces varied from watery (Day 1) or mucoid (Day 4) to bloodstained (Day 11). On Days 7 and 14 it was taken out of the pen into a paddock and appeared blind: the heifer walked into fences, stumbled over objects and lifted her feet abnormally high when stepping over small objects. On these two days, the heifer and a control animal were guided through an obstacle course. On each occasion, the heifer could not cross the first obstacle, while the control animal negotiated the course without colliding into obstacles. The heifer was euthanised on Day 18.

The steer (Bovine No 3) showed watery diarrhoea on Days 1-4. On Day 10 the steer developed blindness: he walked hesitantly with the head held high, tended to stumble and bumped its head against the fence when chased. It was able to see movement and large objects such as thick trees at short distances, but collided with smaller tree trunks while walking. On Days 22, 24, 31 and 38 the steer was driven through the obstacle course and collided into the objects. Its vision remained impaired until Day 74 when euthanasia was performed.

In Bovine No. 2, there was a loss of the menace response in both eyes, but pupillary light reflexes were normal. Ophthalmoscopy did not reveal visible ocular abnormalities in Bovines Nos. 1 and 2. There were also no lesions in the retina or optic papilla and no cause for the blindness was apparent.

Three sheep (Nos. 1-3) given 1,25 – 5 g/kg plant material developed diarrhoea and anorexia and died within 2-3 days. Sheep Nos. 4 and 5 that were dosed with 1 g/kg toxic plant material were euthanised. Sheep No. 4 became depressed and developed a watery diarrhoea on Day 2. On Day 3 it became recumbent and stopped eating, while the faeces became greenish and mucoid and blackish on Day 4. The sheep was euthanised on Day 4. Sheep No. 5 developed a severe watery diarrhoea on Day 2, while the faeces became dark green and mucoid and contained blood spots on Day 3. On Day 7, the diarrhoea stopped and it started eating. None of the sheep showed blindness throughout the course of the experiment.

Pathology

Macroscopical findings

Gross lesions in two cattle (Nos. 1, 2) and four sheep (Nos. 1-4) dosed with *O. prasinum* were most prominent in the intestines. There was mild to moderate congestion and some degree of oedema of the mucosae of the abomasum and small and large intestines, and occasional mucosal petechiation (Sheep Nos. 1-3). The nature of the intestinal content varied from watery to mucoid and bloodstained. Non-specific lesions included pulmonary oedema and congestion and mild hepatosis in some animals.

Histopathological findings

Examination of the brain of the six bovine revealed spongiform changed which varied from moderate in two animals intoxicated by *O. saundersiae* and one animal poisoned by *O. prasinum* (No. 2) to mild in the others. Lesions were symmetrical and most conspicuous in the white matter of the cerebral cortex and white matter tracts in selected areas in the cerebrum, especially the optic radiation and internal capsule (Table 2). In these areas there was mild vacuolation of the grey matter. The optic chiasm and intracranial optic nerves were minimally affected. The vacuoles in the neuropil were round, oval or elongated, uni- or multilocular, 5-40 μm in diameter and empty (Figs. 6-8). In the internal capsule, coalescence of vacuoles formed large spaces and some were traversed by thin myelin septa as demonstrated in sections stained with LFB/H. A few vacuoles were in the neuropil adjacent to the perikaryon of neurons. In the cattle with mild spongy changes, only few small scattered vacuoles in the midbrain were evident. Mild swelling of astrocytes and distention of perivascular spaces in areas most severely affected were seen (Fig. 6). There was no evidence of axonal degeneration, myelinolysis, or inflammation.

Table 2: Distribution and severity of vacuolation in the brain of cattle with *Ornithogalum saundersiae* (n=3) and *O. prasinum* (n=3) poisoning.

Moderately and Consistently affected	**Mildly/minimally and inconsistently affected**
Optic radiation	Optic tracts
Radiation of corpus callosum	Optic chiasma
Internal capsule	Thalamus
External capsule	Globus pallidus
Putamen	Lateral geniculate nucleus
Cerebral white matter	Reticular formation
	Optic nerve

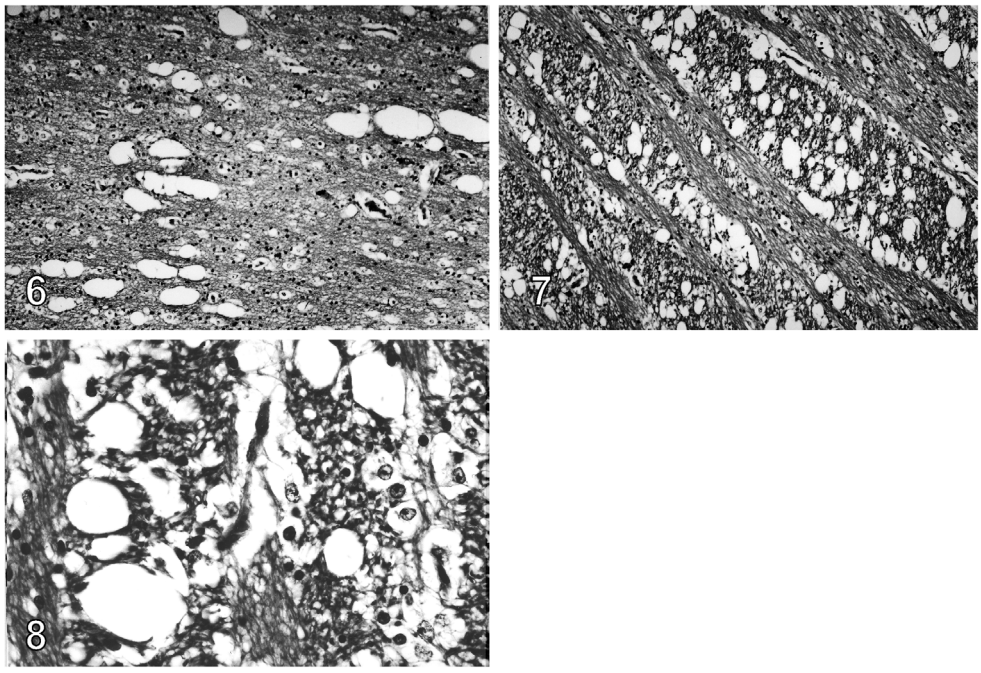

Fig. 6: Cerebrum. Bovine. Field case with *O. saundersiae* poisoning. There is vacuolation of the white matter of the midbrain with some coalescence of vacuoles. HE.
Fig. 7: Bovine No. 1 with *O. prasinum* poisoning. There is widespread vacuolation in the white matter of the optic radiation. HE.
Fig. 8: Bovine No. 1 with *O. prasinum* poisoning. Higher magnification to illustrate vacuolation of the neuropil and perivascular areas. HE.

No significant changes were detected in the eyes of the cattle. Moderate congestion of the mucosa of the small and large intestines and moderate tubular necrosis and hyalin cast formation in the kidney cortices were noted in Bovine No. 1.

In the sheep dosed with *O. prasinum*, histology of brain tissue revealed no significant lesions. The mucosa of abomasum and small and large intestines was congested in three animals (Sheep Nos. 1-3).

Ultrastructural findings

Three types of vacuoles were evident in the brain. The first type was in the white matter and was located within the outer portion of myelin sheaths (Fig. 9). These vacuoles were empty, surrounded by few myelin lamellae and were formed by splitting of myelin lamellae at the intraperiod lines. The second type of vacuoles were mainly located in perivascular areas in the white matter and were interpreted

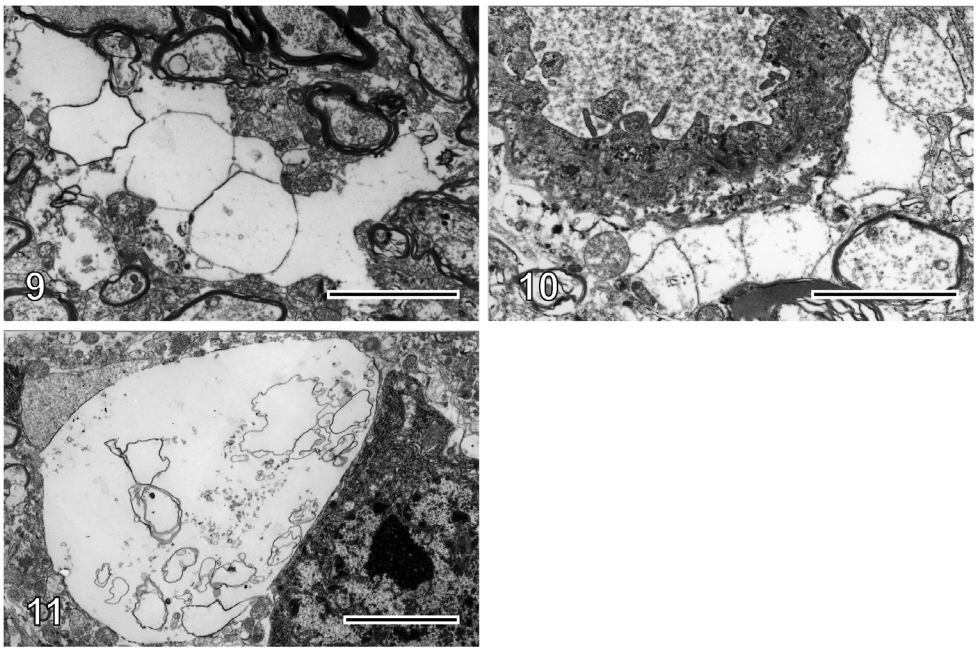

Fig. 9: Transmission electron micrograph. Cerebrum. Bovine No. 2. There is intramyelinic vacuolation (arrow) and distention of the extracellular space. Bar = 4 μm.
Fig. 10: Transmission electron micrograph. Cerebrum. Bovine No. 2. Note distention of astrocytic processes in perivascular area. Bar = 2 μm
Fig. 11: Transmission electron micrograph. Cerebrum. Bovine No. 3. Perineuronal vacuolation compressing the adjacent neuron. The vacuole is membrane-bound and contains irregularly membranous structures and blebs. Bar = 4 μm

as swollen astrocytic processes (Fig. 10). Distended processes exhibited dispersal of cytoplasmic organelles and often contained granular material, small numbers of mitochondria and glycogen granules, and occasional myelin lamellae. Widened extracellular spaces were seen in some affected areas. The nuclei of astrocytes were not affected. The third type of vacuoles occurred in the grey matter within dendrites. They were few, membrane-bound and contained irregular membranous structures and blebs and a small amount of granular material (Fig. 11). These vacuoles sometimes compressed the adjacent neuronal cytoplasm. No abnormalities were seen in endothelial cells and vessel walls.

Discussion

In this study, poisoning of cattle and sheep with *O. saundersiae* and *O. prasinum* consistently produced diarrhoea varying from watery to mucoid and bloodstained.

This is in agreement with previous observations [7]. The diarrhoea typically started within 24 hours of ingestion of plant material, and persisted for up to 18 days in Bovine No. 2. Death may occur if appropriate treatment is not given. Histologically, lesions in the abomasum, small and large intestines were restricted to congestion, possibly reflecting a direct toxic effect of the plant toxin(s) on the intestinal mucosa.

In some cattle with diarrhoea, blindness develops 6-10 days after the onset of clinical signs. The incidence of blindness seemed to vary: 4/11 cattle exposed to *O. saundersiae* were considered blind by the owner while 3/3 animals dosed with *O. prasinum* showed blindness. The herd on the farm, however, was not examined clinically for blindness and the farmer may not have noticed some animals with impaired vision. Blindness has not been reported previously in sheep [7] and we could neither induce significant pathology in the nervous system nor blindness in the sheep that were euthanised 8 and 21 days following ingestion of toxic plant material.

Blindness with normal pupillary size and light responses as well as the absence of appreciable lesions in the eyes of affected cattle in this study, implicated a bilateral defect in the central visual pathway especially involving the optic radiations and/or visual cortex [4]. In these cattle, lesions in selected areas of the cerebrum including optic radiation, optic chiasma, optic tracts, and lateral geniculate nucleus were present. Ultrastructurally, accumulation of excessive fluid within myelin lamellae, astrocytic cytoplasm and extracellular spaces was demonstrated. The intramyelinic vacuoles resulted from splitting of myelin lamellae at the intraperiod lines.

The mechanism of myelin splitting is unresolved. It has been demonstrated as the basic morphological change in status spongiosis in a variety of nervous conditions such as experimental intoxication by hexachlorophene [14,26], triethyl tin [6], cuprizone [25], isonicotinic acid hydrazide [15], and aniline [22], closantel overdosage [Van der Lugt], ammonia intoxication in calves [3], chronic copper toxicity in sheep [21], ingestion of the plants *Helicrysum argyrosphaerum* [27], *Stypandra imbricata* [17] and *S. glauca* [29] in small stock, and diplodiosis associated with the fungus *Stenocarpella maydis* (= *Diplodia maydis*) [23] in sheep.

Swelling of astrocytes and extracellular accumulation of fluid indicate vasogenic oedema [18] or may represent a non-specific, secondary reaction to the intramyelinic oedema [9]. In human spongy degeneration of the central nervous system (Canavan's disease), it has been suggested that astrocytes play a primary role in the excessive fluid accumulation while the myelin swelling is regarded a secondary lesion [1,5].

Few vacuoles in the grey matter were located within dendrites and contained membranous profiles and some granular material. Similar type of vacuoles originating from dendrites has been reported in bovine spongiform encephalopathy [16]. In chinkerenchee poisoning the pathological significance of these vacuoles is unknown.

Three groups of toxic or potentially toxic substances have been extracted from *Ornithogalum* spp. Six novel cholestane glycosides from *O. saundersiae* were isolated and characterised [10,11,12,13, 19]. These compounds, designated saundersiosides A-H, were identified by a team of scientists from Japan in search for new immunosuppressive agents. The glycosides had a potent inhibitory effect on the proliferation of peripheral blood lymphocytes of a human patient with chronic renal failure and showed an additional cytostatic activity against leukaemia HL-60 cells [13, 20]. Other compounds isolated from chinkerinchee plants are prasinosides from *O. prasinum* and *O. thyrsoides* [2,28) and cardiac glycosides, more specifically cardenolides, from *O. magnum* and *O. umbellatum* (Ferth and Kopp, 1992 and Komissarenko, 1974 quoted by Botha)[2]. The toxicity of cholestane glycosides and prasinosides in ruminants however, has not been evaluated. Recent evidence incriminated *O. toxicarium* as the cause of a 'krimpsiekte-like' syndrome in sheep and goats in Namibia [2].

Chinkerinchee poisoning should be differentiated from other conditions in cattle characterised by blindness secondary to pathology of the central nervous system (this condition in which there is blindness but no lesions in the eyes is sometimes designated as amaurosis). Polioencephalomalacia (PEM) associated with thiamine deficiency and lead poisoning may both produce blindness with no lesions in the eyes. In PEM, lesions are mainly located in the parietal-occipital areas of the cerebrum while the cerebral cortices are most commonly affected in lead poisoning. Cattle may also appear blind in salt poisoning [24].

In summary, chinkerinchee poisoning in cattle and sheep manifests as an acute diarrhoeic, often fatal disease, and may lead to temporary or permanent blindness in cattle. The blindness is associated with myelin vacuolation in the central visual pathways. The intramyelinic vacuoles are formed due to splitting of myelin lamellae at the intraperiod line. There is no significant pathology in the eyes and optic nerves.

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References

1. Adachi M, Schneck L, Cara J, Volk BW (1973) Spongy degeneration of the central nervous system (Van Bogaert and Bertrand type; Canavan's disease): a review. Hum Pathol 4:331-437
2. Botha CJ, Schultz RA, Van der Lugt JJ, Archer C (2000) A krimpsiekte-like syndrome in small stock poisoned by *Ornithogalum toxicarium* Archer & Archer. J S Afr Vet Assoc 71:6-9

3. Cho DY, Leipold HW (1977) Experimental spongy degeneration in calves. Acta Neuropathol (Berl) 39:115-127
4. DeLahunta A (1983) Veterinary Neuroanatomy and Clinical Neurology, 2nd edn, WB Saunders Company, Philadelphia, pp 279-303
5. Gambetti P, Mellman WJ, Gonatas NK (1969) Familial spongy degeneration of the central nervous system (Van Bogaert-Bertrand disease): an ultrastrucural study. Acta Neuropathol (Berl.) 12:103-115
6. Jacobs JM, Crertier JE, Cavanagh JB (1977) Acute effects of triethyl tin on the rat myelin sheath. Neuropathol Appl Neurobiol 3:169-181
7. Kellerman TS, Coetzer JAW, Naudé TW (1988) Plant Poisonings and Mycotoxicosis of Livestock in Southern Africa, 1st edn. Oxford University Press, Cape Town
8. Kellerman TS, Naudé TW, Fourie N (1996) The distribution, diagnosis and estimated economic impact of plant poisonings and mycotoxicosis in South Africa. Onderstepoort J Vet Res 63:65-90
9. Kreutzberg GW, Blakemore WF, Graeber MB (1997) Cellular pathology of the central nervous system. In: Graham DI, Lantos PL (eds) Greenfield's Neuropathology, 6th edn, Arnold, London, pp 85-140
10. Kubo S, Mimaki Y, Sashida Y, Nikaido T, Ohmoto T (1992) New polyhydroxylated cholestane glycosides from the bulbs of *Ornithogalum saundersiae*. Chem Pharm Bull (Tokyo) 40:2469-2471
11. Kuroda M, Mimaki Y, Sashida Y (1999) Saundersiosides C-H, rearranged cholestane glycosides from the bulbs of *Ornithogalum saudersiae* and their cytostatic activity on HL-60 cells. Phytochemistry 52:435-443
12. Kuroda M, Mimaki Y, Sashida Y (1999) Cholestane rhamnosides from the bulbs of *Ornithogalum saudersiae*. Phytochemistry 52:445-452
13. Kuroda M, Mimaki Y, Sashida Y, Hirano T, Oka K, Dobashi A (1995) A novel 16,23-epoxy-5β-cholestane glycoside with potent inhibitory activity on proliferation of human peripheral blood lymphocytes from *Ornithogalum saudersiae* bulbs. Chem Pharm Bull 43:1257-1259
14. Lampert P, O'Brien J, Garrett R (1973) Hexachlorophene encephalopathy. Acta Neuropathol (Berl) 23:326-333
15. Lampert PW, Schochet SS (1986) Electron microscopic observations on experimental spongy degeneration of the cerebellar white matter. J Neuropathol Exp Neurol 27:210-220
16. Liberski PP, Yanagihara R, Wells GAH, Gibbs CJ, Gajdusek DC (1992) Comparative ultrastructural neuropathology of naturally occurring bovine spongiform encephalopathy and experimentally induced scrapie and Creutzfeldt-Jakob disease. J Comp Path 106:361-381
17. Main DC, Slatter DH, Huxtable CR, Constable IC, Dorling PR (1981) Stypandra imbricata ("blindgrass") toxicosis in goats and sheep - clinical and pathological findings in 4 field cases. Aust Vet J 57:132-135
18. Manz HJ (1974) The pathology of cerebral oedema. Human Pathology 5:291-313
19. Mimaki Y, Kuroda M, Kameyama A, Sashida Y, Hirano T, Oka K, Koike K, Nikaido T (1996) A new cytotoxic cholestane bisdesmoside from *Ornithogalum saudersiae* bulbs. Biosci Biotechnol Biochem 60:1049-1050
20. Mimaki Y, Kuroda M, Sashida Y, Hirano T, Oka K, Dobashi A (1996)Three novel rearranged cholestane glycosides from *Ornithogalum saudersiae* bulbs and their

cytostatic activities on leukemia HL-60 en MOLT-4 cells. Tetrahedron Lett 37:1245-1248
21. Morgan KT (1973) Chronic copper toxicity of sheep: an ultrastructural study of spongiform leucoencephalopathy. Res Vet Sci 15:88-95
22. Okazaki Y, Yamashita K, Sudo M, Tsuchitani M, Narama I, Yamaguchi R, Tateyama S (2001) Neurotoxicity induced by a single oral dose of aniline in rats. J Vet Med Sci 63:539-546
23. Prozesky L, Kellerman TS, Swart DP, Maartens BP, Schultz RA (1994) Perinatal mortality in lambs exposed to cultures of *Diplodia maydis* (= *Stenocarpella maydis*) during gestation. A study of the central nervous system lesions. Onderstepoort J Vet Res 61:247-253
24. Summers BA, Cummings JF, De Lahunta A (1995) Veterinary Neuropathology. Mosby: St. Louis, pp 1-67, 214-236
25. Suzuki K, Kikkana Y (1969) Status spongiosis of CNS and hepatic damage induced by cuprizone (biscyclohexanone oxalyldihydrazone). Am J Pathol 54:307-325
26. Towfighi J (1980) Hexachlorophene In: Spencer PS, Schaumburg HH (eds) Experimental and Clinical Neurotoxicology, Baltimore/London: Williams & Wilkens, pp 440-455
27. Van der Lugt JJ, Olivier J, Jordaan P (1996) Status spongiosis, optic neuropathy, and retinal degeneration in *Helichrysum argyrosphaerum* poisoning in sheep and a goat. Vet Pathol 33:495-502
28. Van der Westhuizen D (1996) Structural studies on the toxic principle of *Ornithogalum thyrsoides*. MSc Thesis, University of Pretoria
29. Whittington RJ, Searson JE, Whittaker SJ, Glastonbury JRW (1988) Blindness in goats following ingestion of Stypandra glauca. Aust Vet J 65:176-181

Chapter ***3.5***

CHRONIC LIVER DISEASE AND HEPATIC ENCEPHALOPATHY IN CATTLE CAUSED BY *CROTALARIA SPARTIOIDES*

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Abstract

Anorexia, emaciation and mortalities were reported in cattle on farms in the northern Cape Province, South Africa. Based on circumstantial evidence and the presence of chronic liver lesions at necropsy, *Crotalaria spartioides* was incriminated as the possible cause. This plant typically grows in deep sand on dunes in the Kalahari, a semi-arid area in the northern Cape Province. The toxicosis was reproduced in an 18-month-old Friesland ox, which was dosed with plant material at rate of 10g/kg/day for 17 consecutive days. The animal was euthanised on Day 69 and gross changes comprised a smaller than normal liver with accentuated lobulation, polypoid and nodular thickenings of the mucosa of the gall bladder wall and oedema of hepatic lymph nodes. The lungs were greyish and of slightly firmer consistency. Light microscopical examination of tissues from four field cases and the experimental case revealed intra- and interlobular fibrosis, biliary ductular hyperplasia, megalocytosis of hepatocytes, nodular hyperplasia and changes compatible with veno-occlusive disease in the liver as a result of chronic pyrrolizidine alkaloid poisoning. Other lesions included interstitial pneumonia, epithelialization and medial hypertrophy of arteries in the lungs and megalocytosis of tubular epithelial cells in the kidneys. In the brain there was status spongiosis of the white matter especially involving the junction of white and grey matter characteristic of hepatic encephalopathy. The pyrrolizidine alkaloid, senkirkine, and two minor pyrrolizidine alkaloid fractions were isolated from *C. spartioides* plant material. These findings confirmed chronic pyrrolizidine alkaloid poisoning caused by *C. spartioides* as the cause of disease and mortalities in the cattle.

Key words: cattle, *Crotalaria spartioides*, pyrrolizidine alkaloids, senkirkine, chronic liver disease, status spongiosis, hepatic encephalopathy

Introduction

A number of *Senecio* and *Crotalaria* spp. in southern Africa contain pyrrolizidine alkaloids which may cause intoxication in a variety of domestic animals including cattle and horses [7,12,24,25,26]. Seneciosis is the most important non-photosensitizing hepatotoxicosis of stock in the Region [12]. More than 250 *Senecio* spp. have been identified in South Africa although the toxicity of most of these plants has not been confirmed. Only scant information is available on the toxic effects of *Crotalaria* spp. in domestic animals and most reports date back to the early 1900s. Four syndromes in animals have been documented due to *Crotalaria* intoxication, namely 'jaagsiekte' in horses and mules, 'stywesiekte' in cattle, loss of wool in sheep, and hepatic damage in cattle caused by *C. spartioides*.

Crotalaria dura, *C. globifera* (both called wild lucern or 'jaagsiektebossie') and *C. juncea* (sunn-hennep) are associated with a lung condition in horses and mules known locally as 'jaagsiekte'. The intoxication, first described in 1908, is characterised by fever, polypnoea, dyspnoea, subcutaneous and pulmonary emphysema, interstitial pneumonia and chronic liver disease [28]. The pyrrolizidine alkaloid, dicrotaline, was subsequently isolated from *C. dura* and *C. globifera* [16]. This intoxication is now rarely reported in the Region.

'Stywesiekte' (meaning stiffsickness) is a term used to describe several unrelated conditions in cattle clinically characterised by difficulty in walking with or without apparent stiffness. The term is used for phosphate deficiency in cattle, three-day-stiffsickness, and a toxicosis following the ingestion of the plant *C. burkeana* (stywesiektebossie) and possibly *C. barkae* and *C. steudneri* [12]. Initially, cattle lie down frequently, walk with difficulty and have warm, painful hooves, and the condition clinically is virtually indistinguishable from acute laminitis. In the chronic stage the hooves grow out abnormally and are eventually shed or wear off whereafter the feet return to normal [12].

Ingestion of large quantities of *C. juncea* in sheep may result in loss of wool [24,25].

The fourth syndrome is called 'duinetering', a sporadic intoxication of cattle associated with *C. spartioides* (dune bush). The plant occurs in the northern Cape Province of South Africa, southwestern Botswana and southern Namibia (Fig. 1), but typically grows in deep sand on dunes in the Kalahari, a semi-arid area in the northern Cape Province (Fig. 2). Mature plants are rather unpalatable and poisoning in cattle occurs when animals are forced to graze it in times of drought. Little is known on the toxicity of dune bush. Following an outbreak of liver disease in cattle in the Kalahari in 1969, toxic plant material fed to cattle induced cirrhosis consistent with chronic pyrrolizidine alkaloid poisoning [12].

In this publication the pathology of naturally occurring *C. spartioides* intoxication in cattle, the results of a dosing trial of toxic plant material in a bovine and the extraction and identification of the toxin from the plant are described. Preliminary results on one of the field cases have been reported previously [12].

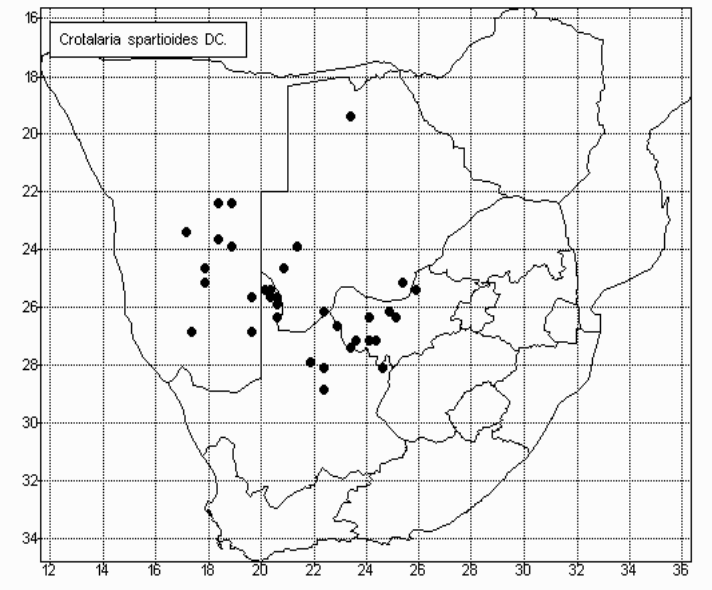

Fig 1: Distribution of *Crotalaria sparthioides* (courtesy Botanical Research Institute, Pretoria).

History of natural cases

Farm 1: A farmer in the Postmasburg district, northern Cape Province, reported the death of *c*. 70 cattle out of a herd of 200 animals over a period of one year. Goats and sheep on the farm were not clinically affected. Most affected cattle were less than 3-4 years of age but there were no mortalities in calves up to six months of age. Animals died after being clinically ill for a period varying from one week to two months. Typically, cattle became thin and progressively weaker and anorexic. Some were dyspnoeic and anaemic. A necropsy on one affected animal revealed an emaciated and anaemic carcass and mild ascites. The liver had a much firmer than normal consistency and a greyish-brown colour and the gall bladder wall was markedly oedematous. No other significant macroscopical changes were evident. Tissues from the animal and another carcass were collected in 10% buffered formalin and submitted for histopathological examination.

A suspected diagnosis of chronic crotalariosis was made based on the observation that approximately 80% of the available grazing consisted of *C. spartioides* and that the plant had been extensively eaten by the cattle. *Crotalaria spartiodes* was identified by staff of the Botanical Research Institute (BRI), Pretoria, South Africa.

Farm 2: The second outbreak occurred on a farm in the Kuruman district, northern Cape Province. Fourteen out of 127 cattle had died or had been euthanised at the

Fig. 2: *Crotalaria spartioides* growing in its natural habitat on sand dunes in the Kalahari, northern Cape Province, South Africa.

time a field investigation was done. Affected cattle were between 6-30 months old and had been grazing *C. spartiodes* almost exclusively during the winter months. The plant was identified by staff of the BRI, Pretoria. According to the farmer, the cattle became sick approximately six months after being introduced to the camps where *C. spartiodes* grew in abundance. Clinical signs included anorexia, emaciation, weakness and difficulty in rising and walking. Adult cattle with their calves, which received supplementation, did not show signs of disease. Gross lesions in two affected cattle comprised cachexia, moderate hydropericardium and ascites, greyish-brown discoloration and increased consistency of the liver and oedematous distension of the wall of the gall bladder and abomasum. Based on circumstancial evidence and necropsy findings, *C. spartiodes* poisoning was suspected. Formalin-fixed tissue specimens of two animal were submitted to confirm the diagnosis.

Materials and methods

Extraction and identification of toxin

Plant material was collected on Farm 2 at the time of the outbreak, and from leaves and stems a crude alkaloidal extract was obtained using standard extraction methods [18]. The extract was examined by thin layer chromatography, gas chromatography and nuclear magnetic resonance spectroscopy (NMR).

Field cases

Formalin-fixed tissues from four affected cattle (two from each farm) were embedded in paraffin wax, cut at 4-5 μm and stained with haematoxylion and eosin. Sections of selected paraffin blocks of the liver were also stained by a modified Masson's trichrome (MT) method (Luna, 1968).

Dosing trial

A batch of *C. spartiodes* plant material was collected on Farm 2, transported to the laboratory, dried and milled, and administered per rumen fistula at a rate of 10g/kg/day for 17 consecutive days to an 18-month-old Friesland ox. At twice-weekly intervals until termination of the trial, the following parameters were determined on venous blood: total plasma protein (P-TPP) by the Biuret method, the albumin/globulin ratio with a Beckman serum protein electrophoresis kit on agarose gel and the activities of aspartate transaminase (AST, EC 2.6.1.1) and gamma-glutamyltransferase (GGT, EC 2.3.2.2) using Boehringer Mannheim test kits. The albumin/globulin ratios were quantified using a Model CDS-200 Beckman Densitometer and enzyme activities were measured at 25°C.

The ox was euthanised 69 days after commencement of dosing toxic plant material by means of an intravenous overdose of pentobarbitone sodium. A detailed necropsy was performed and tissue specimens including liver, lungs, kidneys and brain were collected and fixed in 10% buffered formalin for light microscopy. Tissues were processed as for the field cases.

Results

Extraction and identification of toxin

The extract contained a 96% concentration of the pyrrolizidine alkaloid, senkirkine (identified by one- and two-dimensional NMR), and two minor unidentified pyrrolizidine alkaloid fractions.

Dosing trial

Clinical signs of intoxication became evident in the ox from Day 34 of the experiment and included depression, intermittent feed refusal (especially green feed), a marked decrease in weight and normocytic, normochromic anaemia. Indications of hepatic damage were manifested by a gradual three-fold increase in serum AST activity at Day 17 with levels returning to baseline values at Day 34 and a four-fold increase in serum GGT activity, which after peaking on Day 20, declined progressively to previous baseline levels by Day 45. A noticeable inversion in the serum albumin:globulin ration occurred (0,5).

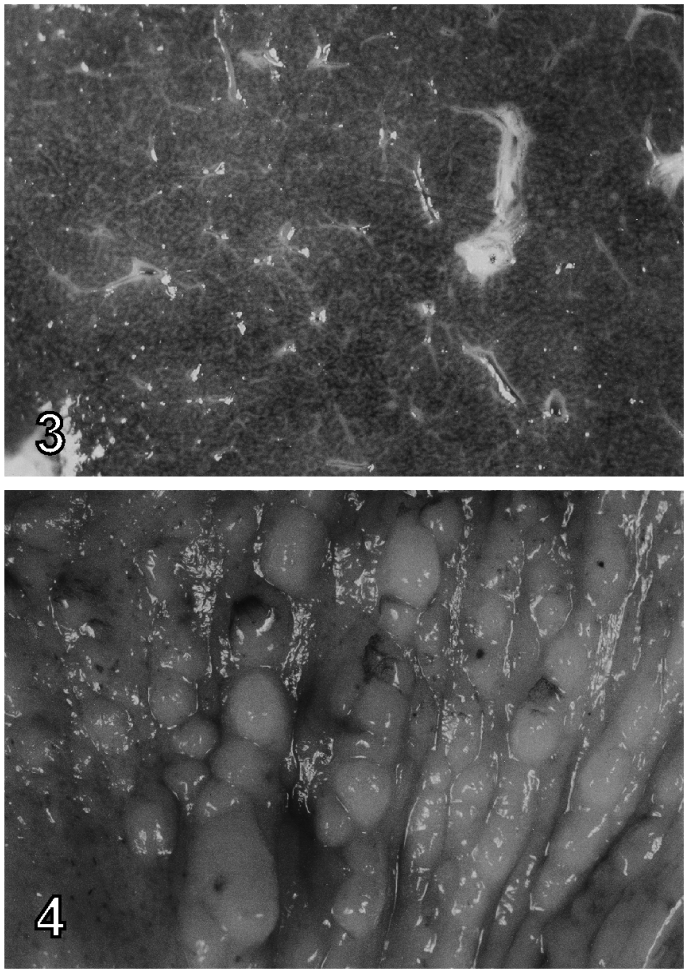

Fig. 3: Liver. Cut section of liver from bovine with experimental *Crotalaria spartioides* poisoning. Note accentuated lobulation.
Fig. 4: Gall bladder. Bovine with experimental *Crotalaria spartioides* poisoning. The mucosa is covered by polypoid and nodular thickenings.

Pathology

Gross lesions of experimental animal: The post-mortem revealed a carcass in poor condition. The abdominal cavity contained approximately 100 ml straw-coloured fluid. The liver was pale-staining and moderately smaller than normal and the capsule of Glisson was thickened and contained prominent lymphatics. The liver was firm and cut section revealed accentuated lobulation (Fig. 3). The gall bladder wall was diffusely oedematous and the mucosa showed opaque, polypoid and nodular thickenings (Fig. 4). Hepatic lymph nodes were oedematous. The wall of

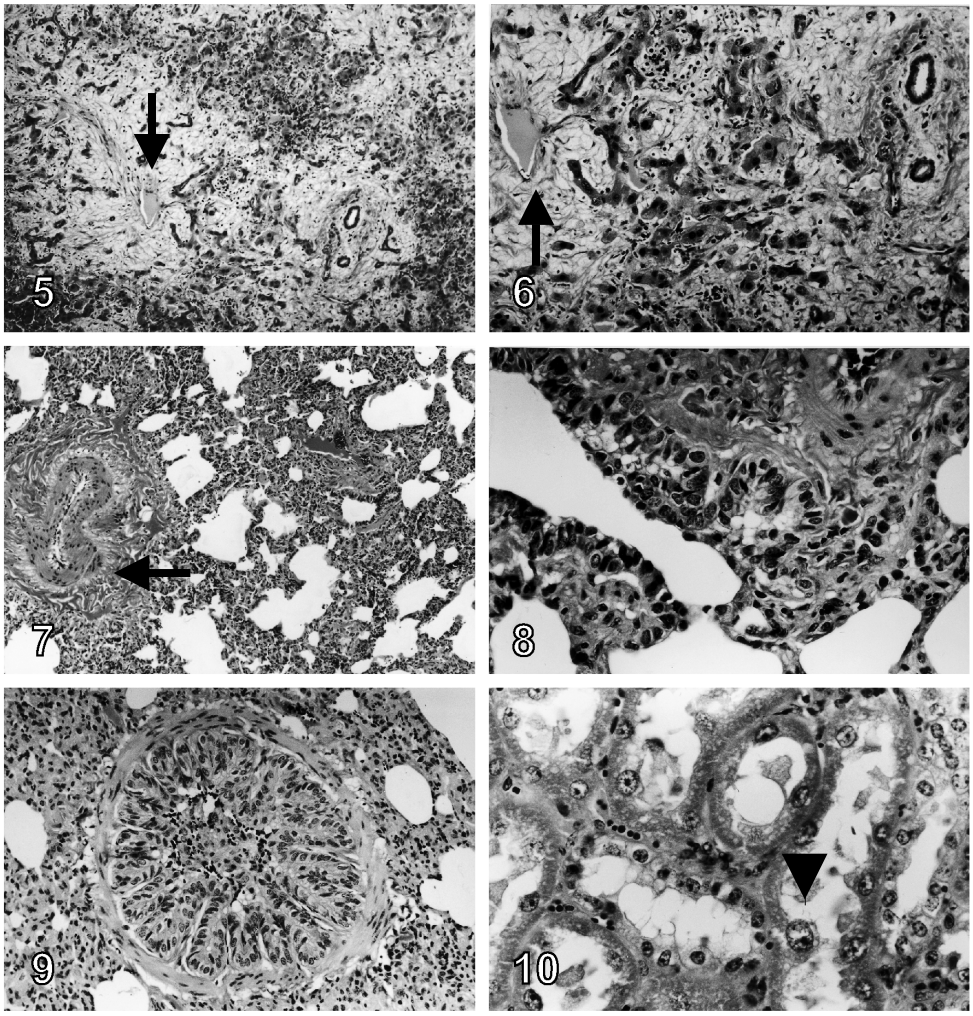

Fig. 5: Liver. Field case of *Crotalaria spartioides* poisoning. There is widespread intra- and interlobular fibrosis causing distortion of architecture. Arrow denotes central vein. HE.
Fig. 6: Liver. Field case of *Crotalaria spartioides* poisoning. Higher magnification to illustrate fibrous tissue dissecting lobules thereby isolating individual and groups of hepatocytes. Note biliary ductular proliferation (arrow denotes central vein). HE
Fig. 7: Lung. Field cases of *Crotalaria spartioides* poisoning. Note distention of alveolar walls, arterial medial hypertrophy (arrow) and periarterial fibrosis. HE.
Fig. 8: Lung. Bovine with experimental *Crotalaria spartioides* poisoning. Higher magnification of lung to illustrate distention of alveolar walls by inflammatory cells and smooth muscle hyperplasia. HE.

Fig. 9: Lung. Bovine with experimental *Crotalaria spartioides* poisoning. A bronchiole is lined by folded, hyperplastic epithelium and the lumen is occluded by sloughed epithelial cells and inflammatory cells. HE.
Fig. 10: Kidney. Note megalocytosis of a tubular epithelial cell (arrowhead). HE

the small and large intestines was moderately distended due to oedema and the lungs were pale-greyish and had a slightly firmer consistency than normal.

Light microscopical pathology of field and experimental animals: Lesions in the liver and gall bladder, lung, kidneys and brain in the field cases and the experimental bovine were similar in nature.

Liver and gall bladder: The lobular architecture was distorted due to portal and intralobular fibrosis and biliary ductular hyperplasia (Figs. 5, 6). Fibrous tissue strands dissecting into the lobules resulted in isolation of individual and groups of hepatocytes. There was also fibrosis around central veins and loss of centrilobular hepatocytes, while partial occlusion of central veins in several lobules was noted. Megalocytosis, scattered necrosis of hepatocytes, multifocal nodular regeneration and bile canaliculi distended with bile were seen. Glisson's capsule was moderately thickened by fibrosis and contained dilated lymphatics. In the wall of the gall bladder the epithelium was folded and the lamina propria-submucosa was oedematous containing several lymphoid follicles.

Lungs: The interstitium was expanded due to the infiltration of moderate numbers of macrophages, lymphocytes and few scattered eosinophils, and fibrosis (Figs. 7,8). Small bronchioles were lined by hyperplastic epithelium two or more cell layers thick that was folded and caused partial occlusion of their lumens (Fig. 9). In these bronchioli, there was also necrosis of individual epithelial cells, scant neutrophil infiltration of the mucosa, the accumulation of cellular debris in some lumens and smooth muscle hyperplasia. Medium sized arteries revealed hypertrophy of the tunica media and perivascular fibrosis (Fig. 7).

Kidneys: A small number of epithelial cells in proximal and distal tubules and, to a lesser extent collecting tubules, revealed cytomegaly and karyomegaly (Fig. 10). Some affected tubules were occluded while others contained finely granular eosinophilic material in their lumens.

Brain: Widespread status spongiosis of the white matter, especially involving the junctions of myelinated fibres that are interspersed with grey matter, was noted (Figs 11, 12). The spongy changes were less severe in the grey matter and were characterised by numerous, ovoid to elongated, sometimes confluent, 5-40 μm diameter, empty vacuoles (Fig. 13). The cerebral cortex, cerebellar white matter and peduncle, thalamus, basal ganglia and internal capsule were consistently affected. Alzheimer type II astrocytes were not observed. In the experimental animal where the entire brain was available for examination, the vacuolation in the white matter had a bilateral symmetrical distribution. There were no lesions indi-

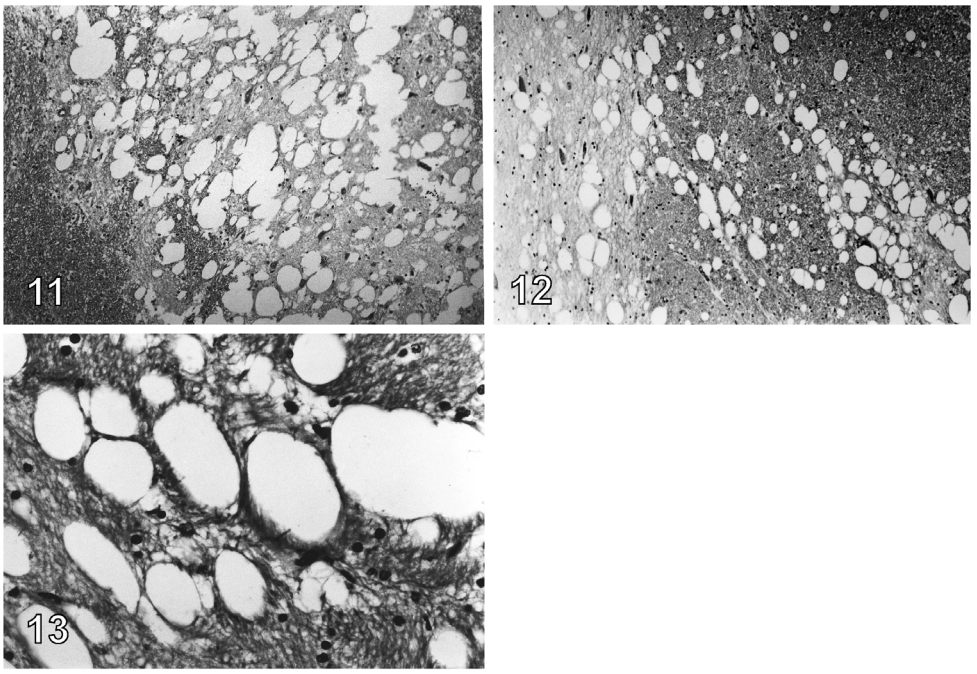

Fig. 11: Cerebrum. Field cases of *Crotalaria spartioides* poisoning. There is severe vacuolation of the white matter of the cerebral cortex. HE.
Fig. 11: Cerebrum. Bovine with experimental *Crotalaria spartioides* poisoning. Status spongiosis in the cortex at the junction of white and grey matter. HE.
Fig. 12: Cerebrum. Bovine with experimental *Crotalaria spartioides* poisoning. Higher magnification of vacuolated white matter. Myelin vacuoles are ovoid to elongated, sometimes confluent, and empty. HE.

cating demyelination or inflammation in the nervous system of the affected animals.

Discussion

This study confirmed that the outbreaks of disease and mortalities in cattle on farms in the northern Cape Province were chronic *C. sparthioides* poisoning on the basis of histopathological lesions and chemical analysis. Liver pathology in the field and experimental cases were consistent with cirrhosis due to chronic pyrrolizidine alkaloid poisoning and comprised fibrosis, biliary ductular hyperplasia, megalocytosis of hepatocytes and nodular regeneration [13]. In addition, intimal fibrous thickening with partial occlusion of central veins typical of veno-occlusive disease (VOD) [13] was present. This is a distinctive lesion but is not specific for any disease and has also been reported in aflatoxicosis [21] and

in captive cheetah that had died due to a variety of causes [5]. The hypoalbuminaemia and normal concentrations of liver enzymes towards the end of the dosing trial can be explained by the chronic nature of the intoxication.

Toxic effects in livestock suffering from chronic exposure to pyrrolizidine alkaloids may vary considerably with the animal species and nature of the alkaloids involved [18]. In addition to acute or chronic liver damage, alkaloids of *Crotalaria* spp may be pneumotoxic and nephrotoxic in animals (Hooper 1987) as has been demonstrated in sheep ingesting *C. mucronata* [14] and pigs exposed to *C. retusa* [10,23]. Both these plant species contain the alkaloid, monocrotaline. Lesions in the lungs of the cattle in this study comprised interstitial pneumonia with epithelialization and arterial medial hypertrophy. Hooper reported that pulmonary vascular disease in pyrrolizidine alkaloid poisoning is not characteristic in domestic animals as it is in rats [9]. Oxygen radicals may play an important role in mediating monocrotaline-induced pulmonary hypertension in rats [2].

Brain lesions in the field and experimental cases in our study were characterised by spongy changes, which were especially prominent at the junctions of white and grey matter. These lesions are consistent with hepatic encephalopathy (HE) in domestic animals [27]. HE has been previously reported in chronic seneciosis [4,8,9,13]. The electron microscopical changes in spontaneous HE have not been studied in detail, but in experimental ammonia infusion in calves the spongiosis resulted from splitting of myelin lamellae at the intraperiod line [3]. In our cases, we did not find astrocytes with swollen, pale, irregular-shaped nuclei typical for Alzheimer type II astrocytes. These astrocytes are seen in a variety of metabolic encephalopathies including hepatic encephalopathy in humans [6,22] but are not necessarily present in HE in domestic animals [27]. The significance of these astrocytes in domestic animals is questionable as similar cells have observed in normal sheep, cattle and horses [8,29]. Immunohistochemical staining to evaluate expression of glial fibrillary acidic protein (GFAP) in these cells may be needed to distinguish Alzheimer type II changes from hypertrophy of astrocytes [19].

Other chronic liver conditions leading to spongy changes in the central nervous system in ruminants include aflatoxicosis [17], facial eczema [29], copper poisoning [11,20] and fascioliosis and lupinosis [8]. In addition, acute poisoning with the plant *Cestrum laevigatum* induced a spongy change in the brain of sheep [30].

On both farms in this study, most affected cattle were less than 3-4 years of age. This observation corresponded with previous reports indicating that chronic pyrrolizidine alkaloid poisoning tends to affect younger animals with only sporadic mortalities occurring in older animals [31]. Calves up to six months of age did not show clinical signs of intoxication which may be explained by the fact that calves in the herds were generally weaned between 6-9 months of age and adult cattle with their calves were being supplemented on one farm (Farm 2). Walker and Kirkland however, reported mortalities in calves as young as 3,5 months with *Senecio lautus* poisoning [31].

The pyrrolizidine alkaloid, senkirkine, and two minor alkaloid fractions were extracted from *C. sparthioides*. Senkirkine, characterised by Briggs and co-workers in 1965 [1], has been isolated from several plant species, mainly *Senecio* and *Crotalaria spp.* [18]. It is well-known that plants generally do not contain a single but several pyrrolizidine alkaloids, which may or may not be closely related. The alkaloids in a given plant may also vary considerably depending on the stage of growth, season and locality [18].

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References

1. Briggs LH, Cambie RC, Candy BJ, O'Donovan GM, Russell RH, Seelye RN (1965) Alkaloids of New Zealand *Senecio* species. J Chem Soc 18:2429-2498
2. Chen MJ, Chiang LY, Lai YL (2001) Reactive oxygen species and substance P in monocrotaline-induced pulmonary hypertension. Toxicol Appl Pharmacol 171:165-173
3. Cho DY, Leipold HW (1977) Experimental spongy degeneration in calves. Acta Neuropathol (Berl.) 39:115-127
4. Finn JP, Tennant B (1974) Hepatic encephalopathy in cattle. Cornell Vet 64:136-153
5. Gosselin SJ, Loudy DL, Tarr MJ, Balistreri WF, Setchell KDR, Johnston JO, Kramer LW, Dresser BL (1988) Veno-occlusive disease of the liver in captive cheetah. Vet Pathol 25:48-57
6. Harper C, Butterworth R (1997) Nutritional and metabolic disorders. In: Graham DI, Lantos PL (eds) Greenfield's Neuropathology, 6th edn, Arnold, London, pp 601-655
7. Henning MW (1932) Virus and deficiency diseases and plant poisonings. In: Animal Diseases in South Africa Vol II, Central News Agency Ltd., South Africa, pp 790-796
8. Hooper PT (1975) Spongy degeneration in the central nervous system of domestic animals I. Morphology. Acta Neuropathol (Berl.) 31:325-334
9. Hooper PT (1978) Pyrrolizidine alkaloid poisoning – pathology with particular reference to differences in animal and plant species. In: Keeler RF, Van Kampen KR, James LF (eds), Effects of poisonous plants on livestock, Academic Press, New York, 161-176
10. Hooper PT, Scanlan WA (1977) *Crotalaria retusa* poisoning of pigs and poultry. Aust Vet J 53:109-114
11. Ishmael J, Gopinath C, Howell JM (1971) Experimental chronic copper toxicity in sheep. Res Vet Sci 12: 358-366
12. Kellerman TS, Coetzer JAW, Naudé TW (1988) Plant Poisonings and Mycotoxicosis of Livestock in Southern Africa, 1st edn. Oxford University Press, Cape Town
13. Kelly WR (1993) The liver and biliary system. In: Jubb KVF, Kennedy PC, Palmer NC (eds), Pathology of Domestic Animals, 4th edn, Vol 2, pp 319-406
14. Laws L (1968) Toxicity of *Crotalaria mucronata* to sheep. Aust Vet J 44:453-455

15. Luna LG (ed) (1968) Manual of histological staining methods of the Armed Forces of Institute of Pathology. 3rd edn, New York: McGraw-Hill
16. Marais JSC (1944) Dicrotaline: the toxic alkaloid from *Crotalaria dura* (Wood and Evans) and *Crotalaria globifera* (E. Mey.). Onderstepoort J Vet Sci Anim Ind 20:61-65
17. Markson LM, Terlicki S (1968) The aetiology of cerebrocortical necrosis. Brit Vet J 124:309-315
18. Mattocks AR (1986) Chemistry and Toxicology of Pyrrolizidine Alkaloids. Academic Press, London
19. Montgomery DL (1994) Astrocytes: form, functions, and role in disease. Vet Pathol 31:145-167
20. Morgan KT (1973) Chronic copper toxicity of sheep: an ultrastrucural study of spongiform leucoencephalopthy. Res Vet Sci 15: 83-95
21. Newberne PM, Butler WH (1969) Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals: a review. Cancer Res 29:236-250
22. Norenberg MD (1994) Astrocyte responses to CNS injury. J Neuropathol Exp Neurol 53:213-220
23. Ross AJ (1977) Effects of feeding diets containing *Crotalaria retusa* L. seed to growing pigs. J Agric Sci 89:101-105
24. Steyn DG, de Kock G (1932) Crotalariosis in sheep. 18th Rep Dir Vet Serv Anim Ind, Union of South Africa, pp 947-953
25. Steyn DG (1934) The Toxicology of Plants in South Africa, Johannesburg, South Africa, pp 247
26. Steyn DG (1949) Vergiftiging van Mens en Dier, Van Schaik Bpk, Pretoria
27. Summers BA, Cummings JF, De Lahunta A (1995) Veterinary Neuropathology. Mosby: St. Louis
28. Theiler A (1918) Jaagziekte in horses. 7th and 8th Rep S Afr Dep Agric Vet Serv, pp 59-108
29. Thompson KG, Lake DE, Cordes DO (1979) Hepatic encephalopathy associated with chronic facial eczema. N Z Vet J 27:221-223
30. Van der Lugt JJ, Nel PW, Kitching JP (1992) Experimentally-induced *Cestrum laevigatum* (Schlechtd.) poisoning in sheep. Onderstepoort J Vet Res 59:135-144
31. Walker KH, Kirkland PD (1981) *Senecio lautus* toxicity in cattle. Aust Vet J 57:1-7

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

1. Jubb KVF, Huxtable CR (1993) The Nervous System. In: Jubb KVF, Kennedy PC, Palmer N (eds) Pathology of Domestic Animals 4th edn Academic Press, Inc, San Diego, pp 267-439

Chapter ***4.2***

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

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-

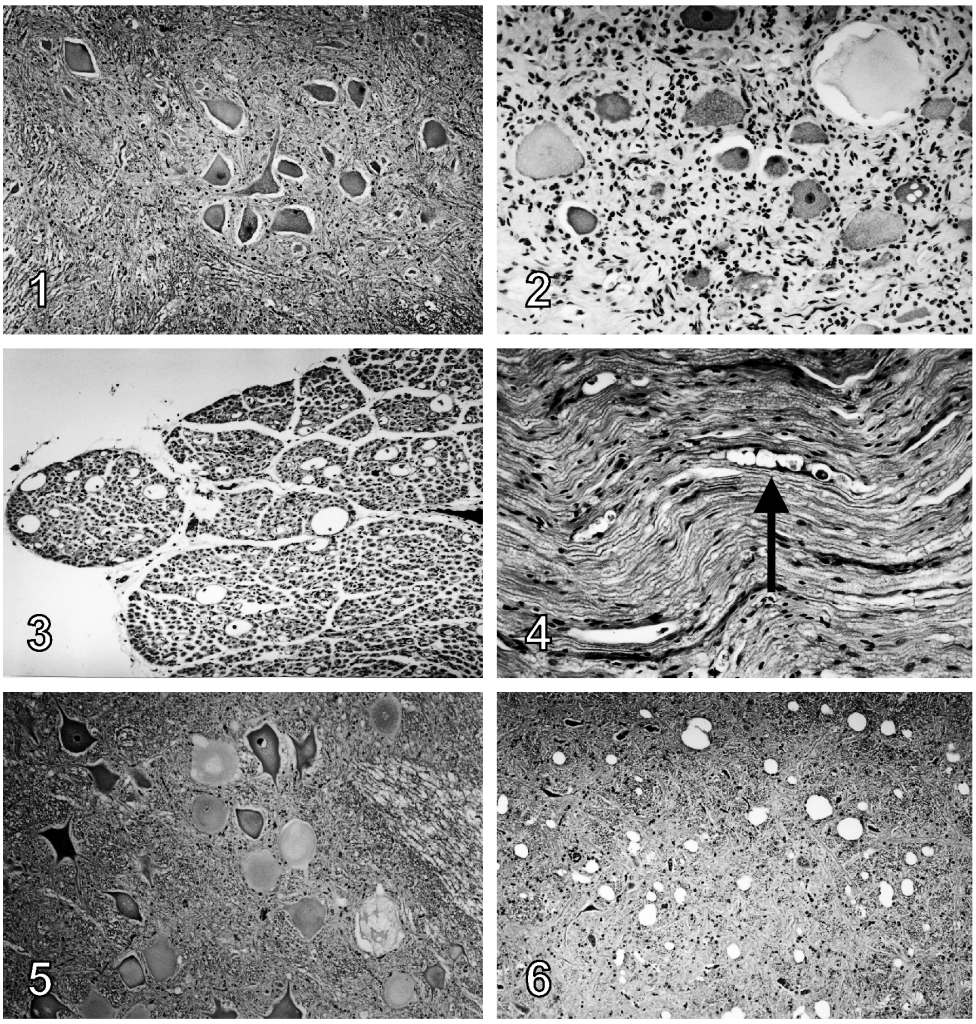

Fig. 1: Spinal cord. Chromatolysis and eccentric nuclei in ventral horn neurons. HE.
Fig. 2: Dorsal root ganglion. Note chromatolysis of neurons and gliosis. HE
Fig. 3: Ventral nerve roots, lumbar spinal cord. There is myelin swelling of scattered nerve fibres. HE.
Fig. 4: Peripheral nerve (sciatic nerve). 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.

vidual 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, ambiguus 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.

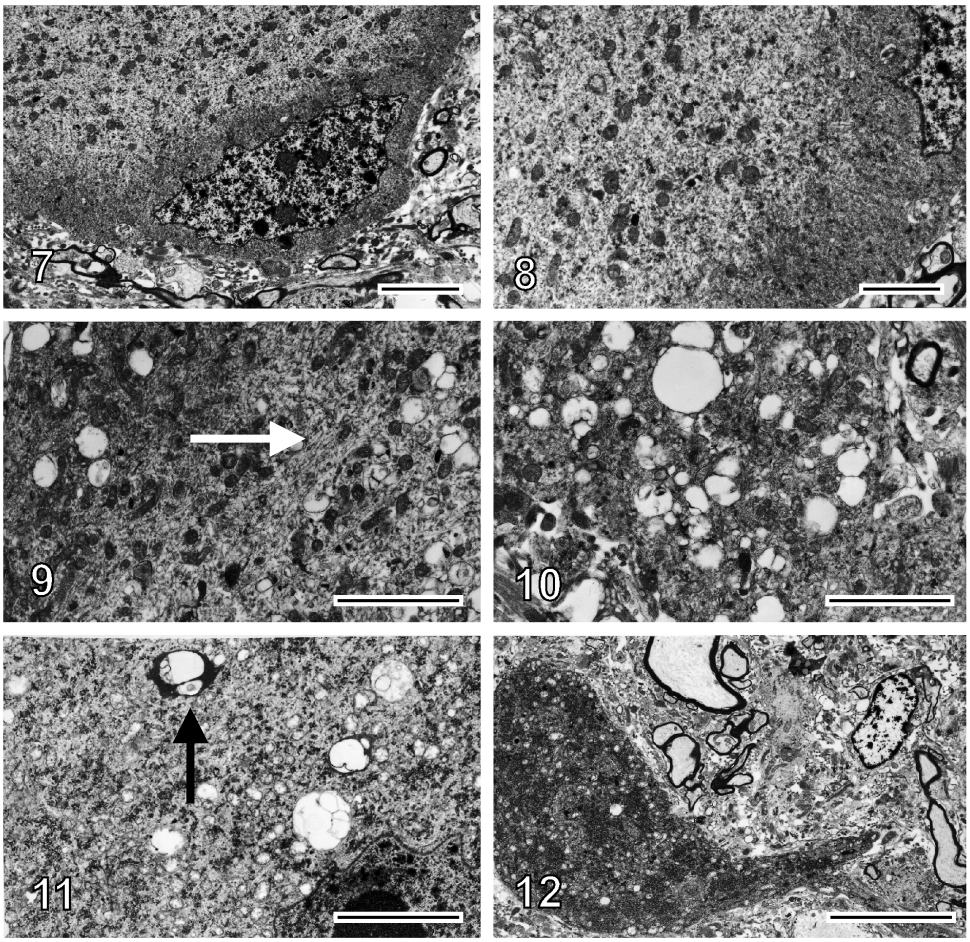

Fig. 7: Transmission electron micrograph. Spinal cord. The central cytoplasm is pale, depleted of Nissl substance and contains increased numbers of mitochondria. The peripheral cytoplasm is granular and the nucleus eccentric. Bar = 5 μm.
Fig. 8: Transmission electron micrograph. Spinal cord. Higher magnification 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.

Fig. 12: Transmission electron micrograph. Spinal cord. A neuron with condensed, slightly vacuolated cytoplasm. The nucleus is absent. Bar = 10 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

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

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References

1. Barlow RM (1963) Further observations on swayback I. Transitional pathology. J Comp Pathol 73:51-60
2. Barron KD, Dentinger MP, Nelson LR, Mincy JE (1975) Ultrastructure of axonal reaction in red nucleus of cat. J Neuropathol Exp Neurol 34:222-248

3. Bergel F, Morrison AL, Moss AR, Klein R, Rinderknecht H, ward JL (1943) An antibacterial substance from *Aspergillus clavatus* and *Penicillium claviforme* and its probable identity with patulin. Nature 152:720
4. Clardy J, Springer JP, Buchi G, Matsuo K, Wightman R (1975) Tryptoquivaline and tryptoquivalone, two tremorgenic metabolites of *Aspergillus clavatus*. J Chem Soc 97: 663-665
5. Cancilla PA, Barlow RM (1966) Structural changes of the central nervous system in swayback (enzootic ataxia) of lambs II. Electron microscopy of the lower motor neuron. Acta Neuropathol (Berl) 6:251-259
6. Cancilla PA, Barlow RM (1966) Structural changes of the central nervous system in swayback (enzootic ataxia) of lambs IV. Electron microscopy of the white matter of the spinal cord. Acta Neuropathol (Berl) 6:294-300
7. Gilmour JS, Inglis DM, Robb J, MacLean M (1989) A fodder mycotoxicosis of ruminants caused by contamination of a distillery by-product with *Aspergillus clavatus*. Vet Rec 124: 133-135
8. Glinsukon T, Yuan SS, Wightman R, Kitaura Y, Bchi G, Shank RC, Wogan GN, Christensen CM (1974) Isolation and purification of cytochalasin E and two tremorgens from *Aspergillus clavatus*. Plant Foods Man 1:113-119
9. Hahn CN, Mayhew IG, de Lahunta A (2001) Central neuropathology of equine grass sickness. Acta Neuropathol (Berl) 102:153-159
10. Jeffrey M, Wilesmith JW (1992) Idiopathic brainstem neuronal chromatolysis and hippocampal sclerosis: a novel encephalopathy in clinically suspect cases of bovine spongiform encephalopathy. Vet Rec 131:359-362
11. Jiang CS, Huang SS, Chen MZ, Yu Z, Chen L H, Sang Y Z (1982) Studies on mycotoxicosis in dairy cattle caused by *Aspergillus clavatus* from mouldy malt sprouts. Acta Vet Zoot Sinica 13:247-254 (Abstract no 3943, Veterinary Bulletin 53)
12. Kellerman TS, Coetzer JAW, Naudé TW (1988) Plant Poisonings and Mycotoxicosis of Livestock in Southern Africa. Oxford University Press, Cape Town, pp 67-69
13. Kellerman TS, Newsholme SJ, Coetzer JAW, Van der Westhuizen GCA (1984) A tremorgenic mycotoxicosis of cattle caused by maize sprouts infested with *Aspergillus clavatus*. Onderstepoort J Vet Res 51:271-274
14. Kellerman TS, Pienaar JG, Van der Westhuizen GCA, Anderson LAP, Naude T W (1976) A highly fatal tremorgenic mycotoxicosis of cattle caused by *Aspergillus clavatus*. Onderstepoort J Vet Res 43:147-154
15. Lieberman AR (1971) The axon reaction: a review of the principal features of perikaryal responses to axon injury. Int Rev Neurobiol 14:49-124
16. Shlosberg A, Zadikov I, Perl S, Yakobson B, Elad D, Papoport E (1992) A lethal mass neurotoxicosis in sheep caused by *Aspergillus clavatus* contaminated feed. In: James LF, Keeler RF, Bailey EM, Cheeke PR, Megarty MP (eds) Poisonous Plants: Proceedings of the Third International Symposium, Iowa State University Press: Ames, Iowa, pp 397-402
17. Shlosberg A, Zadikov I, Perl S, Yakobson B, Varod Y, Elad D, Rapoport E, Handji V (1991) *Aspergillus clavatus* as the probable cause of a lethal mass neurotoxicosis in sheep. Mycopathologia 114: 35-39
18. Summers BA, Cummings JF, De Lahunta A (1995) Veterinary Neuropathology. Mosby: St. Louis, pp 208-350
19. Suzuki T, Takeda M, Tanabe H (1971) A new mycotoxin produced by *Aspergillus clavatus* . Chem Pharm Bull (Tokyo) 19:1786-1788

20. Thomas PK, Scaravilli F, Belai A (1993) Pathologic alterations in cell bodies of peripheral neurons in neuropathy. In: Dyck PJ, Thomas PK (eds) Peripheral Neuropathy 3rd edn WB Saunders Company: Philadelphia, pp 476-513
21. Van der Lugt J J, Kellerman T S, Van Vollenhoven A, Nel P W (1994) Spinal cord degeneration in adult dairy cows associated with the feeding of sorghum beer residues. J S Afr Vet Assoc 65:184-188
22. Van der Vyver FH, Kellerman TS, Bastianello SS, De Wet JAL, Joubert JPJ, Faul A (1985) Valsiekte (Falling disease): a nervous disorder in lambs suspected of being caused by the plant *Chrysocoma tenuifolia*. J S Afr Vet Assoc 56:65-68
23. Wells GAH, Hancock RD, Cooley WA, Richards MS, Higgens RJ, David GP (1989) Bovine spongiform encephalopathy: diagnostic significance of vacuolar changes in selected nuclei of the medulla oblongata. Vet Rec 125:521-524
24. Wouda W, Borst GHA, Gruys E (1986) Delayed swayback in goat kids, a study of 23 cases. Vet Q 8:45-56

Chapter ***4.3***

SPINAL CORD DEGENERATION IN ADULT DAIRY COWS ASSOCIATED WITH THE FEEDING OF SORGHUM BEER RESIDUES

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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*

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.

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

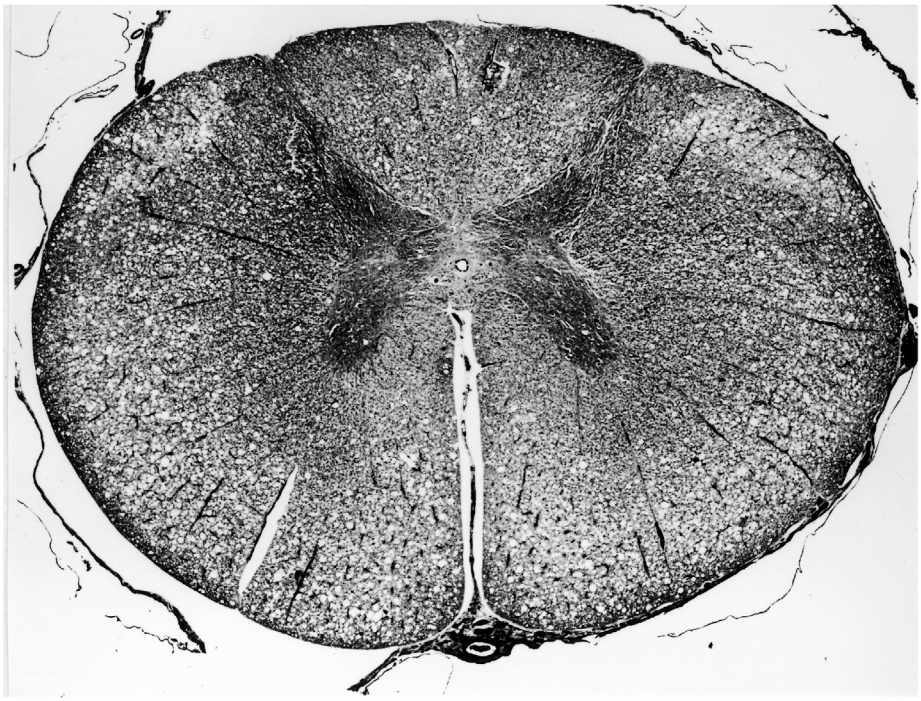

Fig. 1: Transverse section of thoracic spinal cord. There is extensive vacuolation and myclin depletion of the lateral and ventral funiculi with relative sparing of the dorsal funiculi and white matter adjacent to the grey matter. Luxol fast blue periodic acid-Schiff haematoxylin.

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

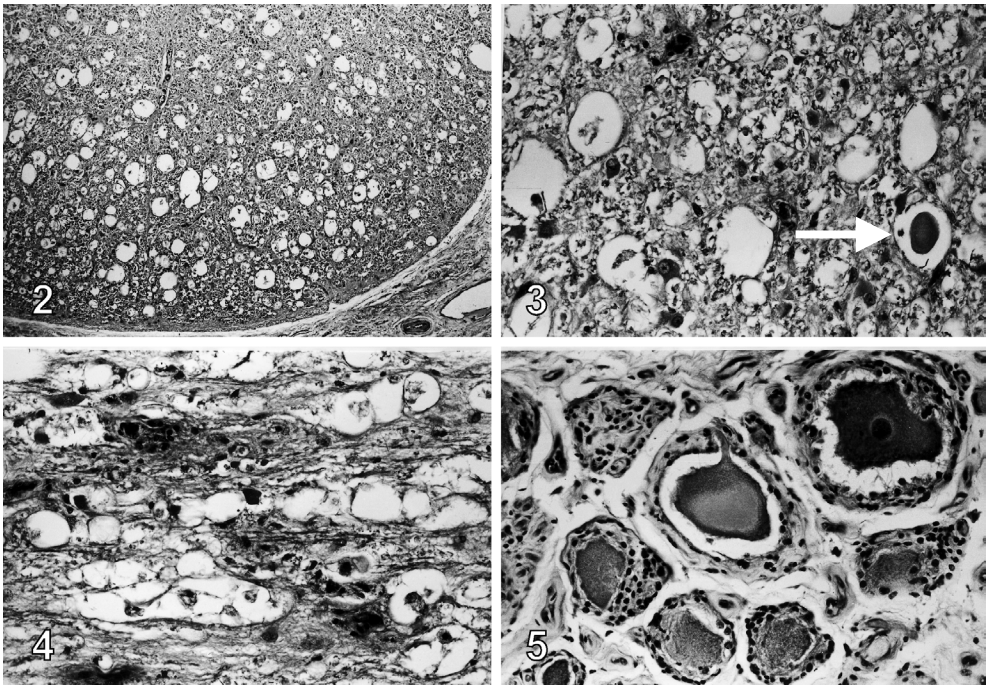

Fig. 2: Higher magnification of the peripheral white matter in the ventral funiculi of the thoracic spinal cord. Note ballooned myelin sheaths giving the white matter a vacuolar appearance. Luxol fast blue periodic acid-Schiff haematoxylin.
Fig. 3: Dilated myelin sheaths are empty or contain spheroids (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.

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References

1. Basson PA, Kellerman TS, Albl P, Von Maltitz LJF, Miller ES, Welman W G (1975) Blindness and encephalopathy caused by *Helichrysum argyrosphaerum* DC. (Compositae) in sheep and cattle. Onderstepoort J Vet Res 42:135-148
2. Beck BE, Wood CD, Whenham G R (1977) Triaryl phosphate poisoning in cattle. Vet Pathol 14:128-137
3. Gilmour JS, Inglis DM, Robb J, MacLean M (1989) A fodder mycotoxicosis of ruminants caused by contamination of a distillery by-product with *Aspergillus clavatus.* Vet Rec 124: 133-135
4. Hooper PT, Best SM, Campbell A (1974) Axonal dystrophy in the spinal cords of cattle consuming the cycad palm, *Cycas* media. Aust Vet J 50:146-149
5. Jiang CS, Huang SS, Chen MZ, Yu Z, Chen L H, Sang Y Z (1982) Studies on mycotoxicosis in dairy cattle caused by *Aspergillus clavatus* from mouldy malt sprouts. Acta Vet Zoot Sinica 13:247-254 (Abstract no 3943, Veterinary Bulletin 53)
6. Jubb KVF, Huxtable CR (1993) The nervous system. In: Jubb K V F, Kennedy P C, Palmer N (eds) Pathology of Domestic Animals, 4th edn Vol. 1 Academic Press Inc. San Diego, pp 267-439
7. Kellerman TS, Coetzer JAW, Naude TW (1988) Plant poisonings and mycotoxicosis of livestock in southern Africa. Oxford University Press, Cape Town
8. Kellerman TS, Newsholme SJ, Coetzer JAW, Van der Westhuizen GCA (1984) A tremorgenic mycotoxicosis of cattle caused by maize sprouts infested with *Aspergillus clavatus.* Onderstepoort J Vet Res 51:271-274
9. Kellerman TS, Pienaar JG, Van der Westhuizen GCA, Anderson LAP, Naude T W (1976) A highly fatal tremorgenic mycotoxicosis of cattle caused by *Aspergillus clavatus.* Onderstepoort J Vet Res 43:147-154
10. Myburgh JG, Fourie N, Van der Lugt JJ, Kellerman TS, Cornelius ST, Ward C (1994). A nervous disorder in cattle caused by the plants *Ficus ingens and Ficus cordata* subsp. *salicifolia.* Onderstepoort J Vet Res 61:171-176
11. Nikov S, Simov I, Koroleva V, Zhelev V (1965) Poisoning of cows by mouldy, germinated barley. Nauchi trudove. Vissh veterinarnomeditsinski institut 'Prof G Pavlov' 15: 169-180 (Abstract no 3433, Veterinary Bulletin 36)
12. Prozesky L, Pienaar JG (1977) Amaurosis in sheep resulting from treatment with rafoxanide. Onderstepoort J Vet Res 44:257-260
13. Raisbock MF, Rottinghaus GE, Kendall JD (1991) Effects of naturally occurring mycotoxins on ruminants. In: Smith JE, Henderson RS (ed.) Mycotoxins and Animal Foods. CRC Press Inc. Boca Raton, pp 647-677
14. Sanders DE, Koestner A (1980) Bovine neonatal ataxia associated with hypocupremia in pregnant cows. J Am Vet Med Assoc 176: 728-730
15. Shlosberg A, Zadikov I, Perl S, Yakobson B, Varod Y, Elad D, Rapoport E, Handji V (1991) *Aspergillus clavatus* as the probable cause of a lethal mass neurotoxicosis in sheep. Mycopathologia 114: 35-39
16. Tomov A (1965) *Aspergillus clavatus, the* cause of poisoning of cows fed malt sprouts. Veterinary Medicine Nauki, Sofia 2: 997-1003 (Abstract no 4687, Veterinan, Bulletin 36)
17. Van der Vyver FH, Kellerman TS, Bastianello SS, De Wet JAL, Joubert JPJ, Faul A (1985) Valsiekte (Falling disease): a nervous disorder in lambs suspected of being caused by the plant *Chrysocoma tenuifolia.* J S Afr Vet Assoc 56:65-68

CHAPTER 5 : SUMMARISING DISCUSSION

In this study the clinical signs and pathology of five plant poisonings and a mycotoxicosis affecting the nervous system of domestic ruminants in southern Africa are described. For comparative purposes, an inherited storage disease (β-mannosidosis) and a drug-induced neurotoxicosis (closantel overdosage) are also presented. **Chapter 1** outlines the aims of this thesis, namely

- to document the pathology of those conditions where detailed descriptions were lacking,
- to study the light and transmission electron microscopical lesions from a differential diagnostic perspective, and,
- to consider the possible causative mechanisms of the nervous lesions.

Lysosomal storage diseases (Chapter 2)

A short summary on the classification of the lysosomal storage diseases in domestic animals is given in **Chapter 2.1**. In **Chapter 2.2** a novel lysosomal storage disease in goats induced by the plant *Ipomoea carnea* is reported [17]. Analysis of plant material confirmed the presence of swainsonine and two glycosidase inhibitors, namely, calystegine B_2 and calystegine C_1. The storage disease caused by *I. carnea* is analogous to the lysosomal storage disease in ruminants induced by ingestion of locoweeds *(Astragalus and Oxytropis* spp.*)* and poison peas *(Swainsona* spp) [19,23,41,58]. These plant species also contain swainsonine, an indolizidine alkaloid, which is an inhibitor of lysosomal α-mannosidase and mannosidase II [18,36]. This group of plant-induced storage diseases closely mimics inherited α-mannosidosis in animals. In **Chapter 2.3** β-mannosidosis is documented in Hereford calves, a breed not previously known to be afflicted by this inherited disorder. The condition was biochemically confirmed by an absence of lymphocyte β-mannosidase activity in one of the affected calves.

Comparison of the two storage diseases described in this thesis highlighted two important aspects pertinent to their recognition and diagnosis. Firstly, they are a diverse group of multisystemic disorders with variable phenotype. The Hereford calves with β-mannosidosis manifested severe clinical disease, hydrocephalus and evidence of myelin deficit in the brain. Similar findings in β-mannosidosis in Salers calves [1,8,35] and Nubian goats [29,37] have been reported. In contrast, goats exposed to *I. carnea* showed less severe clinical signs while specific gross lesions were restricted to asymmetry and atrophy of the cerebellum in 1/6 animals. It has been documented that the severity of disease expression is influenced by the severity of the enzyme deficit [36]. If a mutant gene defect is such that mutant enzyme is not synthesized as in β-mannosidosis, the absence of enzyme activity will result in severe, early onset disease. In the plant-induced storage diseases there is most likely some residual enzyme activity resulting in less severe disease.

Secondly, in β-mannosidosis and *I. carnea* intoxication, storage vacuoles are empty in routinely prepared sections for light microscopy, indicating that the storage material is soluble in water and/or organic solvents. It is therefore not possible to diagnose these conditions based on morphological or staining characteristics of the storage material only. Confirmation of the diagnosis of these disorders therefore depends on biochemical analysis to determine the enzyme defect and the stored substrate. In the case of the plant-induced storage diseases, identification of the chemical compound(s) biosynthesized by the plant provides valuable additional information to support the diagnosis.

This study showed that in chronic *I. carnea* intoxication there is degeneration and loss of cerebellar Purkinje neurons, which probably accounted for the irreversible clinical signs in some of the chronic cases. Purkinje neurons are particularly vulnerable to injury in other storage diseases [56]. It is not known how the accumulation of lysosomal substrate perturbs cell function. Possible explanations that have been put forward are mechanical impairment of intracellular transport; a cytotoxic effect of the accumulated substrate; spheroid formation that affects inhibitory γ-aminobutyric acid (GABA)ergic fibres; and disturbances in synaptic function [56].

'Maldronksiekte' in cattle, caused by ingestion of the plant *Solanum kwebense*, is characterised by degeneration, necrosis and loss of cerebellar Purkinje cells and, to a lesser extent, neurons in cerebellar nuclei. Affected Purkinje neurons were either swollen, pale and finely vacuolated or shrunken and more eosinophilic than normal. In the present study, some Purkinje cells and neurons in cerebellar nuclei stained strongly with *Canavalia ensiformis* (ConA) agglutinin and weakly with *Triticum vulgaris* (WGA) and succinyl-WGA (S-WGA) agglutinins (**Chapter 2.4**). Ultrastructurally, small numbers of membranous bodies were found in the cytoplasm of degenerated Purkinje cells. Low levels of calystegines but not swainsonine and other known glycosidase inhibitory alkaloids were detected in one sample of plant material collected from one of the incriminated farms. The condition is clinically and pathologically virtually identical to *S. fastigiatum* poisoning, a condition believed to be a lysosomal storage disease (probably a gangliosidosis) based on the presence of membranous cytoplasmic bodies in Purkinje cells [48]. When compared to other storage diseases, two pathological features of intoxication by *S. kwebense* and *S. fastigiatum* are, however, unusual, namely the predominance of lesions in Purkinje cells and the degeneration and loss of most Purkinje cells in the cerebellum of affected cattle. 'Maldronksiekte' is therefore classified as an acquired cerebellar cortical degeneration morphologically resembling cerebellar and cerebral cortical degeneration in miniature Poodles [14]. The condition may indeed represent a plant-induced intoxication that does not result from an enzymatic lysosomal defect but in which Purkinje cells are unable to metabolise a plant toxin or cellular substrate [56]. Acquired cerebellar cortical degeneration induced by toxic plants is not restricted to cattle. A syndrome characterised by degeneration and loss of Purkinje cells has been associated with the ingestion of *Solanum cinereum* in goats in Australia [7].

The question is why Purkinje cells are the predominantly cell type affected in cerebellar cortical degeneration. Some studies in domestic animals revealed striking morphological abnormalities in degenerated Purkinje cells but the pathogenesis for the selective involvement of these neurons remains unclear. Morphological changes in injured Purkinje cells in cerebellar cortical degeneration comprised accumulations of tubulovesicular profiles of agranular ER in Aberdeen Angus cattle [5] and cytoplasmic vacuoles and lamellar bodies originating from ER and stacked cisternae of presumed ER-origin in dogs [14,44]. In spinocerebellar ataxia type 6 (SCA6) in humans, a condition in which Purkinje cells are particularly affected, a molecular basis of selective Purkinje neuron degeneration has been reported [33,49]. This condition is an autosomal dominant neurodegenerative disease associated with the expansion of trinucleotide (CAG) repeat coding polyglutamine in the alpha 1A (P/Q-type)-voltage dependant calcium channel. The mutant proteins which are produced form cytoplasmic aggregates and altered protein-protein interactions result in excessive channel activity, intracellular Ca^{2+} overload and cell death. The term 'channelopathy' has been coined for this condition [57]. Whether or not apoptosis is involved in neuronal degeneration in cerebellar cortical degeneration needs further study.

Toxic myelinopathies (Chapter 3)

In this chapter four toxic myelinopathies are described; three are induced by plants (*Helichrysum argyrosphaerum, Ornithogalum prasinum* and *O. saundersiae* and *Crotalaria sparthioides*) and one is caused by overdosage of the anthelmintic compound closantel. As an encompassing term, myelinopathies denote non-inflammatory conditions of the central or peripheral nervous system in which the primary event relates to some disorder of myelin formation, maintenance, or stability [38]. In **Chapter 3.1** a classification of myelinopathies based on morphological criteria in domestic animals is given and explained. **Chapter 3.2** documents the clinical syndrome and pathology induced by the plant *Helichrysum argyrosphaerum* in sheep and a goat and in **Chapter 3.3** the effects of overdosage by the halogenated salicylanilide closantel in sheep and a goat are described. The two intoxications are clinically and pathologically indistinghuishable and are characterised by blindness, ataxia, nystagmus, widespread spongy changes (status spongiosis) in the central nervous system, optic neuropathy and retinal degeneration. The status spongiosis is caused by splitting of myelin lamellae at the intraperiod line, which is consistent with myelin oedema. Oedema of myelinated fibres leads to compression of the optic nerves in the bony canal and eventually results in Wallerian degeneration and fibrosis of the nerves. In both poisonings, toxic retinal degeneration was present. There have been some conflicting interpretations of the site of retinal injury in salicylanilide poisoning. Necrosis of the ganglion cells [6,9,47] and degeneration of the outer retinal layers [26,52] have been reported as primary lesions. Results of our study showed involvement of the photoreceptor layer in both conditions possibly targeting the outer segments in poisoning with *H. argyrosphaerum* and of the rods in salicylanilide overdosage.

Chinkerinchee poisoning (poisoning by *Ornithogalum prasinum* and *O. saundersiae*) is described in **Chapter 3.4**. Diarrhoea was consistently present in cattle and sheep, while blindness occurred in a high proportion (4/11 and 3/3) of cattle. The blindness is associated with a central visual pathway lesion characterised by bilateral status spongiosis of the cerebral white matter especially affecting the optic radiation and internal capsule. Transmission electron microscopy revealed splitting of myelin lamellae at the intraperiod lines and swollen astrocytic processes in the affected white matter. It would appear that this intoxication is an unique example of white matter myelin oedema causing blindness in cattle.

Toxicity with *Crotalaria sparthioides* in cattle induced hepatic encephalopathy secondary to cirrhosis (**Chapter 3.5**). The pyrrolizidine alkaloid senkirkine was isolated from toxic plant material. Brain lesions were bilateral symmetrical and most profound at the junction of the white and grey matter (consistently affecting the cerebral cortex, cerebellar white matter and peducle, thalamus, basal ganglia and internal capsule) in three natural and one experimentally intoxicated bovines. There are few published reports on the ultrastructural changes in hepatic encephalopathy in domestic animals. In one experimental study in which calves were infused with ammonia, swelling of myelin sheaths and splitting of myelin lamellae at the intraperiod lines was demonstrated [11].

The pathogenesis of hepatic encephalopathy is still largely unclear although there is agreement on the important role of neurotoxins, especially ammonia [3,45]. In human medicine, much research is directed to study alterations of astrocyte morphology and function and it has been proposed that hepatic encephalopathy is an example of an ‘astrocytopathy’ or ’gliopathy’ [45]. Astrocytes are the only cells in the brain to contain glutamine synthetase and are accordingly a major site of cerebral ammonia detoxification. It is proposed that astrocyte swelling, induced by amongst others ammonia, hyponatraemia, some neurotransmitters, tumour necrosis factor-α and benzodiazepines, is an early event in hepatic encephalopathy [4,28,45]. An increase in astrocyte water content may then activate extracellular regulated protein kinases, elevates intracellular calcium concentration, upregulates the peripheral type benzodiazepine receptor and affects multiple ion channels and amino acid transport.

Status spongiosis denotes spongy vacuolation of nervous tissue noticeable by light microscopy [2]. Transmission electron microscopy is often required to determine the morphological basis of the vacuolation [56]. The vacuoles usually contain fluid and may be present within myelin sheaths, processes in the neuropil, or the cytoplasm of astrocytes or oligodendrocytes [2]. In the four toxic myelinopathies described in this thesis (Chapter 3), vacuoles within the nervous system were formed within myelin sheaths due to splitting of myelin lamellae the intraperiod lines. This is the most location of fluid in status spongiosis [40]. The intramyelinic vacuoles can be considered to occur in the embryonic extracellular space since the intraperiod line is formed by apposition of the external surface of the plasma membrane of the myelin forming cells [46]. Experimentally it has been

shown that this space can be penetrated by various markers [46]. The integrity of the intraperiod line is believed to be maintained by proteolipid protein (PLP) [20] but whether splits can be produced by an over- or an underproduction of PLP is not clear [10]. The distribution of the vacuolation varied despite having a similar morphological basis. The vacuolation was mainly periventricular in *H. argyrosphaerum* poisoning and overdosage with closantel, located predominantly in the optic radiation and internal capsule in chinkerinchee poisoning, and involved myelinated bundles of fibres that are interspersed with the gray matter in hepatic encephalopathy.

***Aspergillus clavatus* neuromycotoxicosis (Chapter 4)**

In **Chapter 4.1** the light and transmission electron microscopical changes of acute *Aspergillus clavatus* intoxication in cattle is reported. In this neuromycotoxicosis there is chromatolysis particularly of motor neurons in the ventral horns throughout the spinal cord and of neurons in selected brain stem nuclei including the spinal tract nucleus of the trigeminal nerve and the dorsal nucleus of the vagus nerve. At the ultrastructural level, changes in chromatolytic neurons were characterised by disintegration of Nissl bodies, abundant free ribosomes and increased numbers of mitochondria and dense bodies consistent with the axonal reaction. Swollen axons were present in close proximity to affected nerve cell bodies and they contained axoplasmic debris and degenerated organelles compatible with Wallerian degeneration. Wallerian degeneration was more prominent in proximal than in distal portions of the peripheral nerves. The nature and distribution of lesions in the nervous system in this condition indicate a neuronopathy/axonopathy. In **Chapter 4.2** chronic *Aspergillus clavatus* intoxication in cattle fed sorghum beer residues is described. Lesions were characterised by extensive, bilaterally symmetrical Wallerian degeneration in ascending and descending tracts throughout the length of the spinal cord and focal neuronal degeneration in the spinal cord grey matter.

The question is why there is a selective vulnerability of specific populations of neurons, in this case motor neurons, to injury. Recent studies on the pathogenesis of motor neuron diseases in humans, especially amyotrophic lateral sclerosis (ALS), shed some light on factors that render neurons more vulnerable. Various potential contributors to disease have been proposed and are briefly discussed:

- **Damage to the cytoskeleton**: motor neurons are amongst the largest cells in the nervous system. They often have long axonal processes and those supplying the muscles of the distal lower limb in humans may be up to one metre in length [53]. Such neurons would not only have high-energy demands and a high metabolic rate, but also need a robust cytoskeleton with a high neurofilament content. Neurofilament proteins maintain cell shape and axonal calibre and play a significant role in axonal transport. Abnormal assembly and accumulation of neurofilaments in cell bodies and proximal axons in motor neurons in ALS are characteristic. It is not known however, whether accumulation of neurofilament protein occurs as a consequence of axonal transport blockage or whether the neurofilament abnormality directly causes

impairment of axonal transport. Evidence to support the latter hypothesis has come from studies with transgenic mice: animals which overexpressed two of the three neurofilament subunit proteins of different weights (neurofilament [NF]-light and neurofilament [NF]-heavy) developed motor neuron disease as did mice which expressed mutations in one of the subunits of neurofilaments [39].

Several motor neuron diseases exhibiting neurofilament accumulation in neurons have been reported in domestic animals. These include spinal muscular atrophy in Brittany Spaniels [12] and Rottweilers [55] and motor neuron disease in Yorskshire [30] and Hampshire pigs [43] and in horned Hereford [51] and brown Swiss cattle [22]. The condition in Brittany Spaniels, which is inherited as an autosomal dominant trait, has been considered as a model for the study of ALS. Cork and co-workers [13] found inappropriate phosphorylation of neurofilaments in neuronal perikarya in six diseases associated with neurofilament accumulation, namely, spinal muscular atrophy in Brittany Spaniels, motor neuron disease in horned Hereford ('Shaker' calves), pigs and rabbits, 'swayback' in lambs, and myelopathy in zebras. The significance of this cytoskeletal abnormality remains unclear. Some neuron diseases in animals, however, do not display neurofilament accumulation and despite detailed studies the pathogenesis is still unclear in conditions such as progressive neurogenic muscular atrophy in Pointers [32] and spinal muscular atrophy in German Shepherds [15].

- **Excitotoxicity** : Glutamate is the principal excitatory neurotransmitter in the nervous system. Glutamate is normally released into the synaptic cleft where postsynaptic receptors are activated. The excitatory signal is terminated by removal of the neurotransmitter by transported proteins located on glial and neuronal cells. Excessive stimulation of glutamate receptors may induce derangement of intracellular calcium homeostasis and the production of free radicals – the term 'excitotoxicity' has been coined for this process - leading to cellular degeneration and necrosis [50].

  Why then are motor neurons selectively affected if the glutamate neurotransmitter system is widely distributed in the human central nervous system? An important finding is that in motor neurons which are vulnerable to degeneration, impairment of the excitatory amino acid transporter 2 (EAAT2) is evident which may lead to abnormally high levels of glutamate in extracellular and cerebrospinal fluid [50].
- **Oxidative stress** : Mutations in the gene encoding the enzyme Cu/Zn superoxide dismutase have been reported in patients suffering from motor neuron disease [39,54]. This enzyme catalysis the conversion of intracellular superoxide radicals, produced during normal cellular metabolism, to hydrogen peroxide, which is then eliminated by other free radical scavenging enzymes. The mutant enzyme may metabolise biochemical compounds such as hydrogen peroxide and peroxynitrate abnormally, resulting in increased formation of highly damaging hydroxyl radicals and the formation of nitrotyrosine residues

on intracellular proteins. Cu/Zn superoxide dismutase is an ubiquitous enzyme but has high expression in motor neurons compared to other cells of the nervous system. Why then are motor neurons more susceptible to injury in motor neurons disease? It has been proposed that cells such as neurons in which this enzyme has a high level of expression are more vulnerable to genetic or post-translational alterations interfering with the function of these proteins [53,54].

- **Calcium binding proteins** : Calcium is essential for many normal cellular processes including neurotransmission and the regulation of intracellular second messenger systems. Intracellular regulation is maintained by a complex system of compartmentalisation and transportation involving binding to calcium buffering proteins, including parvalbumin and calbinding D28k. These buffering proteins play an important role in protecting neurons from calcium-mediated injury following for example activation of glutamate receptors [50,53]. Human motor neurons that are vulnerable in ALS do not express these calcium-binding proteins while less susceptible motor neuron groups do [50]. Deficiency of calcium binding proteins therefore may play an essential role in the pathogenesis of neuronal degeneration in motor neuron disease.

Vacuolation of nervous tissue and cells in veterinary neuropathology

A histological feature of all the conditions that are described in this thesis is the presence of vacuolation of varying severity in neurons and/or neuropil in the central nervous system of affected animals. In veterinary neuropathology, the diagnostic significance of spongy changes in the neuropil and intracytoplasmic neuronal vacuolation, irrespective of their cause, has been highlighted by the recent epidemics of spongiform encephalopathies in bovine, feline and exotic ungulates [60]. The initial diagnosis of these conditions often relies on histopathology, as was the case in the bovine spongiform encephalopathy (BSE) outbreak in the United Kingdom [61]. Histological examination of brain material of suspect cases is used as an essential method of surveillance for BSE [16].

Accurate interpretation of vacuolation in nervous tissue may be difficult since spongiform changes in the neuropil can be readily obscured by autolysis [21] and by disruption of tissues during routine processing [59]. It may even be caused by certain tissue processing procedures [62]. Perikaryal vacuolation, in contrast, is usually preserved even in the presence of tissue artefacts [59].

Other conditions in ruminants besides those reported in this thesis that may exhibit neuronal vacuolation. In cattle, neuronal vacuolation and spongy changes of the white matter has occasionally been reported in rabies [24]. Idiopathic neuronal vacuolation was described in a 6-day-old calf [27]. The red nucleus and habenular nucleus in cattle may exhibit non-specific, perikaryonal vacuolation. These vacuoles are single or multiple and empty and are regarded as incidental findings in healthy cattle [25,42].

In a study on the differential diagnoses of BSE, non-specific vacuolation of the substantia nigra in 47/225 cattle brains was noted. Of these 16 had additional

lesions such as abscessation, non-suppurative meningoencephalitis or chronic hippocampal atrophy. In some cases the vacuolation extended rostrally to involve the thalamic radiation and the internal capsule, particularly the globus pallidus, or the cerebellar roof. The vacuoles were of different size and located at the junction of the grey and white matter, indicating myelin oedema [34]. In a subsequent report, white matter spongy changes resembling intramyelinic vacuolation was seen in 25,5% (129/506) symptomless 7-year-old cattle. The vacuolation was most often present in the substantia nigra and in 15,4% (78/506) the vacuolation was bilaterally symmetrical [25]. Idiopathic brainstem neuronal chromatolysis and hippocampal sclerosis in cattle is a differential diagnosis for *A. clavatus* neuromycotoxicosis. Non-specific white matter and neuronal vacuolation has also been described in sheep [31,63].

References

1. Abbitt B, Jones MZ, Kasari TR, Storts RW, Templeton JW, Holland PS, Castenson PE (1991) β-Mannosidosis in twelve salers calves. J Am Vet Med Assoc 198:109-113
2. Adornato B, Lampert P (1971) Status spongiosis of nervous tissue. Acta Neuropathol (Berl) 19:271-289
3. Albrecht J (1998) Roles of neuroactive amino acids in ammonia neurotoxicity. J Neurosci Res 51:133-138
4. Albrecht J, Dolińska M (2001) Glutamine as a pathogenic factor in hepatic encephalopathy. J Neurosci Res 65:1-5
5. Barlow RM (1981) Morphogenesis of cerebellar lesions in bovine familial convulsions and ataxia. Vet Pathol 18:151-162
6. Borges AS, Mendes LCN, De Andrade AL, Machado GF, Peiro JR (1999) Optic neuropathy in sheep associated with overdosage of closantel. Vet Human Toxicol 41: 378-380
7. Bourke CA (1997) Cerebellar degeneration in goats grazing *Solanum cinereum* (Narrawa burr). Aust Vet J 75:363-365
8. Bryan L, Schmutz S, Hodges SD, Snyder FF(1993) Bovine β-mannosidosis: pathologic and genetic findings in Salers calves. Vet Pathol 30:130-139
9. Button C, Jerrett I, Alexander P, Mizon W (1987) Blindness in kids associated with overdose of closantel. Aust Vet J 64:226
10. Coetzee T, Suzuki K, Popko B (1998) New perspectives on the function of myelin galactolipids. Trends Neurosci 21:126-130
11. Cho DY, Leipold HW (1977) Experimental spongy degeneration in calves. Acta Neuropathol (Berl.) 39:115-127
12. Cork LC, Price DL, Griffin JW, Sack GH (1990) Hereditary canine spinal muscular atrophy: canine motor neuron disease. Can J Vet Res 54:77-82
13. Cork LC, Troncoso JC, Klavano GG, Johnson ES, Sternberger LA, Sternberger NH, Price DL (1988) Neurofilamentous abnormalities in motor neurons in spontaneously occurring animal disorders. J Neuropathol Exp Neurol 47:420-431
14. Cummings JF, deLahunta A (1988) A study of cerebellar and cerebral cortical degeneration in Miniature Poodle pups with emphasis on the ultrastruture of Purkinje cells. Acta Neuropathol (Berl) 75:261-271

15. Cummings JF, George C, deLahunta A, Valentine BA, Bookbinder PF (1989) Focal spinal muscular atrophy in two German Shephard pups. Acta Neuropathol (Berl) 79:113-116
16. Davis AJ, Jenny AL, Miller LD (1991) Diagnostic characteristics of bovine spongiform encephalopathy. J Vet Diagn Invest 3:266-271
17. De Balogh Katinka KIM, Dimande AP, Van der Lugt JJ, Molyneux RJ, Naudé TW, Welman WG (1999) A lysosomal storage disease induced by *Ipomoea carnea* in goats in Mozambique. J Vet Diagn Invest 11:266-273
18. Dorling PR, Huxtable CR, Colegate SM (1980) Inhibition of lysosomal a-mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens.* Biochem J 191:649-651
19. Dorling PR, Huxtable CR, Vogel P (1978) Lysosomal storage in *Swainsona* spp. toxicosis: an induced mannosidosis. Neuropathol Appl Neurobiol 4:285-295
20. Duncan ID, Hammang JP, Trapp BD (1987) Abnormal compact myelin in the myelin-deficient rat. Absence of proteolipid protein correlates with a defect in the intraperiod line. Proc Natl Acad Sci USA 84:6287-6291
21. Edwards A, Kruckenberg S, Leipold H (1982) Sequence of histologic alterations in the bovine brain and spinal cord undergoing postmortem autolysis. Proc Annu Meet Am Assoc Vet Lab Diagn 25:315-344
22. El-Hamidi M, Leipold HW, Vestweber JGE, Saperstein G (1989) Spinal muscular atrophy in brown Swiss calves. J Vet Med [A] 36:731-738
23. Everist SL (1981) Poisonous plants of Australia, 2nd edn, Angus & Robertson, Sydney, Australia, pp 966
24. Foley GL, Zachary JF (1995) Rabies-induced spongiform change and encephalitis in a heifer. Vet Pathol 32:309-311
25. Gavier-Widen D, Wells GAH, Simmons MM, Wilesmith JWW, Ryan J (2001) Histological observations on the brains of symtomless 7-year-old cattle. J Comp Path 124:52-59
26. Gill PA, Cook RW, Boulton JG, Kelly WR, Vanselow B, Reddacliff LA (1999) Optic neuropathy and retinopathy in closantel toxicosis of sheep and goats. Aust Vet J 77:259-261
27. Hamir AN, Habecker P, Jenny A, Hutto D, Stack MJ, Chaplin MJ, Stasko J (2001) Idiopathic disseminated intracytoplasmic neuronal vacuolation in a neonatal Holstein calf born in the USA. J Vet Diagn Invest 13:349-351
28. Häussinger D, Kircheis G, Fischer R, Schliess F, vom Dahl S (2000) Hepatic encephalopathy in chronic liver disease: a clinical manifestation of astrocyte swelling and low-grade cerebral oedema? J Hepatol 32:1035-1038
29. Healy PJ, Seaman JT, Gardner IA, Sewell CA (1981) β-Mannosidase deficiency in Anglo Nubian goats. Aust Vet J 57:504-507
30. Higgins RJ, Rings DM, Fenner WR, Stevenson S (1983) Spontaneous lower motor neuron disease with neurofibrillary accumulation in young pigs. Acta Neuropathol (Berl) 59:288-294
31. Hooper PT (1999) Incidental lesions in the brains of sheep and goats. Aust Vet J 77:398-399
32. Izumo S, Ikuta F, Igata A, Osame M, Yamauchi C, Inada S (1983) Morphological study on the hereditary neurogenic amyotrophic dogs: accumulation of lipid compound-like structures in the lower motor neurons. Acta Neuropathol (Berl) 61:270-276

33. Ishikawa K, Owada K, Ishida K, Fujigasaki H, Shun Li M, Tsunemi T, Ohkosi N, Toru S, Mizutani T et al (2001) Cytoplasmic and nucelar polyglutamine aggregates in SCA6 Purkinje cells. Neurology 56:1753-1756
34. Jeffrey M A (1992) Neuropathological survey of brains submitted under the Bovine Spongiform Encephalopathy Orders in Scotland. Vet Rec 131:332-337
35. Jolly RD, Thompson KG, Bayliss SL, Vidler BM, Orr MB, Healy PJ (1990) β-Mannosidosis in a Salers calf: a new storage disease of cattle. N Z Vet J 38:102-105
36. Jolly RD, Walkley SU (1997) Lysosomal storage diseases: an essay in comparative pathology. Vet Pathol 34:527-548
37. Jones MZ, Cunningham JG, Dade AW, Alessi DM, Mostosky UV, Vorro JR, Benetiz JT, Lovell KL (1983) Caprine β-mannosidosis: clinical and pathological features. J Neuropathol Exp Neurol 42:268-285
38. Jubb KVF, Huxtable CR (1993) The Nervous System. In: Jubb KVF, Kennedy PC, Palmer N (eds) Pathology of Domestic Animals 4th edn Academic Press, Inc, San Diego, pp 267-439
39. Julien J (2001) Amyotrophic lateral sclerosis: unfolding the toxicity of the misfolded. Cell 104:581-591
40. Kreutzberg GW, Blakemore WF, Graeber MB (1997) Cellular pathology of the central nervous system. In: Graham DI, Lantos PL (eds) Greenfield's Neuropathology, 6th edn, Arnold, London, 85-140
41. Locke KB, MeEwan DR, Hamdorf IJ (1980) Experimental poisoning of horses and cattle with *Swainsona canescens var. homiana.* Aust Vet J 56:379-383
42. McGill IS, Wells GAH (1993) Neuropathological findings in cattle with clinically suspect but histologically unconfirmed bovine spongiform encephalopathy (BSE). J Comp Path 108:241-260
43. Montgomery DL, Gilmore WC, Litke LL (1989) Motor neuron disease with neurofilamentous accumulations in Hampshire pigs. J Vet Diagn Invest 1:260-262
44. Montgomery DL, Storts RW (1984) Hereditary striatonigral and cerebello-olivary degeneration of the Kerry Blue Terrier. II. Ultrastructural lesions in the caudate nucleus and cerebellar cortex. J Neuropath Exp Neurol 43:263-275
45. Norenberg MD (1996) Astrocytic-ammonia interactions in hepatic encephalopathy. Semin Liver Dis 16:245-253
46. Peters A, Palay SL, Webster DeF H (1991) The Fine Structure of the Nervous System. Oxford University Press: New York & Oxford
47. Prozesky L, Pienaar JG (1977) Amourosis in sheep resulting from treatment with rafoxanide. Onderstepoort J Vet Res 44: 257-260
48. Riet-Correa F, Méndez MC, Schild AL, Summers BA, Oliveira JA (1983) Intoxication by *Solanum fastigiatum* var. *fastigiatum* as a cause of cerebellar degeneration in cattle. Cornell Vet 73:240-256
49. Robitaille Y, Lopes-Cendes I, Becher M, Rouleau G, Clark AW (1997) The neuropathology of CAG repeat diseases: review and update of genetic and molecular features. Brain Pathol 7:901-926
50. Rothstein JD (1996) Excitotoxicity hypothesis. Neurology 47:S19-S26
51. Rousseaux CG, Klavano GG, Johnson ES, Shnitka TK, Harries WN, Snyder FF (1985) 'Shaker' calf syndrome: a newly recognized inherited neurodegenerative disoprder of horned Hereford calves. Vet Pathol 22:104-111
52. Seawright AA (1989) Animal Health in Australia, Volume 2, 2nd edn, Chemical and Plant Poisonings, Australian Government Publishing Service, Canberra, pp 331-332

53. Shaw PJ, Eggett CJ (2000) Molecular factors underlying selective vulnerability of motor neurons to neurodegeneration in amyotrophic lateral sclerosis. J Neurol 247:I17-I27
54. Shaw PJ, Chinnery RM, Thagesen H, Borthwick G, Ince PG (1997) Immunocytochemical study of the distribution of free radical scavenging enzymes Cu/Zn superoxide dismutase (SOD1), Mn superoxide dismutase (Mn SOD) and catalase in the normal human spinal cord and in motor neuron disease. J Neurol Sci 147:115-125
55. Shell LG, Jortner BS, Leib MS (1987) Familial motor neuron disease in Rottweiler dogs: neuropathological studies. Vet Pathol 24:135-139
56. Summers BA, Cummings JF, De Lahunta A (1995) Veterinary Neuropathology. Mosby: St. Louis, pp 1-67
57. Toru S, Murakoshi T, Ishikawa K Saegusa H, Fujigasaki H, Uchihara T, Nagayama S, Osanai M, Mizusawa H, Tanabe T (2000) Spinocerebellar ataxia type 6 mutation alters P-type calcium function. J Biol Chem 275:10893-10898
58. Van Kampen KR, James LF (1969) Pathology of locoweed poisoning in sheep. Pathol Vet 6:413-423
59. Wells GAH, Hancock RD, Cooley WA, Richards MS, Higgens RJ, David GP (1989) Bovine spongiform encephalopathy: diagnostic significance of vacuolar changes in selected nuclei of the medulla oblongata. Vet Rec 125:521-524
60. Wells GAH, McGill IS (1992) Recently described scrapie-like encephalopathies of animals: case definitions. Res Vet Sci 53:1-10
61. Wells GAH, Scott AC, Johnson CT, Gunning RF, Hancock RD, Jeffrey M, Dawson M, Bradley R (1987) A novel progressive encephalopathy in cattle. Vet Rec 121:419-420
62. Wells GAH, Wells M (1987) Neuropil vacuolation in brain: a reproducible histological artifact. J Comp Path 101:355-362
63. Zlotnik I, Rennie JC (1958) A comparative study of the incidence of vacuolated neurons in the medulla from apparently healthy sheep of various breeds. J Comp Path 68:411-415

Samenvatting in het Nederlands

Het subcontinent van zuidelijk Afrika, een gebied dat Zuid-Afrika, Namibia, Botswana, Mozambique, Zimbabwe, Lesotho en Swaziland omvat, is bekend vanwege haar grote verscheidenheid en pracht aan flora. Ironisch is echter dat meer dan 600 planten toxisch zijn, waarvan een groot percentage potentieel giftig is voor herkauwers. Naast planten zijn er ook enkele schimmels die ziekte en sterfte onder de levende have kunnen veroorzaken.

In *hoofdstuk 1* van dit proefschrift wordt ter inleiding een korte samenvatting gegeven van de economisch meest belangrijke plant- en schimmelvergiftigingen van herkauwers in zuidelijk Afrika. Een aantal van deze planten en schimmels veroorzaken neurologische verschijnselen in runderen, schapen en geiten. Met uitzondering van de schimmel *Stenocarpella maydis*, die diplodiosis veroorzaakt, komen vergiftigingen met aantasting van het zenuwstelsel bij deze diersoorten in het boven beschreven gebied sporadisch voor en zijn onvolledig bestudeert. In dit proefschrift wordt ingegaan op een aantal vergiftigingen en stapelingsziekten die het zenuwstelsel van runderen, schapen en geiten in zuidelijk Afrika aantasten. Naast gedetailleerde beschrijvingen van het klinische beeld en de pathologie, pogen we het mechanisme van die morfologische laesies in het zenuwstelsel in de toestanden te karakteriseren. Met histologisch onderzoek blijkt dat deze intoxicaties vacuolisatie van myeline en/of het cytoplasma van cellen in het zenuwstelsel veroorzaken, vergelijkbaar met de laesies die bij de meeste stapelingsziekten worden waargenomen.

In *hoofdstuk 2* wordt een nieuwe stapelingsziekte bij geiten, veroorzaakt door consumptie van de plant *Ipomea carnea*, gedocumenteerd. Kenmerkend voor het toxische effect is de aanwezigheid van cytoplasmatische vacuolisatie in neuronen en glia cellen in de hersenen, in neuronen van plexi in de dunne darmen, in tubulusepitheelcellen in de nieren en in macrophagen–fagocyten in de milt en lymfeknopen. Chemisch onderzoek toonde aan dat de plant de mannosidase-inhibitor swainsonine en twee glycosidase inhibitoren calystegine B_2 and calystegine C_1 bevat. Deze stapelingsziekte vertoont klinische en pathologische overeenkomsten met andere door planten-geïnduceerde α-mannosidoses. Ter vergelijking wordt β-mannosidosis, een genetische bepaalde lysosomale stapelingsziekte, beschreven en wel voor het eerst in Simmentaler kalveren. Deze afwijking werd bevestigd door het aantonen van de afwezigheid van het enzym β-mannosidose in lymfocyten in een aangetast kalf. β-Mannosidosis presenteert zich met een ernstig klinisch beeld en macroscopische pathologie in de hersenen hetgeen waarschijnlijk duidt op een totale afwezigheid van enzymactiviteit. Dit in tegenstelling tot *Ipomea carnea* vergiftiging waar de enzymactiviteit waarschijnlijk slechts gedeeltelijk geremd wordt door de inhibitor swainsonine en de ziekte derhalve minder ernstig tot expressie komt.

In dit hoofdstuk worden tevens de resultaten van een onderzoek naar de histologie, elektronmicroscopie en immuunhistochemie van ‘maldronksiekte’ beschreven.

Deze aandoening van de kleine hersenen in runderen wordt veroorzaakt door de plant *Solanum kwebense*. Purkinje cellen worden hierbij selectief aangetast. Ze zijn of gezwollen en gevacuoliseerd of gekrompen en eosinofiel waarna een hoog percentage verdwijnt. Deze twee vormen van degeneratie van Purkinje cellen lijken sterk op cerebellaire corticale degeneratie. Met elektronenmicroscopisch onderzoek werden kleine aantallen membraneuze en lamellaire structuren in het cytoplasma van sommige gedegenereerde Purkinje cellen aangetoond. Het aantal van deze structuren was echter onvoldoende om eenduidig van een klassieke lysosomale stapelingsziekte te spreken. Ook het verspreidingspatroon van de laesies en het feit dat aangetaste Purkinje cellen in de loop van de tijd verdwijnen maakt een stapelingsziekte onwaarschijnlijk. Een middels immuunhistochemie zichtbaar gemaakte positieve cytoplasmatische reactie met het agglutinine van *Canavalia ensiformis* (ConA) in sommige Purkinje cellen toonde daarentegen aan dat er α-mannosyl residuen in de afwijkende cellen aanwezig waren. Bij het proces van neuronale degeneratie bleken apoptotische veranderingen geen rol te spelen (TUNEL assay) .

In *hoofdstuk 3* worden vier toxische myelinopathien beschreven: Drie daarvan worden geïnduceerd door planten (*Helichrysum argyrosphaerum*, *Ornithogalum prasinum* en *O. saundersiae* en *Crotalaria sparthioides*) en één, bestudeerd ter vergelijking, is het gevolg van overdosering van het wormmiddel closantel, een gehalogenizeerde salicylanalide. In vergiftiging met *C. sparthioides*, een plant die het pyrrolizidine alkaloid senkirkine bevat, is de pathologie in de hersenen kenmerkend voor hepatische encephalopathie als gevolg van chronische fibrose van de lever. Alle vier intoxicaties veroorzaken status spongiosis in de witte stof van de hersenen en elektronenmicroscopisch onderzoek toont aan dat de myeline zwelling veroorzaakt word door splitsing van de myelineschede ter plaaste van de intraperiodische lijn. De vacuoles, waarschijnlijk met vocht gevuld, ontstaan in de embryonische extracellulaire spatie maar de pathogenese hiervan is onbekend. Histologisch was de verspreiding van de status spongiosis verschillend: in vergiftiging met *H. argyrosphaerum* en closantel vooral periventriculair, in *O. prasinum* en *O. saundersiae* vooral in de optic radiation en internal capsule en met *C. sparthioides* karakteristiek in gemyeliniseerde bundels waar deze vermengd zijn met de grijze stof. Het toxine van *H. argyrosphaerum* en closantel veroorzaken ook een primaire retinopathie met aantasting van die photoreceptorlaag. Door het toxine van *H. argyrosphaerum* worden waarschijnlijk de buitenste segmenten aangetast en in closantel de staafjes.

In *hoofdstuk 4* wordt de pathologie van acute *Aspergillus clavatus* vergiftiging in runderen beschreven. De intoxicatie wordt gekenmerkt door chromatolyse van neuronen in het ruggenmerg en in bepaalde kernen in de hersenstam. Elektronmicroscopisch vertoont de pathologie enkele overeenkomsten maar ook verschillen met de ‘axonale reactie’. De hier beschreven intoxicatie kan het best geclassificeerd worden als een neuronopathie/axonopathie waarbij primaire schade aan het neuron, zichtbaar als chromatolyse, gepaard gaat met secundaire of simultane axonale degeneratie. Runderen die sorghum bier residuen (‘maroek’)

aten ontwikkelde een toestand die hoogst waarschijnlijk chronische *A. clavatus* vertegenwoordigd. Een bilaterale symmetrische dilatatie van myeline, zwelling en verlies van axonen en Wallerse degeneratie in het ruggenmerg werd bij de aangetaste dieren waargenomen.

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List of publications

1. Van der Lugt JJ, Coetzer JAW, Jordaan P, Marlow KHB (1985) Suspected Tyzzer's disease in two foals. Journal of the South African Veterinary Association 56:107-108
2. Fourie N, Van der Lugt JJ, Newsholme SJ, Nel PW (1987) Acute *Lantana camara* toxicity in cattle. Journal of the South African Veterinary Association 58:173-178
3. Ehret WJ, Potgieter FT, De Waal DT, Van der Lugt JJ, Van der Vyver FH (1987) Chronic splenitis as a possible complicating factor in calves inoculated with an attenuated redwater vaccine. Journal of the South African Veterinary Association 58:131-134
4. Van der Lugt JJ, Kriek JC (1988) Chlamydiosis in a springbok *(Antidorcas marsupialis)*. Journal of the South African Veterinary Association 59:33-37
5. Van der Walt ML, Van der Lugt JJ (1988) The isolation of *Campylobacter hyointestinalis* from a pig in South Africa. Onderstepoort Journal of Veterinary Research 55:85-87
6. Marasas WFO, Kellerman TS, Gelderblom WCA, Coetzer JAW, Thiel PG, Van der Lugt JJ (1988) Leukoencephalomalacia in a horse induced by fumonisin B_1 isolated from Fusarium monoliforme. Onderstepoort Journal of Veterinary Research 55:197-203
7. Van der Lugt JJ, Prozesky L (1989) The pathology of blindness in new-born calves caused by hypovitaminosis A. Onderstepoort Journal of Veterinary Research 56:99-109
8. Trichard CJV, Basson PA, Van der Lugt JJ, Jacobsz EP (1989) An outbreak of contagious bovine pleuropneumonia in the Owambo Mangetti area of South West Africa/Namibia: microbiological, immunofluorescent, pathological and serological findings. Onderstepoort Journal of Veterinary Research 56:277-284
9. Hunter P, Van der Lugt JJ, Gouws JJ (1990) Failure of an Actinomyces pyogenes vaccine to protect sheep against an intravenous challenge. Onderstepoort Journal of Veterinary Research 57:239-241
10. Van der Lugt JJ, Van der Merwe HE (1990) Amoebic meningoencephalitis in a sheep. Journal of the South African Veterinary Association 61:33-36
11. Van der Lugt JJ, Nel PW, Kitching JP (1991) The pathology of *Cestrum laevigatum* (Schlechtd.) poisoning in cattle. Onderstepoort Journal of Veterinary Research 58:211-221
12. Gerdes GH, Van der Lugt JJ (1991) Electron microscopic evidence of a papillomavirus and a parapoxvirus in the same lesion. The Veterinary Record 128:594-595
13. Van Rensburg IBJ, Petrick WP, Van der Lugt JJ, Smit MME (1992) Multiple inhereted eye anomalies including persistent hyperplastic tunica vasculosa lentis in Bouvier des Flandres. Progress in Veterinary and Comparative Ophthalmology 2:133-139
14. Van der Lugt JJ, Nel PW, Kitching JP (1992) Experimentally-induced *Cestrum laevigatum* (Schlechtd.) poisoning in sheep. Onderstepoort Journal of Veterinary Research 59:135-144
15. Van der Lugt JJ, Carlyon JF, De Waal DT (1992) Cutaneous leishmaniasis in a sheep. Journal of the South African Veterinary Association 63:74-77
16. Van der Lugt JJ, Schultz RA, Fourie N, Hon LJ, Jordaan P, Labuschagne L (1992) *Galenia africana* L. poisoning in sheep and goats: hepatic and cardiac changes. Onderstepoort Journal of Veterinary Research 59:323-333

17. Venter EH, Van der Lugt JJ, Gerdes GH (1992) Detection of bluetongue virus RNA in cell cultures and in the central nervous system of experimentally infected mice using in situ hybridization. Onderstepoort Journal of Veterinary Research 60:39-45
18. Van Heerden J, Van der Lugt JJ, Durante E (1993) Experimental vermeersiekte (*Geigeria ornativa* O. Hoffm. poisoning) in sheep. I: An evaluation of diagnostic aids and an assessment of the preventive effect of ethoxyquin. Journal of the South African Veterinary Association 64:76-81
19. Van der Lugt JJ, Van Heerden J (1993) Experimental vermeersiekte (*Geigeria ornativa* O. Hoffm. poisoning) in sheep. II: Histological and ultrastructural lesions. Journal of the South African Veterinary Association 64:82-88
20. Van der Lugt JJ, Jordaan P (1994) Facial paralysis associated with space occupying lesions of cranial nerves in calves. The Veterinary Record 134:579-580
21. Van der Lugt JJ, Randles JL (1993) Systemic herpesvirus infection in neonatal goats. Journal of the South African Veterinary Association 64:169-171
22. Myburgh JG, Fourie N, Van der Lugt JJ, Kellerman TS, Cornelius ST, Ward C (1994) A nervous disorder in cattle caused by the plants *Ficus ingens* var. *ingens* and *Ficus cordata* subsp. *salicifolia*. Onderstepoort Journal of Veterinary Research 61:171-176
23. Staley GP, Van der Lugt JJ, Axsel G (1994) Congenital skeletal malformations in Holstein calves associated with putative manganese deficiency. Journal of the South African Veterinary Association 65:73-78
24. Van der Lugt JJ, Markus MB, Kitching JP, Daly TJM (1994) Necrotic encephalitis as a manifestation of acute sarcocystosis in cattle. Journal of the South African Veterinary Association 65:119-121
25. Last RD, Jardine JE, Smit MME, Van der Lugt JJ (1994) Application of immunoperoxidase techniques to formalin-fixed brain tissue for the diagnosis of rabies in southern Africa. Onderstepoort Journal of Veterinary Research 61:183-187
26. Penrith ML, Van der Lugt JJ, Henton HH, Botha JA, Stroebel C (1994) Mycotic nasal granuloma in three cattle. Journal of the South African Veterinary Association 65:179-183
27. Van der Lugt JJ, Kellerman TS, Van Vollenhoven A, Nel PW (1994) Spinal cord degeneration in adult dairy cows associated with the feeding of sorghum beer residues. Journal of the South African Veterinary Association 65:184-188
28. Van der Lugt JJ (1994) Tyzzer's disease. In: Coetzer JAW, Thomson GR, Tustin RC (ed). Infectious diseases of livestock with special reference to Southern Africa, Cape Town: Oxford University Press Southern Africa, Vol 2:1514-1517
29. Van der Lugt JJ (1994) Leishmaniasis. In: Coetzer JAW, Thomson GR, Tustin RC (ed). Infectious diseases of livestock with special reference to Southern Africa, Cape Town: Oxford University Press Southern Africa, Vol 1:269-272
30. Van der Lugt JJ (1994) Amoebic infections. In: Coetzer JAW, Thomson GR, Tustin RC (ed). Infectious diseases of livestock with special reference to Southern Africa, Cape Town: Oxford University Press Southern Africa, Vol 1:220-221
31. Barnard BJH, Van der Lugt JJ, Mushi WZ (1994) Malignant catarrhal fever (snotsiekte). In: Coetzer JAW, Thomson GR, Tustin RC (ed). Infectious diseases of livestock with special reference to Southern Africa, Cape Town: Oxford University Press Southern Africa, Vol2:946-957
32. Barrowman P, Stoltsz WH, Van der Lugt JJ, Williamson CC (1994) Dourine. In: Coetzer JAW, Thomson GR, Tustin RC (ed). Infectious diseases of livestock with

special reference to Southern Africa, Cape Town: Oxford University Press Southern Africa, Vol 1:206-212
33. Harkness JW, Van der Lugt JJ (1994) Bovine viral diarrhoea and mucosal disease. In: Coetzer JAW, Thomson GR, Tustin RC (ed). Infectious diseases of livestock with special reference to Southern Africa, Cape Town: Oxford University Press Southern Africa, Vol 1:642-650
34. Markus MB, Van der Lugt JJ (1994) Sarcocystosis. In: Coetzer JAW, Thomson GR, Tustin RC (ed). Infectious diseases of livestock with special reference to Southern Africa, Cape Town: Oxford University Press Southern Africa, Vol 1, 253-265
35. Schneider HP, Van der Lugt JJ, Hubschle OJB (1994) Contagious bovine pleuropneumonia. In: Coetzer JAW, Thomson GR, Tustin RC (ed). Infectious diseases of livestock with special reference to Southern Africa, Cape Town: Oxford University Press Southern Africa, Vol 2:1485-1494
36. Van der Lugt JJ, Henton MM (1995) Melioidosis in a goat. Journal of the South African Veterinary Association 66:71-73
37. Michel A, Buchholz GS, Van der Lugt JJ (1995) Monitoring experimental Alcelaphine Herpesvirus - 1 infection in cattle by nucleic-acid hybridization and PCR. Onderstepoort Journal of Veterinary Research 62:109-115
38. Van der Lugt JJ, Coetzer JAW, Smit MME, Cilliers C (1995) The diagnosis of Wesselsbron disease in a new-born lamb by immunohistochemical staining. Onderstepoort Journal of Veterinary Research 62: 143-146
39. Van der Lugt JJ, De Wet SC, Bastianello SS, Kellerman TS, Van Jaarsveld LP (1995) Type C and D botulism in sheep and goats in South Africa. Journal of the South African Veterinary Association 66:77-82
40. Jardine JE, Vogel SW, Van Kleef M, Van der Lugt JJ (1995) Immunohistochemical identification of *Cowdria ruminantium* in formalin-fixed tissue sections from mice, sheep, cattle and goats. Onderstepoort Journal of Veterinary Research 62:277-280
41. Venter IJ, Van der Lugt JJ, Van Rensburg IBJ, Petrick SW (1996) Multiple congenital eye anomalies in Bloodhound puppies. Progress in Veterinary and Comparative Ophthalmology 6:9-13
42. Van der Lugt JJ, Olivier J, Jordaan P (1996) Status spongiosis, optic neuropathy and retinal degeneration in *Helichrysum argyrosphaerum* poisoning in sheep and a goat. Veterinary Pathology 33:495-502
43. Van der Lugt JJ, Coetzer JAW, Smit MME (1996) Distribution of viral antigen in tissues of new-born lambs infected with Rift Valley fever virus. Onderstepoort Journal of Veterinary Research 63:341-347
44. Bain O, Van der Lugt JJ, Kazadi LM (1996) *Stephanofilaria boomkeri* N. sp., as a cause of severe skin disease in pigs in Zaire. Parasite 4:377-381
45. Botha CJ, Van der Lugt JJ, Erasmus GL, Kellerman TS, Schultz R Anitra, Vleggaar R (1997) Krimpsiekte, associated with thalamic lesions, induced by the neurotoxic cardiac glycoside, cotyledoside, isolated from *Tylecodon wallichii* (Harv.) Toelken subsp. *wallichii*. Onderstepoort Journal of Veterinary Research 64:189-194. This work was also presented at the 5th International Symposium on Poisonous Plants (ISOPP5), San Angelo, Texas, May 1997 and published in the proceedings of this conference: Toxic Plants and Other Natural Toxicants, Garland T, Barr AC (eds), CAB International, Oxon, United Kingdom, 1998, 407-412

46. Michel Anita L, Van der Lugt JJ, Bengis RG, De Vos V (1997) Detection of AHV-1 DNA in lung sections from blue wildebeest (*Connochaetes taurinus*) calves by in-situ-hybridization. Onderstepoort Journal of Veterinary Research 64:235-238
47. Paweska JT, Henton MM, Van der Lugt JJ (1997) Experimental exposure of pregnant mares to the asinine-94 strain of equine arteritis virus. Journal of the South African Veterinary Association 68:49-52
48. Hunter P, Swanepoel SP, Esterhuysen JJ, Raath JP, Bengis RG, Van der Lugt JJ (1997) The efficacy of an experimental oil-adjuvanted encephalomyocarditis vaccine in elephants, mice and pigs. Vaccine 16:55-61
49. De Balogh Katinka KIM, Dimande AP, Van der Lugt JJ, Molyneux RJ, Naudé TW, Welman WG (1999) A lysosomal storage disease induced by *Ipomoea carnea* in goats in Mozambique. Journal of Veterinary Diagnostic Investigation 11:266-273. This work was also presented at the 5th International Symposium on Poisonous Plants (ISOPP5), San Angelo, Texas, May 1997 and published in the proceedings of this conference: Toxic Plants and Other Natural Toxicants, T Garland & AC Barr (editors), CAB International, Oxon, United Kingdom, 1998, 428-434
50. Botha CJ, Schultz RA, Van der Lugt JJ, Retief E, Labuschagne L (1999) Neurotoxicity in calves induced by the plant, *Neurembergia hippomanica* Miers var. *violacea* Millán in South Africa. Onderstepoort Journal of Veterinary Research 66:237-244
51. Van Vuuren M, Steinel A, Goosen T, Lane E, Van der Lugt JJ, Pearson J, Truyen U (2000) Feline panleukopeania virus revisited: molecular characteristics and pathological lesions associated with three recent isolates. Journal of the South African Veterinary Association 71:140-143
52. Möhr AJ, Lobetti RG, Van der Lugt JJ (2000) Acute pancreatitis: a newly recognised potential complication of canine babesiosis. Journal of the South African Veterinary Association 71:232-239
53. Botha CJ, Schultz RA, Van der Lugt JJ, Archerd C (2000) A krimpsiekte-like syndrome in small stock poisoned by *Ornithogalum toxicarium* Archer & Archer. Journal of the South African Veterinary Association 71:6-9
54. Dvir E, Van der Lugt JJ (2001) Chronic idiopathic intestinal pseudo-obstruction in an English bulldog. Journal of Small Animal Practice 42: 243-247
55. Booth MJ, Van der Lugt JJ, Van Heerden A, Picard JA (2001) Temporary remission of disseminated paecilomycosis in a German shephard dog treated with ketoconazole. Journal of the South African Veterinary Association 72: 99-104
56. Thompson PN, Van der Lugt JJ, Olivier A (2001) Botryomycosis associated with *Pseudomonas aeruginosa* in the nasopharynx of a cow. The Veterinary Record 149:495-496
57. Möhr AJ, Van der Lugt JJ, Josling D, Picard J, Van der Merwe LL (2002) Primary bacterial enteritis caused by *Providencia alcalifaciens* in 3 dogs. The Veterinary Record 150:52-53

Curriculum vitae

Jaco van der Lugt werd geboren op 20 februari 1958 te Gouda en emigreerde in 1969 naar Zuid-Afrika. De middelbare schoolopleiding volgde hij aan ‘Die Hoërskool DF Malan’ in Bellville, Kaapstad waar hij in 1975 het einddiploma (‘matriek’) haalde. Na twee jaar militaire dienstplicht begon in 1978 de studie diergeneeskunde aan de Faculteit Veeartsenijkunde, Universiteit van Pretoria, Onderstepoort, Zuid-Afrika. De graad BVSc werd in 1983 behaald. Van september 1983 tot december 1995 was hij werkzaam als diagnostische patoloog en onderzoeker binnen de afdeling Pathologie aan het ‘Onderstepoort Veeartseny Instituut’, Pretoria. De graad BVSc (Hons) werd in 1985 behaald en de specialisatie in pathologie (MMedVet(Path)) werd voltooid in 1989. In 1990 volgde hij een voltijdse studie van een jaar in immunologie aan ‘The University of Cape Town’ en werd de graad BSc Med (Hons) behaald. Op 1 januari 1996 werd hij aangesteld als mede-professor in het Departement Pathologie aan de Faculteit Veeartsenijkunde, Universiteit van Pretoria, Onderstepoort en werd op 1 juni 1999 hoofd van hetzelfde departement. Op 1 september 1999 sloot hij aan bij een specialisatiepraktijk in Pretoria onder de naam BothaVanderLugt Veterinêre Patoloë. In juli 2001 emigreerde hij met zijn gezin naar Nederland en is hij sinds 1 augustus 2001 werkzaam als docent/onderzoeker/dierenarts by de hoofdafdeling Pathologie van de Faculteit Diergeneeskunde, Universiteit van Utrecht.