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Short Communication

Cerebellar cortical degeneration in cattle caused by *Solanum kwebense*Jaco J. van der Lugt^{a,*}, Stella S. Bastianello^{b,1}, Anne Marie van Ederen^c, Erna van Wilpe^d^a Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X05, 0110 Onderstepoort, South Africa^b Section of Pathology, Onderstepoort Veterinary Institute, Onderstepoort, South Africa^c Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands^d Electron Microscopy Unit, Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, South Africa

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ABSTRACT

The pathology of maldronksiekte, a sporadic neurological disorder of cattle caused by the ingestion of the plant *Solanum kwebense* in certain parts of South Africa, was studied in three chronic field cases. There was loss of cerebellar Purkinje cells with the remaining neurons either swollen or shrunken and showing degeneration and necrosis. Ultrastructurally, neurons with a swollen perikaryon showed depletion and empty dilated cisternae of granular endoplasmic reticulum. In a few Purkinje cells, the cytoplasm contained small numbers of lamellar and membranous bodies. In the shrunken neurons, the highly condensed cytoplasm contained distended Golgi saccules, dense clusters of granular endoplasmic reticulum and swollen mitochondria. Lectin histochemistry revealed that the cytoplasmic vacuoles in some distended Purkinje cells stained strongly with *Canavalia ensiformis* (ConA) agglutinin and weakly with *Triticum vulgare* (WGA) and succinyl-WGA (S-WGA) agglutinin. The pattern of lectin binding only partially agreed with that reported in calves poisoned with *Solanum fastigiatum*, causing a presumed glycolipid storage disease. Apoptosis was not detected in neurons using a commercial deoxyuridine triphosphate nick-end labelling (TUNEL) method. The pathogenesis of the cerebellar lesions is unknown but the intoxication may have resulted from the inability of neurons, in particular Purkinje cells, to metabolise a plant toxin or cellular substrate.

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Ingestion of the plant *Solanum kwebense* N.E. Br. (Solanaceae) may induce a neurological condition in cattle known in South Africa as maldronksiekte (which means, literally, mad-drunk-disease) (Pienaar et al., 1976). The condition has only been reported sporadically in the parts of the Northern Province where overgrazing may cause replacement of the palatable *Panicum maximum* grass by *S. kwebense* (Kellerman et al., 1988; Fig. 1).

The occurrence of natural cases of maldronksiekte provided an opportunity to study the pathology in more detail. We selected three cattle, ranging in age from 15 to 36 months, which had been on natural grazing and had been showing clinical signs for 3–4 months or more. We report here for the first time the ultrastructural changes in cerebellar Purkinje cells. In addition, lectin histochemistry was applied to sections of the cerebellum and evidence for DNA fragmentation in neurons in the cerebellum was sought using a modified terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick-end labelling (TUNEL) method.

Specimens of cerebellum of the three animals were collected within 10–15 min of euthanasia for transmission electron microscopy. Sections of cerebellum from the affected cattle and a normal 20-month-old bovine were used for lectin histochemical examination. Slides were incubated overnight with each of nine biotinylated lectins (Vector Laboratories) 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 vulgare* [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. Incubation of each lectin with its corresponding sugar served as a control for binding specificity. Incubation of tissue sections with phosphate buffered saline (PBS) alone served as non-specific negative controls.

To detect apoptotic cells in the cerebellum of the affected cattle, a TUNEL method (NeuroTACS II In situ Apoptosis Detection Kit, R&D Systems) was used according to the manufacturer's instructions. Nuclease-generated slides of cerebellum were used as a positive control. As a negative control, TdT was excluded in the protocol.

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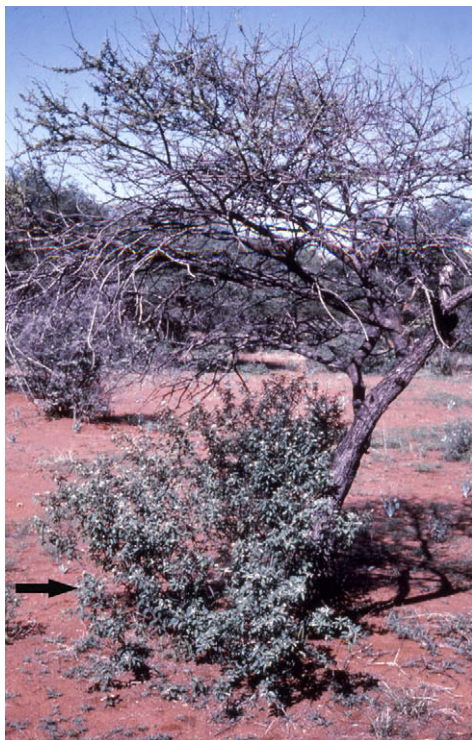


Fig. 1. *Solanum kwebense* (arrow) grows up to 2 m high often in the shade of *Acacia erubescens* (geelhaak) trees.

Gross inspection of the brains revealed a reduced size of the cerebellum in two animals. Histological lesions in the cerebellum in the three animals corresponded to the chronic stage of maldronksiekte (Pienaar et al., 1976; Kellerman et al., 1988). Briefly, we found extensive loss of Purkinje neurons with the Purkinje cell layer absent in many folia. A small number of Purkinje cells were swollen and pale and exhibited fine vacuolation of the perikaryon while a few contained large, irregular vacuoles (Fig. 2) which were PAS-negative. Most of the remaining Purkinje cells were shrunken, deeply eosinophilic and occasionally showed mild cytoplasmic vacuolation. Nuclei in both swollen and shrunken cells were often eccentric and pyknotic or fragmented.

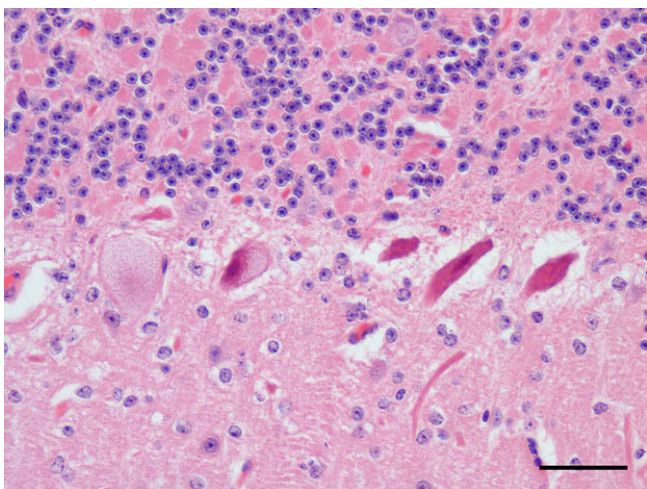


Fig. 2. Cerebellum: Bovine (1). Purkinje cells reveal a fine vacuolar appearance of the perikaryon and nuclear margination (at left) while other cells are shrunken and dark staining (at right). HE. Bar = 50 μ m.

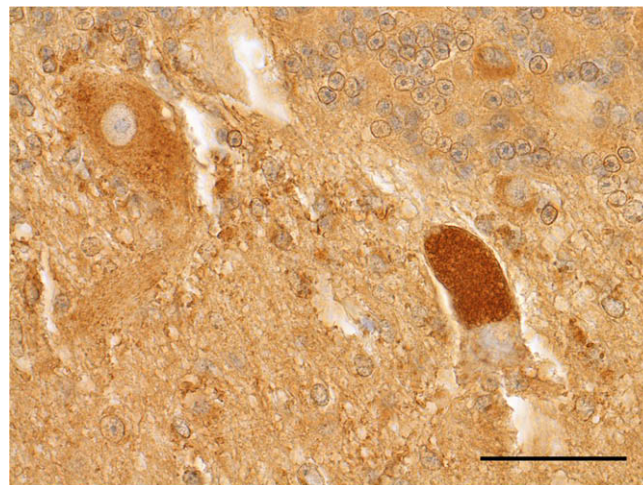


Fig. 3. Cerebellum: Bovine (2). Positive staining of the cytoplasm of two Purkinje neurons after incubation with ConA and counterstaining with Mayer's haematoxylin. Bar = 50 μ m.

The cytoplasm of several swollen and a few shrunken Purkinje neurons, cells in the granular layer (including Golgi cells) and neurons in cerebellar nuclei stained strongly with ConA (Fig. 3) and weakly with WGA and S-WGA. Positive staining with these lectins was also seen multifocally in astrocytes and in macrophages in the white matter. There was no staining with the other lectins. Only an occasional cell in the granular cell layer of the cerebellum of two of the affected animals and the control bovine showed positive, nuclear staining with the TUNEL method.

Electron microscopy revealed that Purkinje neurons with a distended perikaryon had depletion of the granular endoplasmic reticulum especially in the perinuclear cytoplasm. These areas often appeared vacuolated and contained irregular, empty dilated cisternae of endoplasmic reticulum, small numbers of membranous bodies, swollen mitochondria with partial loss of cristae (Fig. 4) and, in few neurons, lamellar bodies. In Purkinje cells that were shrunken, increased density of the cytoplasm, clusters of granular endoplasmic reticulum, aggregates of distended Golgi saccules, and few membranous bodies were seen (Fig. 5). Mitochondria in several affected neurons were swollen and frequently contained membranous vesicles. No storage material was detected.

Maldronksiekte is clinically and pathologically similar to a syndrome found in cattle following ingestion of *Solanum fastigiatum*

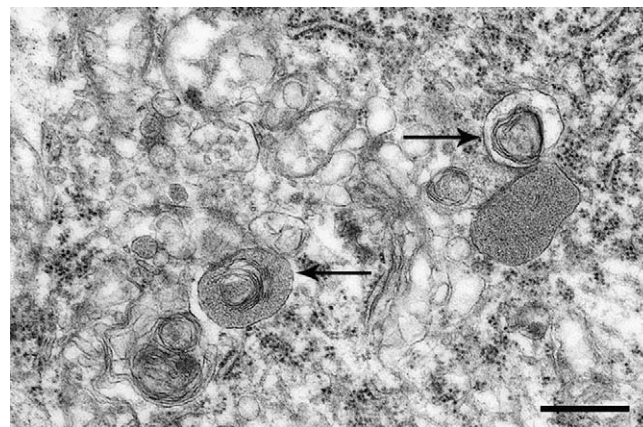


Fig. 4. Transmission electron micrograph. Cerebellum: Bovine (2). The Purkinje neuron contains several membranous bodies within a vacuole or within a lysosomal configuration (arrows). Bar = 1 μ m.

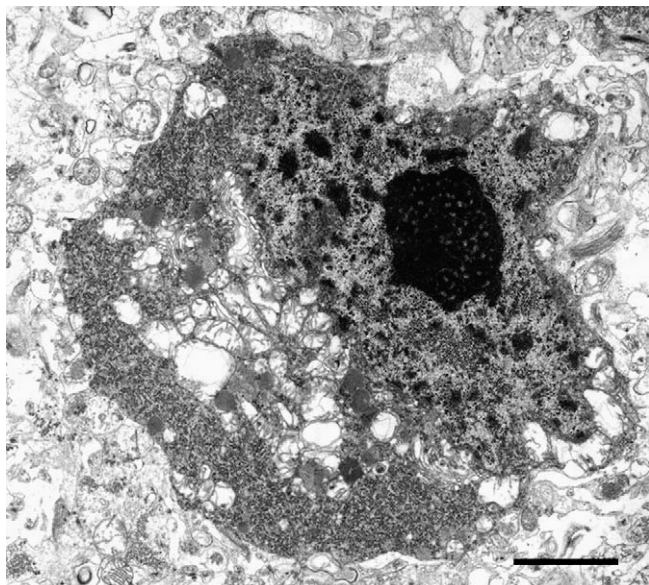


Fig. 5. Transmission electron micrograph. Cerebellum: Bovine (3). Shrunken Purkinje cell; the cytoplasm is markedly increased in density and contains predominantly clusters of granular endoplasmic reticulum at the periphery of the cell body, distended Golgi saccules and swollen mitochondria. Bar = 5 μ m.

var. *fastigiatum* (Riet-Correa et al., 1983; De Barros et al., 1987), *S. bonariensis* (Verdes et al., 2006) and *S. dimidiatum* (Menzies et al., 1979), respectively. Different bodies (lamellar, membranous, vesiculo-membranous and dense) in degenerated Purkinje cells, axons and dendrites have been demonstrated in cattle poisoned by *S. fastigiatum* (Riet-Correa et al., 1983; De Barros et al., 1987). Lamellar bodies are regarded as non-specific (Bestetti and Rossi, 1980) while the presence of membranous bodies gave rise to the hypothesis that *S. fastigiatum* is an induced lysosomal storage disease, probably a gangliosidosis (Riet-Correa et al., 1983). In a more recent publication, it was speculated that the active principle of *S. fastigiatum* forms a complex with lipid material that cannot readily be metabolised by Purkinje cells (De Barros et al., 1987).

In our study, membranous bodies, which may represent storage material, most likely accounted for the positive staining of Purkinje cells with ConA, WGA and S-WGA. 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 (Damjanov, 1987). Stored material with *S. fastigiatum* intoxication reacted strongly with ConA, sWGA, WGA and RCA-I and based on this lectin-binding pattern it was proposed that this intoxicosis represents a glycolipid storage disease (Paulovich et al., 2002). The pattern of lectin staining in maldronksiekte partially agrees with those observed in intoxication with *Sida carpinifolia* (Driemeier et al., 2000), locoweed (*Astragalus lentiginosus*) and swainsonine (*Swainsona galegifolia*) (Alroy et al., 1985). 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. The selective vulnerability of Purkinje cells in maldronksiekte however, is highly unusual for a lysosomal storage disease in which different populations of neurons in the brain are generally affected (Summers et al., 1995). In some affected Purkinje cells in cattle with Maldronksiekte, degenerative changes resembled those reported in apoptosis. DNA fragmentation indicating apoptosis was not demonstrated by a modified TUNEL method.

Leaves and stems of *S. kwebense* were subsequently collected from the source farms and low levels of calystegines were detected in one sample. There were no detectable levels of swainsonine or other known glycosidase inhibitory alkaloids in the plant material. Calystegine B₂ has previously been detected in *S. kwebense* from southern Africa (Nash et al., 1993), in *S. dimidiatum* (Nash et al., 1993; Molyneux et al., 1994), and in *Ipomea carnea* and other *Ipomea* spp. that cause poisoning in livestock (Biastoff and Dräger, 2007). The toxicity of isolated calystegines have however, not been proven. The toxic affects of *Ipomea* plants are most probably attributed to swainsonine rather than the isolated calystegines (Biastoff and Dräger, 2007; Stegelmeier et al., 2008).

In conclusion, maldronksiekte is a plant-induced cerebellar cortical degeneration with selective involvement of Purkinje neurons. The presence of carbohydrate moieties in affected Purkinje cells suggests that there may be an inability of cerebellar neurons to metabolise a plant toxin or cellular substrate.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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