Short Communications

Use of lectin histochemistry to diagnose *Sida carpinifolia* (Malvaceae) poisoning in sheep

A. L. Seitz, E. M. Colodel, M. Schmitz, E. J. Gimenó, D. Driemeier

The plants of the genera Astragalus and Oxytropis in the USA (Molyneux and James 1982, James and Nielsen 1990), *Swainsona* in Australia (Colegate and others 1979) and *Ipomoea* in Mozambique (Balogh and others 1999) and in Brazil (Tokarnia and others 2000) have been reported to produce lysosomal storage disease when consumed by livestock. Such plants have been reported to affect cattle, sheep, goats and horses (Misra and Misra 1965, Laws and Anson 1968, Colegate and others 1979, James and others 1981, James and Panter 1989, Kirkpatrick and Burrows 1990, Balogh and others 1999). The disease is caused by the indolizidine alkaloid swainsonine, which is an inhibitor of the lysosomal enzyme α-mannosidase. The condition induces the storage of mannose-containing oligosaccharides in the lysosomes of several types of cell, especially neurons, hepatocytes and acinar and pancreatic cells (Dorling and others 1980, Agamanolis 1995, Stegelmeier and others 1995, Jolly and Walkley 1997).

A similar syndrome linked to the ingestion of *Sida carpinifolia* has already been described in goats (Driemeier and others 2000) and horses (Loretti and others 2003) in southern Brazil. *S. carpinifolia*, also known as *Sida acuta var. carpinifolia*, frequently dominates improved pastures; although native to tropical America, it has spread throughout the tropics and subtropics (Lorenzi 2000). A recent study identified the alkaloid swainsonine as the toxic agent present in *S. carpinifolia* (Colodel and others 2002).

Light and electron microscopy are used to observe the presence of lysosomal storage diseases (Glew and others 1983); however, lectin histochemistry performed on paraffin-embedded sections enables the identification of specific sugars, providing additional diagnostic information (Alroy and others 1984). Lectins are carbohydrate-binding proteins of non-immune origin, which agglutinate cells and precipitate sugars (0.1 to 0.2 per cent in PBS) for one hour at room temperature, when bound to visualising agents, they allow localisation of complementary carbohydrates (Damjanov 1987).

This short communication reports an occurrence of *S. carpinifolia* poisoning in sheep and describes the microscopic and histochemical findings associated with the lysosomal storage disease.

The cases were observed in April 2001 in a flock of 73 Texel sheep. A neurological disease characterised by ataxia, hypermetria, general incoordination and muscular tremors, especially of the head and neck, was observed in six animals. Large amounts of *S. carpinifolia* were present in the paddock where the sheep had been kept. As there was no reversal of the clinical signs after the animals had been moved away from the contaminated pasture, and their condition continued to deteriorate, they were euthanased (Beaver and others 2001, Booth and McDonald 1992), according to local regulations (CFMV 2002).

At postmortem examination, the only change noted was enlargement of the lymph nodes. Tissue samples from several organs, including the central nervous system, were processed by standard histological methods and stained with haematoxylin and eosin. Microscopically, there was distension and vacuolation of Purkinje cells in the cerebellum (Fig 1) and of neurons in the cerebral cortex, thalamus, mesencephalon and spinal cord. Cytoplasmic vacuolation was also present in the epithelium of the pancreatic acinus and renal tubules, follicular epithelium of the thyroid, in hepatocytes, and in macrophages of the lymphoid tissues. Axonal spheroids were observed in the brain and spinal cord, particularly in the granular layer of the cerebellum.

Lectin histochemistry was conducted on formalin-fixed, paraffin-embedded sections of the cerebellum and pancreas. The lectins used were obtained commercially (E-Y Labs) and are listed in Table 1. After deparaffinisation, the sections were incubated in 0.3 per cent hydrogen peroxide (H$_2$O$_2$) in methanol for 30 minutes at room temperature, rinsed several times in 0.01M phosphate-buffered saline (PBS), pH 7.2, and treated with 0.1 per cent bovine serum albumin in PBS for 15 minutes. Subsequently, they were incubated with biotinylated lectins for one hour, followed by incubation with avidin-biotin-peroxidase complex (Vector Labs) for 45 minutes. The peroxidase was marked by incubation for four to 10 minutes with buffered Tris-HCl, 0.05M, pH 7.6 solution containing 0.02 per cent diaminobenzidine and 0.05 per cent H$_2$O$_2$. All the sections were counterstained with Mayer’s haematoxylin. Each lectin was used at a dilution of 30 µg/ml in PBS, except for *Arachis hypogaea* (Table 1), which was applied at 10 µg/ml. As controls for the lectin histochemical procedure, the lectins were omitted or blocked by incubating them with their blocking controls for the lectin-binding patterns of affected and control sheep are summarised in Table 2.

![FIG 1: Cerebellum of a sheep affected by *Sida carpinifolia* poisoning, showing vacuolation of the Purkinje cells. Haematoxylin and eosin. Bar=25 µm](image)

**TABLE 1: Lectins used in the histochemical studies of a lysosomal storage disease associated with *Sida carpinifolia* poisoning in sheep**

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Acronym</th>
<th>Carbohydrate specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concanavalina ensiformis</td>
<td>Con A</td>
<td>α-D-Man, α-D-Glc</td>
</tr>
<tr>
<td>Glycine max</td>
<td>SBA</td>
<td>α-D-GalNac, β-D-GalNac, α-β-Gal</td>
</tr>
<tr>
<td>Dolichos biflorus</td>
<td>DBA</td>
<td>α-D-GalNac</td>
</tr>
<tr>
<td>Ulex europaeus-1</td>
<td>UEA-1</td>
<td>α-D-Gal</td>
</tr>
<tr>
<td>Trifidium vulgaris</td>
<td>WGA</td>
<td>β-D-GalNac, NeuNac</td>
</tr>
<tr>
<td>Sucecoy-WGA</td>
<td>SWEA</td>
<td>β-(1-3)-D-GalNac, α-(1-3)-D-GalNac</td>
</tr>
<tr>
<td>Ricinus communis-1</td>
<td>PNA</td>
<td>β-D-Fuc, β-D-Fuc(1-3)GalNac</td>
</tr>
<tr>
<td>Bandeiraea simplicifolia</td>
<td>RCA-1</td>
<td>β-D-Gal &gt; α-D-Gal</td>
</tr>
<tr>
<td><strong>Fuc</strong> Fucose, Gal Galactose, GalNac N-acetyl-galactosamine, Glc Glucose, GlcNac N-acetyl-glucosamine, Man Mannose, NeuNac N-acetyl-neuramic acid**</td>
<td>BS-1</td>
<td>α-D-Gal</td>
</tr>
</tbody>
</table>

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A. L. Seitz, DVM, MSc, M. Schmitz, DVM, D. Driemeier, DVM, PhD, Department of Veterinary Pathology, Federal University of Rio Grande do Sul – UFRGS, Porto Alegre, Brazil

E. M. Colodel, DVM, MSc, Department of Veterinary Clinical Medicine (UFMT), Federal University of Mato Grosso – UFMT, Mato Grosso, Brazil

E. J. Gimenó, DVM, MSc, PhD, Institute of Pathology, Faculty of Veterinary Sciences, National University of La Plata, Argentina

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TABLE 2: Intensity of lectin binding in tissues from *Sida carpinifolia*-poisoned and normal sheep. Lectin binding sites were found on affected cells in the cerebellum and pancreas

<table>
<thead>
<tr>
<th>Lectin</th>
<th>SWGA</th>
<th>WGA</th>
<th>UEA-1</th>
<th>PNA</th>
<th>RCA-1</th>
<th>SBA</th>
<th>DBA</th>
<th>Con A</th>
<th>BS-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purkinje cells</td>
<td>3 (0)*</td>
<td>2 (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Granular layer neurons</td>
<td>3 (0)</td>
<td>0 (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Deep cerebellar nuclei</td>
<td>3 (0)</td>
<td>2 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2-3 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Glial cells</td>
<td>3 (0)</td>
<td>3 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (0)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3 (0)</td>
<td>2 (2)</td>
<td>0-2 (0-2)</td>
<td>0-2 (0-2)</td>
<td>1-2 (2)</td>
<td>0-1 (0-1)</td>
<td>0-2 (0)</td>
<td>3 (0-1)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
* Numbers indicate the staining intensity on a subjective estimated scale from 0 Non-reactive to 3 Most reactive. Control results from normal sheep are presented in parentheses.

Sida carpinifolia- and Triticum vulgaris- poisoned goats; the condition in horses has been principally characterised by a digestive disorder (Loretti and others 2003). The consumption by sheep or other livestock of *S carpinifolia* induces the lysosomal storage disease characterised as α-mannosidosis by the method of lectin histochemistry.

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References


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