Spontaneous poisoning of goats by the plant *Ipomoea sericophylla* (Convolvulaceae) in Brazil – a case report

Fábio de S. Mendonça[1], Joaquim Evêncio-Neto[1], Raquel F. de Albuquerque[1], David Driemeir[2], Lázaro M. Camargo[3], Renata G. S. Dória[3], Fabiana M. Boabaid[4], Flávio Henrique B. Caldeira[4], Edson M. Colodel[4]

[1]Department of Animal Morphology and Physiology, Federal Rural University of Pernambuco, Brazil
[2]Department of Veterinary Clinical Pathology, Faculty of Veterinary Medicine, Federal University of Rio Grande do Sul, Brazil
[3]Faculty of Agrarian Science, University of Cuiabá, Mato Grosso, Brazil
[4]Department of Veterinary Medical Clinic, Faculty of Veterinary Medicine, Federal University of Mato Grosso, Brazil

Received March 6, 2010
Accepted September 21, 2010

Abstract

The aim of this study was to report a neurological disease in goats caused by the plant *I. sericophylla*. The epidemiology, clinical signs, histological findings and the results of the lectin histochemistry analysis of a nervous cells and epithelial cells are also reported. Five goats that remained with neurological signs were examined in more detail. Two goats were necropsied. Histological lesions consisted of neurons with thin cytoplasm vacuolation, presence of axonal spheroids and vacuolation in pancreatic acinar cells, thyroid folliculars cells, hepatocytes and renal tubular cells. On lectin-histochemical analysis, cerebellar cells, pancreatic acinar cells and follicular thyroid cells showed positive staining for *Concanavalia ensiformis*, *Triticum vulgaris*, succinylated *Triticum vulgaris* and *Lens culinare*s, which indicate the storage of α-D-mannose, α-D-glucose, β- D-N-acetyl-glucosamine, and acetyl-neuraminic acid. It is concluded that *I. sericophylla* is an important toxic plant that causes lysosomal storage disease in goats at semi-arid region of Pernambuco, Brazil.

Plant poisoning, lysosomal storage disease, ruminant, histochemical identification of lectins, swainsonine

Ipomoea sericophylla Meisn., prostrate herbaceous plant of Convolvulaceae family (Austin and Huáman 1996), is a toxic plant of agropecuary interest mainly in the State of Paraíba, Brazil, where it is pointed out as toxic for goats (Barbosa et al. 2006). It belongs among plants such as *Ipomoea riedelii* (Barbosa et al. 2006), *I. carnea* subsp. *fistulosa* (Ármien et al. 2007), *Turbina cordata* (Dantas et al. 2006), *Sida carpinifolia* (Driemeier et al. 2000), and *Phalaris angusta* (Gava et al. 1999), causing lysosomal storage disease.

Acquired lysosomal storage disease in herbivores is usually induced by the ingestion of plants containing alkaloids, which inhibit lysosomal hydrolases (Agamanolis 1995). Swainsonine is the main alkaloid that inhibits lysosomal α-mannosidase and Golgi mannosidase II. The inhibition of lysosomal α-mannosidase leads to the intralysosomal cumulation of incompletely processed oligosaccharides, resulting in a phenocopy of inherited α-annosidosis. The resulting lysosomal dysfunction is often manifested as neurological disease (Agamanolis 1995).

Alpha-mannosidosis is the most important acquired lysosomal storage disease affecting goats in Brazil. Alpha-mannosidosis also affects other species like sheep, cattle and horses (Loretti et al. 2003). In this type of disease, the lectin histochemistry is particularly useful in identifying the way of stored material in glycoproteinoses because these proteins specifically react with carbohydrate structures and allow the specific identification of sugars *in situ* (Driemeier et al. 2000).

Address for correspondence:
Fábio de Souza Mendonça DVM PhD
Dept. Animal Morphology and Physiology
Federal Rural University of Pernambuco
Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, Recife
PE, 52171-900 Brazil

Phone: +55 (081) 33206387
Fax: +55 (081) 3320-6050
E-mail: mendonca@dmfa.ufrpe.br
http://www.vfu.cz/acta-vet/actavet.htm

The main clinical signs of lysosomal storage disease in herbivores are nervous alterations such as depression, ataxia, hypermetria, intention tremors, lateral march, spastic paresis or weakness. No significant alterations are found during necropsy. Histologically, swelling and thin vacuolation of the perikaria of neurons in all regions of the central nervous system can be visualized (Agamanolis 1995). Other lesions include formations of axonal spheroids at cerebellum and cytoplasmic vacuolation in epithelial cells and macrophages of liver, spleen and lymph nodes (De Balogh et al. 1999).

There are only few studies about the toxicity of *I. sericophylla* in goats (Barbosa et al. 2006). The aim of this study is to report a neurological disease in goats caused by the plant *I. sericophylla*. The epidemiology, clinical signs, histological findings and the results of the lectin histochemistry analysis of a nervous cells and epithelial cells are also reported.

Materials and Methods

Epidemiological data

During the rainy period between the months of March and June of 2009, 15 goats of a total of 45 animals (mostly crossbreed) at the farm of municipality of Sertânia, semi-arid region of Pernambuco, Brazil, showed neurological clinical signs. During the visit at the farm, five of these animals (four females and one male) still showed signs of poisoning. These goats grazed on pasture with *I. sericophylla*. They were examined and two of them were euthanatized and necropsied.

According to information obtained at this farm and other five neighbouring farms in this region, the disease has been occurring only with sporadic cases for several years. In the last five years, the disease has become more frequent, but a veterinary diagnostic was never performed. During the visit at the grazing field, a wide area was invaded by *I. sericophylla* (Plate III, Fig. 1A). It was also verified that the aerial parts of the plants were intensively consumed by the goats.

In order to gather additional information on the occurrence of lysosomal storage disease and to observe the grazing areas of the goats, five other farms in the region were visited.

Neurological examination

The goats were examined according to the behavior and mental state; posture and head coordination; evaluation of abnormalities in the skull nerves function; evaluation of the walking and the posture and HR test (Head Raising), which consists in extending tail-dorsally the head of the animal and holding in that position for 1 minute, and then suddenly releasing it.

Histological examination

Samples of encephalon, spinal cord, thyroid gland, pancreas, liver, kidney, heart, lungs, lymph nodes, spleen, skeletal muscles, intestine and trigeminal ganglion were collected from necropsied animals. These samples were fixed in 10% formaldehyde solution and processed routinely and stained by haematoxylin and eosin (HE). Samples of cerebellum, pancreas and thyroid gland were treated with 0.3% hydrogen peroxide for 30 min at room temperature. After blocking, the samples were submitted to antigen recovery for 15 min in citrate buffer pH 6.0 in a water bath at 100 °C. The sections were incubated with four lectins: *Concanavalia ensiformis* (Con-A, α -D-Man; α-D-Glc), *Triticum vulgaris* (WGA), succinylated *Triticum vulgaris* (sWGA) and *Lens culinares* (LCA) (Biotinylated lectin Kit I e Kit II, BK1000 e BK2000, Vector Laboratories®, CA, EUA). Afterwards the sections were treated with streptavidin-peroxidase conjugate (Dako®, CA, EUA) for 20 min and subjected to disclosure using the chromogen 3,3-diaminobenzidine (DAB, Dako®) or red chromogen (NovaRed, Vector Laboratories®, CA, EUA). Then the sections were counterstained with Mayer's haematoxylin, dehydrated, imbibed in xylene and examined under a light microscope. The lectins were used at the dilution of 5 µg/ml phosphate-buffered saline (PBS), except *Canavalia ensiformis* which was used at the dilution of 0.5 mg/ml PBS (Brooks et al. 1997).

Results

Clinical signs

A detailed description of clinical signs in examined goats is presented in Table 1. Three of the five goats examined showed more intensive neurological symptoms such as frequent head shaking, incoordination with ataxia and dysmetria mainly of hind limbs, besides spastic paresis, weakness and abnormal position (Plate III, Fig. 1B). It was also observed that they lost their balance and fell backward or sideways when frightened. All the examined goats were positive in "head raising test". Two goats with intensive neurological clinical signs were euthanized and necropsy was performed. The other three goats were kept on a pasture

Table 1. Neurological clinical signs in goats poisoned by plant *Ipomoea sericophylla*

Goat	Age (months)	Sex	Breed	Intensity of clinical signs	Outcome of the poisoning
1	18	Male	Nubian	Intensive	Permanent clinical signs
2	21	Female	Crossbreed	Slight	Recovered in 10 days
3	27	Female	Crossbreed	Intensive	Euthanized
4	32	Female	Nubian	Slight	Recovered in 12 days
5	6	Female	Saanen	Intensive	Euthanized

without *I. sericophylla* for 60 days. Two of these goats showed improvement of clinical signs after 10 days and one showed permanent clinical signs.

Pathology

During necropsy no significant alterations were found. Microscopically, neurons were detected with thin cytoplasmatic vacuolation in several areas of the central nervous system, mainly in the Purkinje neurons (Plate III, Fig. 1C), neurons of cerebellar nucleus, bridge and medulla oblongata. Axonal spheroids were visualized in the cerebellar granular layer, white cerebellar substance, cerebellar peduncles and cerebellar nucleus. Vacuolations were also present in neurons of the cerebral cortex and of the spinal cord. There was vacuolation in pancreatic acinar cells, thyroid follicular cells, hepatocytes and renal tubular epithelial cells.

Lectin histochemistry

On lectin-histochemical analysis, Purkinje cells gave a strong positive reaction to *Concanavalia ensiformis* (Con-A) and *Triticum vulgaris* (WGA) lectins (Plate III, Fig. 1D). This same reaction was moderate for the lectins succinylated *Triticum vulgaris* (sWGA) and *Lens culinares* (LCA). The lectins Con-A and WGA had also stained with intensity ranging from moderate to severe in the cytoplasm of neurons of cerebellar granular and molecular layers. The cytoplasm of pancreatic acinar cells and thyroid follicular cells stained strongly with Con-A. The intensity of lectin reaction in the foamy cells of the cerebellum, pancreas and thyroid is expressed in Table 2.

Table 2. Intensity of lectin reaction tested in vacuolated cells of the cerebellum, pancreas and thyroid gland of goats naturally poisoned by plant *Ipomoea sericophylla*

Organ	Type of cell	Lectins*			
		Con A	WGA	sWGA	LCA
Cerebellum	Purkinje cells	3[a]	3	2	2
	Molecular layer neurons	2	2	1	1
	Granular layer neurons	3	2	1	1
Pancreas	Acinar cells	3	2	1	2
Thyroid	Folicular cells	3	2	1	1

[a] Numbers indicate the intensity of subjective marking related to staining intensity and number of stained cells, considering: 0 = without reaction, 1 = light stain, 2 = moderate stain and 3 = strong stain. *Con A (*Concanavalia ensiformis*); WGA (*Triticum vulgaris*); sWGA (succinylated *Triticum vulgaris*); LCA (*Lens culinares*).

Discussion

In the State of Pernambuco, spontaneous poisoning by *Ipomoea sericophylla* in goats had not yet been reported. The diagnosis of poisoning by this plant was based on epidemiological data, clinical signs and mainly on histopathological examination and lectin-histochemical analysis which corresponded to what is described in the poisonings by others plants that cause lysosomal storage disease in goats (Agamanolis et al. 1995). Moreover, detailed

examination of pasture did not reveal the presence of any etiologic agent related to this disease.

An atypical pluviometric index with trimestrial average of 840 mm between the months of March and June was attributed as the determinant factor for higher infestation of pasturage areas of the goats by *I. sericophylla*. The poisoning of goats by this plant has become more frequent and intense.

There is no treatment for acquired lysosomal storage disease in goats. The affected animals must be removed from pastures invaded by plants containing swainsonine for 30 days from the beginning of clinical signs. In these cases, there is a good chance they may fully recover. In experiments with *I. sericophylla* and *I. riedelli*, goats that continued to ingest these plants for 20-38 days after the initial clinical signs, recovered within 4-14 days after removal of the plant (Barbosa et al. 2006). Goats that continued to ingest these plants for 40 days after the initial clinical signs did not recover (Dantas et al. 2007). Our results also indicate that after 40 days, the neuronal loss becomes permanent.

The positivity on lectin-histochemical analysis of cerebellar cells, pancreatic acinar cells and follicular thyroid cells for *Concanavalia ensiformis* (Con A), *Triticum vulgaris* (WGA), succinylated *Triticum vulgaris* (sWGA) and *Lens culinare*s (LCA) indicates the storage of α-D-mannose, α-D-glucose, β-D-N-acetyl-glucosamine, and acetyl-neuraminic acid. The lectins Con A and LCA have specific affinity to α-D-mannose and of α-D-glucose, while the staining observed by WGA and sWGA indicates the accumulation of β-D-N-acetyl-glucosamine and N-acetylneuraminic acid (Goldstein and Hayes 1978). The nervous signs observed in the goats involved in this study reflect mainly the storage of these oligosaccharides and the impediment of cytoplasmic transport in neurons of cerebellum, brainstem and spinal cord.

These alterations origin from the action of indolizidinic alkaloid 1,2,8-triol, denominated swainsonine which inhibits the enzymes' lysosomal alpha-mannosidase and Golgi alpha-mannosidase II complex of neurons (Agamanolis 1995). The same mechanism occurs in the poisonings by plants of the genera *Oxytropis* (Stegelmeier et al. 1995), *Astragalus* (Harris et al. 1988), *Ipomoea* (Ármien et al. 2007) and *Sida carpinifolia* (Driemeier et al. 2000). The alkaloid swainsonine is produced by endophytic fungi. Endophytes were isolated from the leaves, stems, seeds, and flowers of eight populations of the toxic locoweeds *Astragalus mollissimus*, *Oxytropis lambertii*, and *O. sericea*. All cultured endophytes produced the alkaloid swainsonine, which causes locoism (Braun et al. 2003). Probably, endophytic fungi also produce swainsonine in *Ipomoea* specie's.

It is concluded that *I. sericophylla* is an important toxic plant that causes lysosomal storage disease in goats at semi-arid region of Pernambuco, Brazil.

Acknowledgements

We would like to thank the Brazilian agencies CNPQ and FACEPE.

References

Agamanolis DP 1995: The pathology of lysosomal storage diseases. Pathol Annu **30**: 247-285

Armién AG, Tokarnia CH, Peixoto PV, Frees K 2007: Spontaneous and experimental glycoprotein storage disease of goats induced by *Ipomoea carnea* subsp. *fistulosa* (Convolvulaceae). Vet Pathol **44**: 170-184

Barbosa RC, Riet-Correa F, Medeiros RTM, Lima EF, Barros SS, Gimeno ER, Molyneux RJ, Gardner DR 2006: Intoxication by *Ipomoea sericophylla* and *Ipomoea riedelii* in goats in the state of Paraíba, northeastern Brazil. Toxicon **47**: 371-379

Braun K, Romero J, Liddell C, Creamer R 2003. Production of swainsonine by fungal endophytes of locoweed. Mycol Res **107**: 980-988

Brooks SA, Leathem AJC, Schurmacher U 1997: Lectin histochemistry - a concise practical handbook. Bios Scientific Publishers, Oxford, 177p.

Dantas AFM, Riet-Correa F, Gardner DR, Medeiros RMT, Barros SS, Anjos BL, Lucena RB 2006: Swainsonine induced lysosomal storage disease in goats caused by the ingestion of *Turbina cordata* in northeastern Brazil. Toxicon **49**: 111-116

Driemeier D, Colodel EM, Gimeno EJ, Barros SS 2000: Lysosomal storage disease caused by *Sida carpinifolia* in goats. Vet Pathol **37**: 153-159

Gava A, Sousa RS, De Deus MS, Pilati C, Cristani J, Mori A, Neves DS 1999: *Phalaris angusta* (Gramineae) causing neurological disease in cattle in the State of Santa Catarina, Brazil. Pesq Vet Bras **19**: 35-38

Goldstein IJ, Hayes CE 1978: The lectins: carbohydrate-binding proteins of plants and animals. Adv Carbohydr Chem Biochem **35**: 127-340

Harris CM, Campbell BC, Molyneux RJ, Harris TM 1988: Biosynthesis of swainsonine in the diablo locoweed (*Astragalus oxyphysus*). Tetrahedrom Letters **29**: 4815-4818

Ikeda K, Kato A, Adachi I, Haraguchi M, Asano N 2003: Alkaloids from the poisoning plant *Ipomoea carnea*: effects on intracellular lysosomal glycosidase activities in human lymphoblast culture. J Agric Food Chem **51**: 7642-7646

Loretti AP, Colodel EM, Gimeno EJ, Driemeier D 2003: Lysosomal storage disease in *Sida carpinifolia* toxicosis: an induced mannosidosis in horses. Eq Vet J **35**: 434–438

Stegelmeier BL, James LF, Panter KE, Molyneux RJ 1995: Tissue and serum swainsonine concentrations in sheep ingesting *Astragalus lentiginosus* (locoweed). Vet Hum Toxicol **37**: 336-339

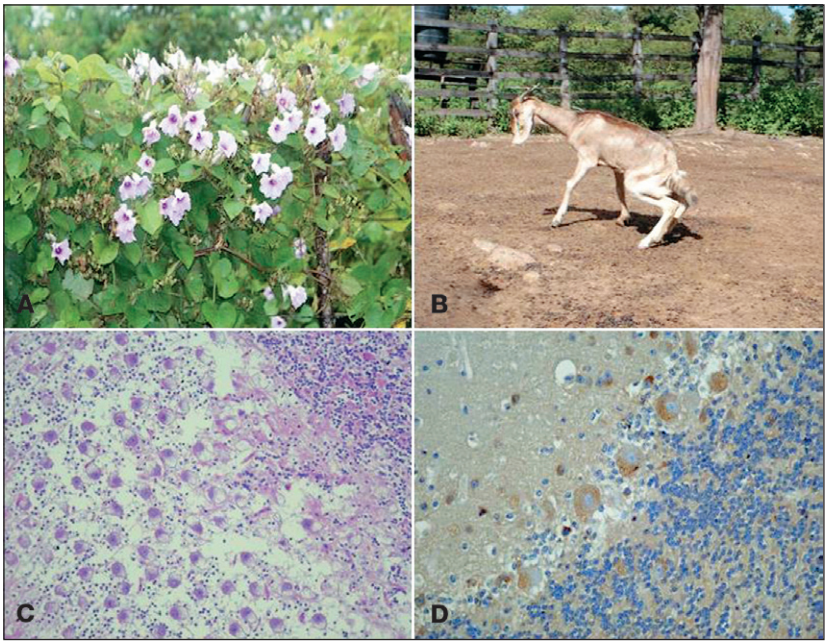

Fig. 1. A. *Ipomoea sericophylla* with inflorescences, May 2009, Municipality of Sertânia, Pernambuco, Northeastern Brazil. B. Goat with paraparesis of hind legs associated with spontaneous poisoning by *Ipomoea sericophylla*. C. Purkinje neurons and neurons of molecular layer with thin cytoplasmatic vacuolation. Obj. × 20. H.E. stain. D. Purkinje cells with strongly positive reaction to *Triticum vulgaris* (WGA). Obj. × 40. Section of formalin-fixed, paraffin-embedded tissue was incubated with WGA and counterstained with Mayer's haematoxylin.