



Case report

Swainsonine-induced lysosomal storage disease in goats caused by the ingestion of *Sida rodrigo* Monteiro in North-western Argentina

Juan Francisco Micheloud^{a, b, *}, Raúl Marín^c, Luis Adrián Colque-Caro^{a, b},
Olga Gladys Martínez^d, Dale Gardner^e, Eduardo Juan Gimeno^f

^a Grupo de Trabajo de Patología, Epidemiología e Investigación Diagnóstica, Área de Sanidad Animal-IIACS/INTA, Salta, Argentina

^b Facultad de Ciencias Veterinarias y Agrarias-UCASAL, Salta, Argentina

^c Cátedra de Producción Bovina, Universidad Nacional de Jujuy, Jujuy, Argentina

^d Cátedra de Diversidad de las Plantas, Universidad Nacional de Salta, Salta, Argentina

^e Poisonous Plants Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Logan, UT, USA

^f Cátedra de Patología General, Facultad de Ciencias Veterinarias-Universidad Nacional de La Plata, Bs As, Argentina

ARTICLE INFO

Article history:

Received 27 November 2016

Received in revised form

19 December 2016

Accepted 21 December 2016

Available online 16 January 2017

Keywords:

Storage disease

Swainsonine

Poisonous plants

Argentinian Northeastern

ABSTRACT

There are numerous poisonous plants that can induce intralysosomal accumulation of glycoproteins and neurologic syndromes. Here we describe for the first time, a disease caused by ingesting *Sida rodrigo* Monteiro in goats in North-western Argentina. The animals showed weight loss, indifference to the environment, unsteady gait and ataxia. Histopathologic studies showed vacuolization in cells of various organs, mainly in the CNS. The material deposited in the cells was positive for LCA (*Lens culinaris* agglutinin), WGA (*Triticum vulgaris* agglutinin), sWGA (succinyl-*Triticum vulgaris* agglutinin) and Con-A (*Concanavalina ensiformis* agglutinin) lectins. Finally, toxic levels of swainsonine were identified in the plant. The present investigation allowed to recognize *S. rodrigo* Monteiro poisoning as a plant induced α -mannosidosis.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Glycogenesis due to the ingestion of plants has been known for years in species of the genera *Swainsona* (in Australia), *Oxytropis* and *Astragalus* in several parts of the world (Huang et al., 2003). In recent years the list of plant species reported to produce this type of poisoning has increased (Cook et al., 2014). Several species of *Ipomoea* and *Turbina* genera's have been reported to contain swainsonine and cause toxicity (de Balogh et al., 1999; Dantas et al., 2006; Armien et al., 2007). To date, only one species of the family Malvaceae, *Sida carpinifolia*, has been shown to be toxic in goats due to the presence of swainsonine (Driemeier et al., 2000; Colodel et al., 2002). *S. carpinifolia* toxicity has been observed also in horses (Loretti et al., 2003), sheep (Seitz et al., 2005), cattle (Furlan et al., 2008) and deer (Pedroso et al., 2009).

Here, we describe an acquired lysosomal storage disease in goats in the Province of Jujuy, Argentina. Epidemiological data and

confirmation of the presence of swainsonine on the ground, suggest that *S. rodrigo* Monteiro is involved in the aetiology. The purposes of this study were to characterize the microscopic lesions and lectin staining pattern observed in tissues in natural poisoning cases.

2. Case reports

2.1. Clinical and epidemiological findings

A neurologic disease was observed in a flock of crossbred goats grazing in a 8 ha pasture field. During summer (January–May) of 2015, a nervous condition was noted in a large number of animals. The affected flock consisted of 196 animals and the outbreak lasted about 60 days being the attack rate of 81% (160/196) and mortality of 51% (100/196). The owner of goats mentioned that the previous year had seen a few animals with similar clinical signs. Due to the high animal load (24,8 animals/ha), the pasture presented low forage supply, with evidence of overgrazing.

The affected animals had varying degrees of ataxia and astasia with paresis and paralysis of the hindquarters (Fig. 1). Signs

* Corresponding author. Área de Sanidad Animal-IIACS, Instituto Nacional de Tecnología Agropecuaria, Cerrillos, Salta, Argentina.

E-mail address: micheloud.juan@inta.gob.ar (J.F. Micheloud).



Fig. 1. Goat with nervous signs. It shows abnormal posture and wide-based stance.

highlighted unsteady gait, tremors of head and neck. By raising the head manually, signs were increased and affected animals had rushed to the ground and unable to rise up despite trying repeatedly. Occasionally, they acquired abnormal positions. Affected animals remained separated from the flock, showed erratic gait and compulsory consumption of *S. rodrigoii*. Abortions and stillbirths were frequent in goats on the farm.

2.2. Pathological findings

The autopsies of 4 clinically affected goats were performed: a male of about 18 months (N1) and three female adult goats (N2, N3 and N4) at different stages of evolution of clinical disease. No macroscopic changes were observed with the exception of absence of subcutaneous and abdominal fat.

After complete post-mortem examination, tissues samples (brain, spinal cord, urinary bladder, liver, kidney, heart, pancreas and thyroid gland) were processed as controls. Sections were stained with hematoxylin and eosin and PAS stain. All animals showed diffuse vacuolization of different tissues that tested negative to PAS. These results are summarized in Table 1. Changes in nervous system are compiled in Table 2. Pericarian vacuolation, axonal swelling and axonal torpedoes were observed at the cerebellar cortex (Fig. 2A and C).

Table 1
Histopathological findings in tissues.

Animal	Thyroid Gland	Urinary Bladder	Liver	Kidney	Heart	Pancreas
N1	+++	+++	++	+++	++	++
N2	+++	+++	+++	+++	++	+++
N3	+++	+++	++	+++	+	++
N4	+++	+++	+++	+++	+	+++

– = No lesion + = Minors lesion ++ = moderate +++ = severe.

Table 2
Histopathological findings in the nervous system.

Animal	Córtex	Basal nuclei	Cerebellum	Choroid plexus
N1	++	++	+++	++
N2	++	+++	+++	+++
N3	+	+++	+++	++
N4	++	+++	+++	++

– = No lesion + = Minors lesion ++ = moderate +++ = severe.

2.3. Lectin histochemistry

Selected sections of brain were submitted to a lectin histochemistry technique as previously described (Driemeier et al., 2000; Ríos et al., 2015). Sections of cerebellum from healthy goats were used as controls. Most of the lectins tested negative, while LCA, WGA, Con A and sWGA labelled the stored material in vacuoles of cerebellar neurons (Fig. 2B and D).

2.4. Botanical identification and analysis of swainsonine

The predominant plant in the pasture was *Sida rodrigoii* Monteiro (Griseb.) Krapov (Fig. 3). It was registered in the Herbarium of the National University of Salta with the Number to MCNS 12894. Part of the plant material from each area was collected, pooled to obtain a sample composed of several grazing areas. The swainsonine (SW) concentration (expressed in %/dry matter) was determined using the method described by Gardner et al. (2001). The sample of *S. rodrigoii* Monteiro was positive for swainsonine (0.033% dry matter).

3. Discussion

The clinical and pathological findings, along with the history and the high swainsonine levels found in *S. rodrigoii* Monteiro, allow us to attribute the severe clinical cases observed to the consumption of this plant. Swainsonine level were about 0.033% of SW/dry matter in a collected sample. This level of swainsonine was much higher than those mentioned in *S. carpinifolia* (Colodel et al., 2002) but similar to other species of the genera *Astragalus* and *Oxitropis* (Ralphs et al., 2008). Stegelmeier et al. indicated that doses of swainsonine equal or above 0.2 mg/kg/day for at least 21 days can produce irreversible neurological disease (Stegelmeier et al., 1995). However levels of swainsonine above 0.001% are considered harmful for livestock (Molyneux et al., 1994). The high incidence and mortality observed in this outbreak could be due to the low forage availability, the high animal load and the abundance of the plant in the grazing area. The owner mentioned that intoxicated animals develop a preference for consuming the plant. This fact, has been mentioned in other plant species capable of inducing lysosomal storage disease (Tokarnia et al., 2012).

The observed vacuolation in tissues is a typical characteristic of poisoning by species containing swainsonine (James et al., 1969; Stegelmeier et al., 1995; Tokarnia et al., 2012). The alpha-mannosidosis results from deficient activity of the enzyme alpha-mannosidase and are characterized by intracellular accumulation of oligosaccharides (mainly mannose) (Tulsiani and Touster, 1983). In addition to nervous system, many other tissues could be affected (Elbein, 1989; Stegelmeier et al., 1995, 2001; Driemeier et al., 2000; McLain et al., 2004; Dantas et al., 2006; Stegelmeier et al., 2007; Ríos et al., 2015). In this cases, nervous, pancreatic, hepatic, thyroidal, urinary and myocardial lesions agree with those produced by other plant species that induce lysosomal accumulation due to the presence of swainsonine (James et al., 1969; Van Kampen and James, 1970; Driemeier et al., 2000; Dantas et al., 2006; Armién et al., 2007). The presence of axonal torpedoes was an important finding in the nervous system, especially in the cerebellar tissue. The torpedoes are a rounded swelling of the proximal portion of the Purkinje cell axons; the swelling is thought to result from the mis-accumulation of normal or abnormal cell constituents in disease states (Louis et al., 2009). Some studies relate the presence of these lesions with the presence of tremors (Yu et al., 2012). This sign is relevant in several poisonous plants that induce storage lysosomal disease (Van Kampen and James, 1969; Van Kampen and James, 1970; Driemeier et al., 2000; Dantas et al., 2006; Tokarnia et al., 2012). Cardiac lesions observed seem to be much less frequent,

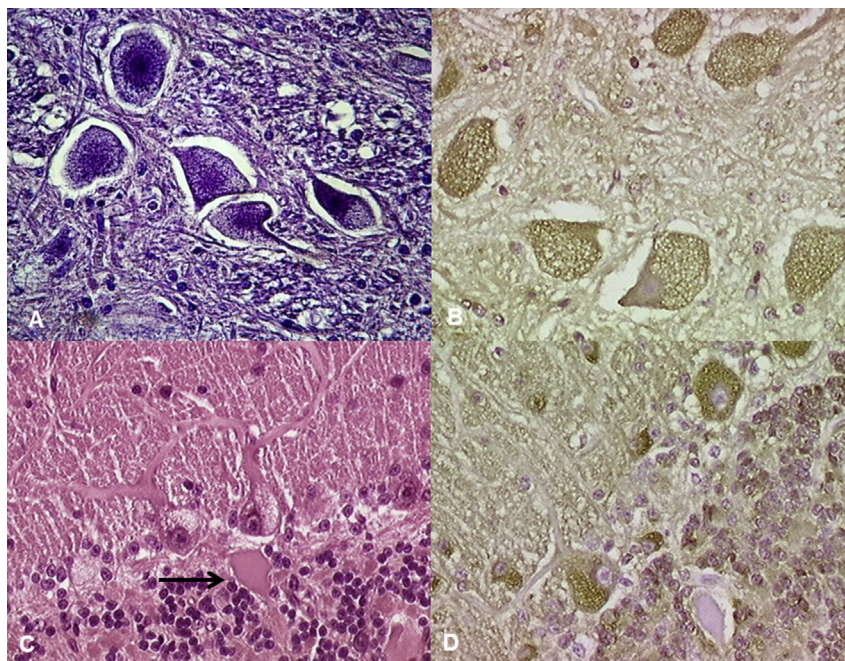


Fig. 2. Cerebellum. Cerebellar basal nuclei. Vacuolation of neurons. Bar 25 μ m. HE. (2. A). LCA labelling of neurons. Lectinhistochemistry. Bar 25 μ m (2. B). Cerebellar cortex. Purkinje cells showed vacuolation of the pericaryon and axonal torpedoes (Arrow). Bar 25 μ m (2. C). HE. WGA labelled the pericaryon of Purkinje cells. Lectinhistochemistry. Bar 25 μ m. (2. D).



Fig. 3. Predominance of *Sida rodrigoii* Monteiro in the suspicious field. Province of Jujuy, North-western Argentina.

although they were described in experimentally poisoned rats (Stegelmeier et al., 1995). Therefore, the myocardium seems to be more resistant to the action of swainsonine than other tissues. In agreement with other cases of α -mannosidosis, PAS staining was negative and the lectinhistochemistry showed positive reactivity with Con-A, WGA, sWGA and LCA in cytoplasmic vacuoles of Purkinje cells (Driemeier et al., 2000; Loretti et al., 2003; Seitz et al., 2005).

A long list of plants are enumerated as toxic due to the presence of swainsonine (Cook et al., 2014). Most of these species belong to the genera: *Astragalus*, *Swaionsa* and *Oxitropis* (James et al., 1969; Van Kampen and James, 1969; Van Kampen and James, 1970; Zhao et al., 2011). Since years several species of *Ipomoea* sp. have been linked to such diseases (de Balogh et al., 1999; Rodríguez et al., 2004; Armien et al., 2007; Tokarnia et al., 2012; Ríos et al., 2015). Dantas et al. (2006) described similar disease from eating *Turbina cordata* in Brazil. All species mentioned above correspond to the

family *Fabaceae* or *Convolvulaceae* (Cook et al., 2014). Some years ago *Sida carpinifolia* was mentioned as a plant capable of inducing lysosomal accumulation in various domestic species (Driemeier et al., 2000; Loretti et al., 2003; Seitz et al., 2005; Furlan et al., 2008; Pedrosa et al., 2009). The latter species belongs to the family *Malvaceae* (Fryxell, 1997). According to these records *Sida rodrigoii* Monteiro is the second species of this family able to induce lysosomal accumulation. This genus is composed of over 200 species. Argentina is home to some 24 species, half of which grow in north western of the country (Krapovickas et al., 2008). Like *Sida carpinifolia*, *Sida rodrigoii* tends to dominate overgrazed areas (Seitz et al., 2005). However its role as undergrowth has not been studied.

Relatively recent studies have identified the presence of endophyte fungi capable of producing swainsonine in several of the species mentioned above (Braun et al., 2003; McLain et al., 2004; Ralphs et al., 2008; Chechen et al., 2014; Cook et al., 2014). The presence of swainsonine in *S. carpinifolia* (Colodel et al., 2002) and in *S. rodrigoii* Monteiro, suggests that the participation of endophyte fungi not yet identified could be the cause. All these evidences indicate that studies should continue on this issue.

Acknowledgments

This study was supported by grants from INTA (Instituto Nacional de Tecnología Agropecuaria) (PNSA-1115055 and SALJU-1232307), Consejo de Investigación de la Universidad Católica de Salta (Res- 676-14) and PICT 2011/1379 (FONCYT). EJG is a senior fellow of CONICET (Consejo Nacional e Investigaciones Científicas y Técnicas). We thank Dr. Hector A. Keller for his contribution to botany identification of *Sida rodrigoii* Monteiro and Histotech. María Guadalupe Guidi for technical assistance.

Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.toxicon.2016.12.011>.

References

- Armién, A.G., Tokarnia, C.H., Peixoto, P.V., Frese, K., 2007. Spontaneous and experimental glycoprotein storage disease of goats induced by *Ipomoea carnea* subsp. fistulosa (Convolvulaceae). *Vet. Path.* 44, 170–184.
- Braun, K., Romero, J., Liddell, C., Creamer, R., 2003. Production of swainsonine by fungal endophytes of locoweeds. *Mycol. Res.* 107, 980–988.
- Chechen, W., Xiaowen, Y., Shanshan, W., Pengshuai, G., Hao, L., Baoyu, Z., 2014. Pathogenesis and preventive treatment for animal disease due to locoweeds poisoning. *Environ. Toxicol. Pharmacol.* 37, 336–347.
- Colodel, E.M., Gardner, D.R., Zlotowski, P., Driemeier, D., 2002. Identification of swainsonine as a glycoside inhibitor responsible for *Sida carpinifolia* poisoning. *Vet. Hum. Toxicol.* 44, 177–178.
- Cook, D., Gardner, D.R., Pfister, J.A., 2014. Swainsonine-containing plants and their relationship to endophytic fungi. *J. Agr. Food. Chem.* 62, 7326–7334.
- Dantas, A.F., Riet-Correa, F., Gardner, D.R., Medeiros, R.M., Barros, S.S., Anjos, B.L., Lucena, R.B., 2006. Swainsonine induced lysosomal storage disease in goats caused by the ingestion of *Turbina cordata* in northeastern Brazil. *Toxicon* 49, 111–116.
- de Balogh, K.K., Dimande, A.P., van der Lugt, J.J., Molyneux, R.J., Naude, W.G., Welman, G., 1999. A lysosomal storage disease induced by *Ipomoea carnea* in goats in Mozambique. *J. Vet. Diagn. Invest.* 11, 266–273.
- Driemeier, D., Colodel, E.M., Gimeno, E.J., Barros, S.S., 2000. Lysosomal storage disease caused by *Sida carpinifolia* poisoning in goats. *Vet. Path.* 37, 153–159.
- Elbein, A.D., 1989. The effects of plant indolizidine alkaloids and related compounds on glycoprotein processing. In: James, L.F., Elbein, A.D., Molyneux, R.J., Warren, C.D. (Eds.), *Swainsonine and Related Glycosidase Inhibitors*. Iowa State University Press, Ames, IA, pp. 155–187.
- Fryxell, P.A., 1997. The American genera of Malvaceae-II. *Brittonia* 49, 204–269.
- Furlan, F.H., Luciolli, J., Veronezi, L.O., Traverso, S.D., Gava, A., 2008. Intoxicação experimental por *Sida carpinifolia* (Malvaceae) em bovinos. *Pesq. Vet. Bras.* 28, 57–62.
- Gardner, D.R., Molyneux, R.J., Ralphs, M.H., 2001. Analysis of swainsonine: extraction methods, detection, and measurement in populations of locoweeds (*Oxytropis* spp.). *J. Agric. Food Chem.* 49, 4573–4580.
- Huang, Y.Q., Zhang, E.Y., Pan, W.F., 2003. Current status of locoweeds toxicity. *Shandong Sci.* 16, 34–39.
- James, L.F., Van Kampen, K.R., Staker, G.R., 1969. Locoweeds (*Astragalus lentiginosus*) poisoning in cattle and horses. *J. Am. Vet. Med. Assoc.* 155, 525–530.
- Krapovickas, A., Tolaba, J.A., Novara, L., 2008. Malvaceae: Aportes Botánicos de Salta- Serie Flora, vol. 8, 1–136.
- Loretti, A.P., Colodel, E.M., Gimeno, E.J., Driemeier, D., 2003. Lysosomal storage disease in *Sida carpinifolia* toxicosis: an induced mannosidosis in horses. *Equine Vet. J.* 35, 434–438.
- Louis, E.D., Yi, H., Erickson-Davis, C., Vonsattel, J.P., Faust, P.L., 2009. Structural study of Purkinje cell axonal torpedoes in essential tremor. *Neurosci. Lett.* 450, 287–291.
- McLain, R.J., Creame, R., Zepeda, H., Strickland, J., Bell, G., 2004. The toxicosis of *Embellisia* fungi from locoweeds (*Oxytropis lambertii*) is similar to locoweeds toxicosis in rats. *J. Anim. Sci.* 82, 2169–2174.
- Molyneux, R.J., James, L.F., Ralphs, M.H., Pfister, J.A., Panter, K.E., Nash, R.J., 1994. Polyhydroxy alkaloid glycosidase inhibitors from poisonous plants of global distribution: analysis and identification. In: Colegate, S.M., Dorling, P.R. (Eds.), *Plant-associated Toxins. Agricultural, Phytochemical and Ecological Aspects*. CAB International, Wallingford, pp. 107–112.
- Pedroso, P.M., Von Hohendorf, R., De Oliveira, L.G., Schmitz, M., Da Cruz, C.E., Driemeier, D., 2009. *Sida carpinifolia* (Malvaceae) poisoning in fallow deer (*Dama dama*). *J. Zoo. Wildl. Med.* 40, 583–585.
- Ralphs, M.H., Creamer, R., Baucom, D., Gardner, D.R., Welsh, S.L., Graham, J.D., Hart, C., Cook, D., Stegelmeier, B.L., 2008. Relationship between the endophyte *Embellisia* spp. and the toxic alkaloid swainsonine in major locoweeds species (*Astragalus* and *Oxytropis*). *J. Chem. Ecol.* 34, 32–38.
- Ríos, E.E., Cholích, L.A., Chilesky, G., García, E.N., Lértora, J., Gimeno, E.J., Guidi, M.G., Mussart, N., Teibler, G.P., 2015. Suspected natural lysosomal storage disease in goats, induced by *Ipomoea carnea* in Argentina. *J. Vet. Med. Sci.* 77, 847–850.
- Rodríguez, R.A., Repetto, A.E., Ortega, H.H., Peralta, C.J., Pensiero, J.F., Ref, P.K., Salvetti, N.R., 2004. Intoxicación en cabras por ingestión de *Ipomoea hieronymi* var *calchaquina* en la Provincia de Catamarca, Argentina. *Vet. Arg.* 21, 332–341.
- Seitz, A.L., Colodel, E.M., Schmitz, M., Gimeno, E.J., Driemeier, D., 2005. *Sida carpinifolia* (Malvaceae) poisoning in sheep (*Short communication*). *Vet. Rec.* 156, 386–388.
- Stegelmeier, B.A., Molyneux, R.J., Elbein, A.D., James, L.F., 1995. The lesions of locoweeds (*Astragalus mollissimus*), swainsonine, and castanospermine in rats. *Vet. Path.* 32, 289–298.
- Stegelmeier, B.L., James, L.F., Hall, J.O., Mattix, M.T., 2001. Neurologic disease in range goats associated with *Oxytropis sericea* (Locoweeds) poisoning and water deprivation. *Vet. Hum. Toxicol.* 43, 302–304.
- Stegelmeier, B.L., Lee, S.T., James, L.F., Gardner, D.R., Panter, K.E., Ralphs, M.H., Pfister, J.A., 2007. The comparative pathology of locoweeds poisoning in livestock, wildlife and rodents. Oxon, Wallingford, U.K. In: Panter, K., Wierenga, T.L., Pfister, J. (Eds.), *Poisonous Plants Global Research and Solutions*, pp. 359–365.
- Tokarnia, C.H., Brito, M., de, F., Barbosa, J.D., Peixoto, P.V., Döbereiner, J., 2012. *Plantas Tóxicas do Brasil para Animais de Produção*, second ed. Editora Heli-anthus, Rio de Janeiro.
- Tulsiani, D.R.P., Touster, O., 1983. Swainsonine, a potent mannosidase inhibitor, elevates rat liver and brain lysosomal α -D-mannosidase, decreases Golgi α -D-mannosidase II, and increases the plasma levels of several acid hydrolases. *Arch Biochem. Biophys.* 224, 594–600.
- Van Kampen, K.R., James, L.F., 1969. Pathology of locoweeds poisoning in sheep. *Pathol. Vet.* 6, 413–423.
- Van Kampen, K.R., James, L.F., 1970. Pathology of locoweeds (*Astragalus lentiginosus*) poisoning in sheep. Sequential development of cytoplasmic vacuolation in tissues. *Pathol. Vet.* 7, 503–508.
- Yu, M., Ma, K., Faust, P.L., Honig, L.S., Cortés, E., Vonsattel, J.P., Louis, E.D., 2012. Increased number of Purkinje cell dendritic swellings in essential tremor. *Eur. J. Neurol.* 19, 625–630.
- Zhao, M.L., Gao, X., Han, B., 2011. Locoweeds poisoning in the native grassland of China. *IJPPR* 1, 41–46.