Toxicon 128 (2017) 1–4

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Case report

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

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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 rodrigoi 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 (*Concanavalia ensiformis agglutinin*) lectins. Finally, toxic levels of swansonine were identified in the plant. The present investigation allowed to recognize *S. rodrigoi Monteiro* poisoning as a plant induced α-mannosidosis.

1. Introduction

Glycogenosis 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; Armién 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. rodrigoi 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

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http://dx.doi.org/10.1016/j.toxicon.2016.12.011
0041-0101/

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. rodrigoi*. 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. Pericarion vacuolation, axonal swelling and axonal torpedoes were observed at the cerebellar cortex (Fig. 2A and C).

Table 1
Histophatological findings in tissues.

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

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

Table 2
Histophatological findings in the nervous system.

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

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

2.3. Lectinhistochemistry

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 rodrigoi 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. rodrigoi 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. rodrigoi 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 swansonine 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-manosidase 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 swansonine (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 misaccumulation 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,