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Intoxication by *Astragalus garbancillo* var. *garbancillo* in llamas

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Abstract. Lysosomal storage diseases are inherited and acquired disorders characterized by dysfunctional lysosomes. Intracytoplasmic accumulation of undegraded substrates leads to impaired cellular function and death. Several plant species are toxic to livestock because of the presence of indolizidine alkaloids, including swainsonine, which cause a storage disease. Swainsonine-induced nervous disease (i.e., locoism) of sheep and cattle is well recognized in several parts of the world, particularly in the western United States and in parts of Australia. Spontaneous intoxication by *Astragalus garbancillo* var. *garbancillo* was suspected in a group of 70 llamas (*Lama glama*) in Jujuy Province, northwestern Argentina. The animals grazed an area dominated by stands of *A. garbancillo* var. *garbancillo*. Clinical signs were staggering, ataxia, hypermetria, and progressive weight loss. The clinical course in individual animals was ~50 d. The main microscopic changes were Purkinje cell degeneration, necrosis, and loss, associated with intracytoplasmic vacuolation, meganeurite formation, and Wallerian degeneration. Specific positive labeling for ubiquitin was observed in axonal spheroids. Composite leaf and stem samples of *A. garbancillo* var. *garbancillo* analyzed by high-performance liquid chromatography contained 0.03% swainsonine. Based on the microscopic lesions, clinical history, and plant analysis, a diagnosis was made of storage disease caused by consumption of swainsonine-containing *A. garbancillo* var. *garbancillo*.

Key words: Argentina; *Astragalus garbancillo* var. *garbancillo*; intoxication; llamas; lysosomal storage disease; swainsonine.

Acquired lysosomal storage diseases are characterized by inhibition of specific lysosomal enzymes, resulting in intracytoplasmic accumulation of undegraded substrates that produce cell alteration and death.[1] Lysosomal storage diseases, particularly the so-called locoism caused by ingestion of plants containing indolizidine alkaloids,[2] are economically important for livestock. Locoism has been reported associated with multiple plants, including species of genera *Astragalus*,[16,24,27] *Sida*,[8,17] *Oxytropis*,[22,26] *Swainsona*,[7,13] and *Ipomoea*.[6,14,15]

The toxicity of plants in genus *Astragalus* is mostly the result of the presence of indolizidine alkaloids. Of these, swainsonine and its *N*-oxide are considered the most important given their ability to inhibit lysosomal α-mannosidase and Golgi α-mannosidase II.[3]

Approximately 70 species of *Astragalus* are native to Argentina.[5,11] One of these is *A. garbancillo*, colloquially known as "garbanzo," of which 2 varieties exist (i.e., *A. garbancillo* var. *mandoni* and *A. garbancillo* var. *garbancillo*). Only *A. garbancillo* var. *garbancillo* is present in Argentina, where it grows at elevations of 1,600–4,500 m above sea level.[12]

Natural intoxication by *A. garbancillo* var. *garbancillo* has been reported in sheep,[16] and a disease with clinical signs and microscopic lesions resembling locoism was induced experimentally in guinea pigs fed this plant (Abam S, et al. Intoxicación experimental con *Astragalus garbancillo var. garbancillo* en cobayos [Experimental poisoning by *Astragalus garbancillo var. garbancillo* in guinea pigs]. X Argentinean Vet Pathol Meeting; 24–26 August 2016; Esperanza, Santa Fe Province, Argentina). Natural or experimental intoxication by this plant has not been described in other animal species. Locoism has not been reported in South American camelids to date.

We describe herein a cluster of cases of natural intoxication of llamas with *A. garbancillo* var. *garbancillo*. The cases occurred in the Puna region, a grassland plateau in northwestern Argentina that has an average elevation of 4,500 m above sea level, a cold, dry climate with wide daily temperature fluctuations, and absolute minimal temperatures of −15°C

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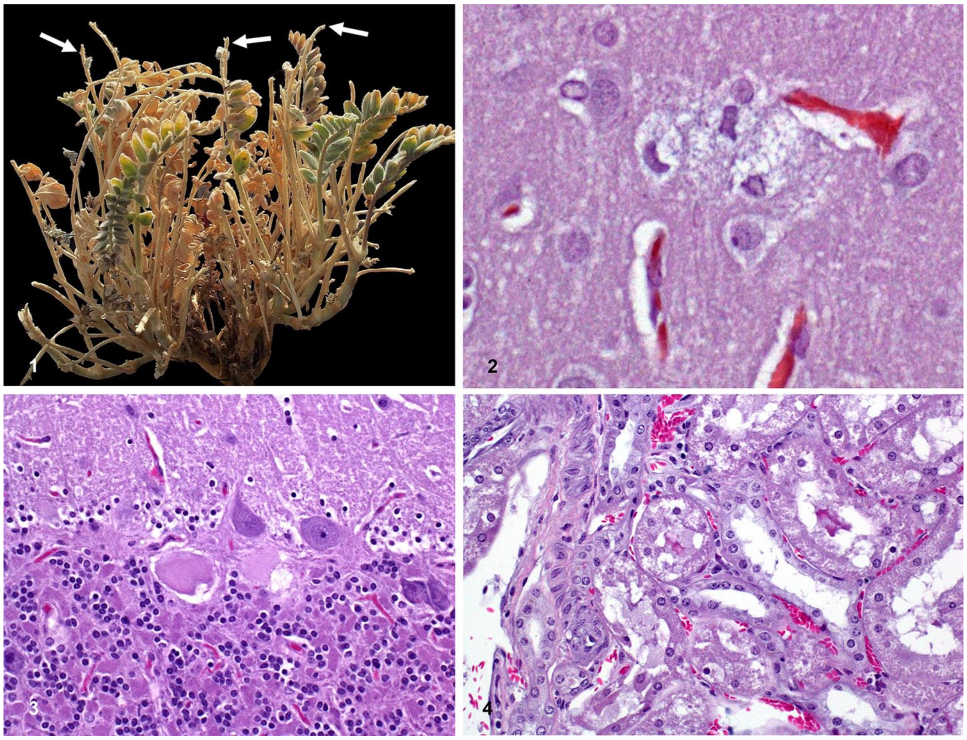

Figures 1–4. Intoxication of llamas by *Astragalus garbancillo* var. *garbancillo*. **Figure 1.** *A. garbancillo* var. *garbancillo* plant, with signs of consumption (arrows). **Figures 2–4.** Tissues of intoxicated llama 1. H&E. **Figure 2.** Fine intracytoplasmic vacuolation of neurons in the cerebral cortex. **Figure 3.** Chromatolysis of Purkinje cells in the cerebellum. **Figure 4.** Fine cytoplasmic vacuolation in renal tubular epithelial cells.

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Llama breeding is the most relevant livestock activity for smallholders in Jujuy Province (Puna region), which has the largest stock of llamas in Argentina (Plan Estratégico Productivo Jujuy 2011–2020. Sector 4: ovino y camélido [Strategic Production Plan Jujuy 2011–2020. Area 4: ovine and camelid]. Ministerio de Producción de la Provincia de Jujuy, Edición: Gabriela Tijman, 243–259. Spanish. Available from: https://drive.google.com/file/d/0By4c_oeuLMchTn-NmSWd2cHdvOFk/view). Intoxication of llamas by *A. garbancillo* var. *garbancillo* has long been suspected in the region, but never confirmed or described, to our knowledge. Given the sociocultural and economic importance of camelids in sectors of Latin America where the plant is present, recognition of this intoxication is important for the health management of these animals.

During the winter of 2013, a neurologic disease in llamas was reported by several smallholders in the Puna region. This is the same area in which locoism caused by *A. garbancillo* var. *garbancillo* was reported in sheep in 2017.[16] One of the authors (R.E. Marin) visited a smallholder premises in the town of Puesto Sey, Jujuy Province (Puna region), at 4,008 m above sea level. The owner had a herd of 70 llamas on a pasture with heavy stands of *A. garbancillo* var. *garbancillo* (Fig. 1). At the time of the visit, 5 adult llamas (4 females and 1 male) had moderate ataxia and marked hypermetria. Two of the 5 had moderate tremors of the head and ears. The owner reported that the 5 animals had these clinical signs for ~30 d. There was no evidence of clinical improvement 20 d later. The producer reported that similar clinical signs affected 5–7% of the adult herd annually, with low reproductive indexes also being observed. The problem was more evident in dry years. He also reported that, once clinical signs were observed, affected animals separated from the herd, walked erratically, and tended to consume *A. garbancillo* var. *garbancillo* compulsively. On rare occasions, they died spontaneously, but most affected animals lost body condition progressively, necessitating euthanasia.

Whole blood and serum were collected from 2 affected llamas, prior to euthanasia with an overdose of sodium pentobarbital. A complete blood cell count and serum chemistry revealed no abnormalities, and no cytoplasmic vacuolation was identified in circulating monocytes in peripheral blood smears.[2]

Postmortem examinations were performed. Both carcasses were in poor nutritional condition, with no fat reserves anywhere in the body, moderate serous atrophy of fat, and mild generalized muscle atrophy. No other significant gross abnormalities were observed. The brains were grossly unremarkable. The content of the whole gastrointestinal tract was scant but otherwise grossly unremarkable.

In addition to whole brains, samples of liver, kidney, spleen, lung, adrenal glands, small and large intestine, heart, and skeletal muscle were collected and fixed in 10% neutral-buffered formalin (pH 7.2) for 24 h. Brains were sliced at 1-cm intervals, and fixed in fresh formalin for an additional 7 d before subsamples were taken from cerebral cortex, corpus striatum, thalamus, midbrain at the level of anterior colliculi, pons, cerebellar peduncles, cerebellum, and medulla oblongata at the level of the obex. Tissue samples were processed routinely for production of 4-μm thick hematoxylin and eosin sections. Selected sections of brain were stained with Holmes, luxol fast blue, and periodic acid–Schiff.

Microscopic changes were similar in both animals. Lesions in the brain consisted of fine intracytoplasmic vacuolation of neurons in basal nuclei, cerebellum, and cerebral cortex (Fig. 2). Most Purkinje cells were absent from the cerebellum, resulting in empty baskets. Persisting Purkinje cells exhibited diffuse central or peripheral chromatolysis (Fig. 3). Swollen axons and diffuse gliosis were present in cerebral cortex and subcortical white matter. Meganeurite formation at axon hillocks was observed in the granular cell layer of the cerebellum.

There were discrete, random foci of hepatocellular necrosis. Hepatocytes exhibited severe, panlobular cytoplasmic microvacuolation. Mild portal lymphoplasmacytic hepatitis was present. The epithelium of proximal convoluted renal tubules had marked fine cytoplasmic vacuolation (Fig. 4).

Immunohistochemistry (IHC) for ubiquitin was performed on brain sections as described previously.[19] Brain from a clinically normal llama was used as a negative control. Additionally, IHC for West Nile virus, rabies virus, and *Chlamydia* spp. was performed on sections of cortex, brainstem, and cerebellum, as described previously.[10,20,25] Positive and negative controls were brain sections from cattle or horses that were PCR positive or negative, respectively, for each of the infectious agents studied. Positive labeling for ubiquitin was observed in axonal spheroids of cerebral cortex and subcortical white matter. IHC for all infectious agents studied was negative. No staining was observed in any of the negative controls.

The pasture in which the animals had grazed was examined the same day that 2 affected llamas were euthanized for our study. The area was sparsely covered by vegetation with ~50% consisting of the aforementioned garbanzo plant. The other ~ 50% of vegetation included *Baccharis tola* ssp. *tola*, *Azorella compacta*, and *Pycnophyllum molle*. Most garbanzo plants had evidence of consumption with subsequent regrowth (Fig. 1).

No supplemental feed was provided to any of the animals. Plant specimens were collected, press-dried, and submitted for identification to the Department of Systematic Botanic of the Agricultural Sciences Faculty, National University of Jujuy, Argentina, where they were identified as *Astragalus garbancillo* var. *garbancillo*.

Additional specimens of *A. garbancillo* var. *garbancillo* were collected in March (fall) and December (spring) 2014, and subjected to extraction and analysis by high-performance liquid chromatography (HPLC)-apci(+) mass spectrometry (MS) using methods described previously for the determination of swainsonine.[4,9] The concentration of swainsonine in a composite sample of leaves and fine stems ground at an early vegetative stage was 0.030% in March 2014 and 0.034% in December 2014.

We diagnosed intoxication by *A. garbancillo* var. *garbancillo* based on clinical signs, microscopic lesions, abundance of these plants with evidence of grazing on the pasture, and determination of swainsonine in plant specimens.[2,3,18] The lesions in brain and kidney of the 2 llamas were almost identical to those described previously in sheep[16] and guinea pigs (Abam S, et al. 2016) spontaneously and experimentally intoxicated, respectively, with *A. garbancillo* var *garbancillo* plants. Changes are compatible with lysosomal storage disease. The positive labeling of ubiquitin in meganeurites is consistent with active degradation of short-lived proteins.[19,29] Positive IHC for this protein has been detected in a variety of chronic neurodegenerative disorders of humans and other animals, including calves with β-mannosidosis,[19] which was the rationale for the use in our study. The concentrations of swainsonine in the analyzed plant samples were higher than the minimum concentration established to produce neurologic damage (0.001%) when plants are consumed for a sufficient time.[7] The concentration of swainsonine in the plants can vary over different seasons of the year. Because we analyzed the plant at a time different from when the clinical event occurred, it is possible that other concentrations of swainsonine were involved.

Swainsonine is an indolizidine alkaloid that acts as an α-mannosidase and mannosidase II inhibitor and alters glycoprotein processing, resulting in a lysosomal storage disease.[2] Swainsonine is present in a number of plant families worldwide including 6 genera (*Ipomoea*, *Turbina*, *Astragalus*, *Oxytropis*, *Swainsona*, and *Sida*) of the *Convolvulaceae*, *Fabaceae*, and *Malvaceae* families. Most of these plants have been associated with locoism in several animal species.[6,8,13-17,24,26,28]

Intoxication by *A. garbancillo* var. *garbancillo* has been suspected in llamas and other South American camelids, but this intoxication has not been confirmed previously. The llamas in our study had a chronic protracted clinical course, with some spontaneous deaths, possibly attributable to intoxication. Anecdotal information from producers from the Puna region suggests that intoxication by *A. garbancillo* var. *garbancillo* in llamas results in chronic disease. This observation distinguishes the syndrome in llamas from *A. garbancillo* var. *garbancillo* intoxication in sheep, in which both chronic and acute forms occur.[16] Because llamas are native to the region in which this episode occurred, it is possible that they are better adapted than other domesticated species.[26]

The vacuolation in hepatocytes and renal tubular epithelium is likely related to swainsonine, given that similar changes have been described in swainsonine-intoxicated sheep,[16,28] rats,[27] and guinea pigs.[13] Swainsonine has also been associated with reproductive problems, including long estrous cycles, abortion, hydrops, and weak neonates in cows and ewes.[21-23] The owners of the llamas in our study reported reproductive failure. It is possible that this, too, was swainsonine-induced. It is noteworthy that vacuolation in monocytes, which has been described previously in some swainsonine-intoxicated animals,[2] was not observed in either of the 2 llamas included in our study. This might be an idiosyncratic response of this animal species to swainsonine.

Declaration of conflicting interests

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