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## Cerebellar degeneration in cattle grazing *Solanum bonariense* (“Naranjillo”) in Western Uruguay

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**Abstract.** Cattle in western Uruguay that were eating *Solanum bonariense* developed periodic episodes of ataxia, hypermetria, hyperesthesia, head and thoracic limb extension, opisthotonus, nystagmus, and falling to the side or backward. Similar clinical signs were experimentally reproduced in cattle by administration of *S. bonariense* via rumen cannula at a dose of 1,024 g/kg body mass. No significant gross lesions were observed in field cases or experimentally induced cases. Spontaneous and induced histologic lesions were similar and included vacuolation, degeneration, and loss of Purkinje cells. Axonal spheroids, microcavitations, and other changes of wallerian-type degeneration in cerebellar white matter were also observed. Ultrastructural changes included increased number of electron-dense residual storage bodies in membrane-bound vesicles in affected Purkinje cells, and similar vesicles and mitochondria in axonal spheroids. No histologic lesions were detected in the other examined tissues. The Purkinje-cell swelling and vacuolation with subsequent cerebellar degeneration are suggestive of Purkinje-cell specific toxin that produces abnormal lysosome function and cell specific axonal transport. This is the first report of *S. bonariense* toxicity.

**Key words:** Cattle; cerebellar degeneration; plant poisoning; *Solanum bonariense*.

Neurological diseases in ruminants caused by ingestion of different *Solanum* species have been described in South Africa, North America, South America, and Australia.<sup>1,6,7,10,11,13,15</sup> *Solanum bonariense* L.<sup>5,8</sup> is a perennial native shrub, particularly common adjacent to the Uruguay River and its tributaries, and in nearby grazing lands of western Uruguay. To our knowledge there have been no previous reports of *S. bonariense* poisoning. The purpose of this study is to describe field cases of *S. bonariense* intoxication, and also to verify its toxicity and pathological lesions by experimentally reproducing toxicity in cattle.

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From December 2000 to June 2001, cattle with neurological disorders were observed on farms in the village of Asencio, 25 km from Mercedes, Uruguay (58°05'W, 33°20'S). *S. bonariense* L. is a native plant and it was particularly abundant in paddocks with native grasses in this area (Fig. 1). Anecdotal accounts from veterinarians and farmers suggested an association between ingestion of the shrub and the neurological disorder.<sup>13</sup> A major feature of the nervous condition in affected cattle was periodic episodes of recumbency with inability to rise without loss of consciousness, lasting up to 1 minute; the animals appeared normal between episodes. Other clinical observations included ataxia, hypermetria, hyperesthesia, staggering gait, muscle tremors, head and thoracic limbs extension, opisthotonus, nystagmus, and in those animals most severely affected, falling to the side or backward. Nervous signs occurred spontaneously or were induced when affected animals became excited or intentionally stressed. A few animals with permanent neurological signs were



**Figure 1.** Aerial parts of *Solanum bonariense*, showing terminal inflorescences at top and fruits at bottom of the stem.

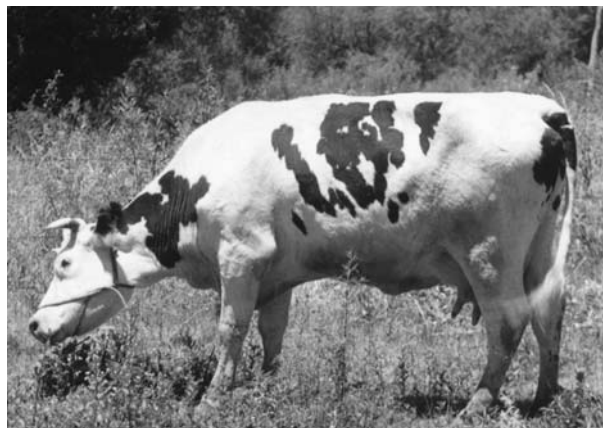
observed to develop dissymmetric gait and “star gazing” attitude (Fig. 2).

On farms near Asencio, dairy cattle (mainly Holstein and Holstein-crosses) appeared to be more affected than were beef breeds (mainly Hereford). Severely affected animals were always over 1 year of age. In one dairy, 160 out of 912 (17.5%) cattle were affected in one episode and mortality was less than 1%.

Plant from pastures with field cases were collected and a voucher specimen was submitted to the Herbarium of Chemical Science School, Universidad de la República, Montevideo, Uruguay (voucher number MVFQ 4259). The plants were identified as *S. bonariense* L. belonging to the family *Solanaceae*. In Uruguay the plant is known as “naranjillo” (literally “little orange”) because of the yellowish or orange color of its ripe fruit during summer. Full botanical descriptions have been previously published (Fig. 1).<sup>5,8</sup>

In the dairy described above, two severely affected Holstein heifers (1 to 2 years old) that had ingested the plant during unknown period were obtained for our studies. Blood samples were taken by jugular venipuncture to measure serum levels of alkaline phosphatase (AP), aspartate transaminase (AST), and gamma glutamyl transferase (GGT). Immediately after that, animals were humanely euthanized and necropsied.

To reproduce *S. bonariense* toxicity, freshly harvested leaves of the plant were administered daily, via rumen cannula at doses of 1% body mass (BM) to 4 Holstein steers (6 to 12 months old) from a *S. bonariense*-free farm. A similar Holstein steer, was given an equivalent amount of chopped hay via rumen cannula as a control. Treated and control animals were kept together in a *S. bonariense*-free paddock at the Veterinary Faculty Experimental Station No 2, San José, Uruguay (56°35'W, 34°40'S). The animals were observed daily. Animals were weighed each week to adjust the plant dose. Blood samples were taken monthly



**Figure 2.** Spontaneously poisoned cow with cerebellar degenerative disorder, showing an altered head posture (“star gazing” attitude) and wide-based stance.

and serum levels of AP, AST, and GGT were measured. Initially, the steers received a daily dose of 1% of BM. The plant induced mild cerebellar signs (including ataxia, hypermetria, hyperesthesia, and staggering gait) in all animals after 128 days. These clinical signs were similar to those noted in field cases. The mean dose of leaves that induced clinical symptoms over a dosing period of 128 days was  $1.024 \pm 0.016$  kg of fresh leaves/kg BM. Thereafter, 1.024 kg/kg BM was considered as the threshold dose for this study.

After the threshold dose was determined, one of the affected animals was euthanized and necropsied (steer 1). Two steers continued to receive incremental doses of the plant until they reached 1.25 times the threshold (total dose: 1.28 kg/kg BM during 160 days; steer 2), 2 times threshold (total dose: 2.048 kg/kg BM during 198 days; steer 3). A fourth animal, steer 4, received a daily dose of 1% of BM of fresh leaves over a dosing period of 61 days, until it reached a dose of 0.5 times threshold (total dose: 0.512 kg/kg BM), without apparent development of cerebellar signs. All animals, including the control, were humanely euthanized by intravenous injection of sodium pentobarbital 24 hours after the last administration of the above doses. At necropsy samples of the central nervous system, spinal ganglia, liver, kidney, heart, lung, and spleen were collected and fixed in 10% neutral buffered formalin. Paraffin-embedded sections were stained with hematoxylin and eosin (HE) or with toluidine blue. Transverse sections were taken from the cervical, thoracic and lumbar spinal cord, medulla, pons, mesencephalon at the level of the caudal colliculi, thalamus, basal nuclei, cortex, cerebellar peduncles, and cerebellum. Duplicate 1-mm<sup>3</sup> tissue block samples from the cerebellum for electron microscopy were fixed in cacodylate-buffered 4% glutaraldehyde, post-fixed in 1.5% osmium tetroxide, dehydrated through alcohols and propylene oxide and embedded in epon. Semi-thin and thin sections were stained with 1% toluidine blue and uranyl acetate/lead citrate respectively.<sup>12</sup> To estimate the changes in Purkinje-cell populations, 10 additional trans-

**Table 1.** Estimates of Purkinje-cell populations and cerebellar lesions in experimental *Solanum* intoxication.

|                     | Cerebellar signs | Purkinje cells per 400x field (as median* value) | Axonal spheroids in white matter per 400x field (as median value) | Presence of microcavitations† in white matter per 400x field |
|---------------------|------------------|--------------------------------------------------|-------------------------------------------------------------------|--------------------------------------------------------------|
| Control             | No               | 3                                                | 0                                                                 | No                                                           |
| (0.5CSIT‡) Steer 4  | No               | 3                                                | *0                                                                | No                                                           |
| (1CSIT‡) Steer 1    | Mild             | 3                                                | *1                                                                | Low                                                          |
| (1.25CSIT‡) Steer 2 | Severe           | *1                                               | -                                                                 | High†                                                        |
| (2CSIT‡) Steer 3    | Severe           | *1                                               | *0                                                                | High†                                                        |

\* Median of each treatment group within columns preceded by asterisks are significantly different from the control using the Mann-Whitney test ( $P < 0.0001$ ). † Microcavitations were the major findings present in white matter instead of axonal spheroids, which were found in steers given lower doses. ‡ CSIT (cerebellar sign-inducing threshold) was a total shrub dose of 1.024 kg of fresh leaves/kg BM.

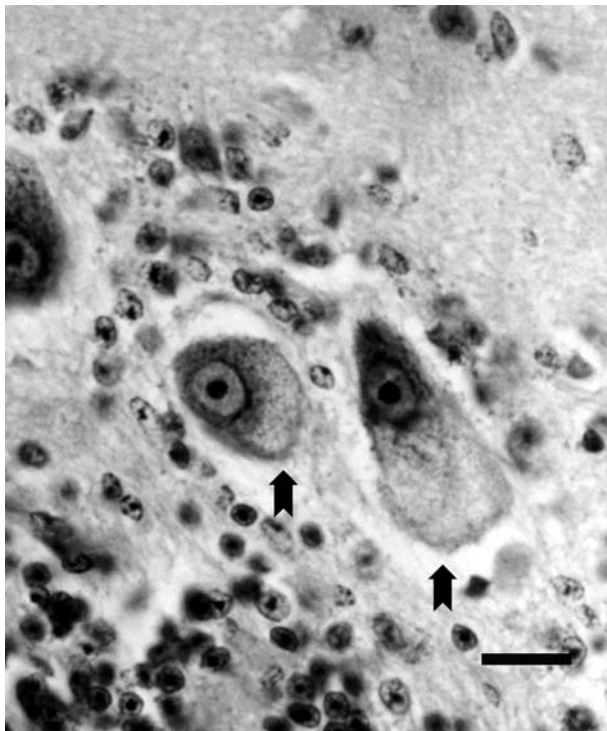
verse sections of the cerebellum from field cases and dosed animals were prepared. Purkinje cells and axonal spheroids in the white matter of 50 microscope fields from each slide at 400 $\times$  were counted and compared by Kruskal-Wallis analysis (Table 1) significant differences between groups were determined at  $\alpha < 0.01$ . Mann-Whitney tests were also used to define differences between individual treatment groups ( $\alpha < 0.01$ ) (SPSS® for Windows v. 11.0 software licensed by Universidade de Santiago de Compostela, Spain).

At necropsy, there were no significant gross lesions in affected natural and experimental poisoned cattle. The microscopic lesions of both were specifically localized in the cerebellum. All the other tissues were normal.

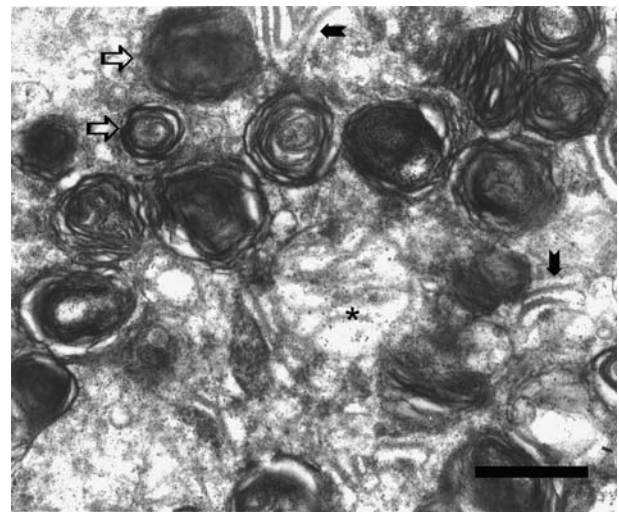
Our estimates of Purkinje-cell population suggests there were fewer Purkinje cells in the heifers poisoned under

natural conditions than the control animal. Perikaryon vacuolation was present in Purkinje neurons. Persisting Purkinje neurons had variable damage: some had swollen, pale, eosinophilic cell bodies with fine vacuolation of perikaryon (Fig. 3); others contained one or more large vacuoles. The nuclei of these cells were distended or pyknotic and displaced to the cell margin. The ultrastructural study of degenerative Purkinje cells showed perikarya filled with heterogeneous residual bodies that consisted of homogeneous areas (possibly lipids) admixed with electron dense granules and membrane debris. Degenerative cells also have lamellar arrays of endoplasmic reticulum without ribosomes (Fig. 4).

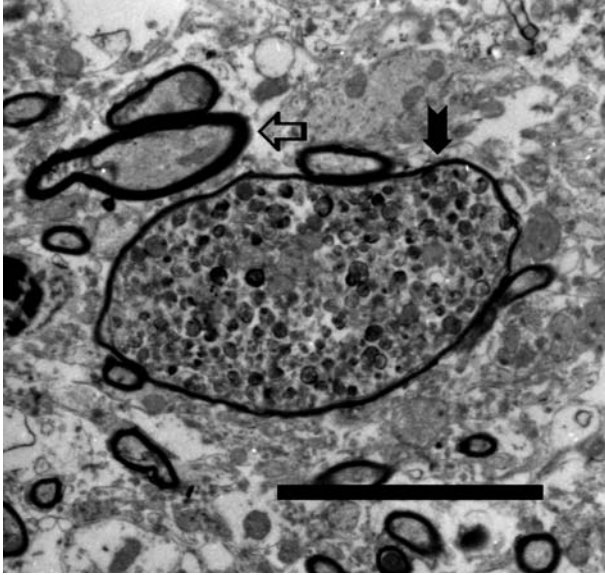
The cerebellum of the naturally poisoned heifers and severely affected dosed steers also had gliosis, axonal spheroids, and macrophages within microcavitations in the white matter of the cerebellar folia (wallerian-type degeneration). Ultrastructurally, these transversely sectioned axonal spheroids showed swollen myelinated axons that contained electron-dense residual bodies, swollen mitochondria, and an increase in the ratio of axoplasm/myelin (Fig. 5).



**Figure 3.** Cerebellum of heifer 1 with naturally occurring *S. bonariense* toxicosis. Note vacuolation of perikarya in Purkinje cells (black arrows). Toluidine blue, 720 $\times$ . Bar = 24  $\mu$ m.



**Figure 4.** Transmission electron micrograph. Cerebellum. Steer 1. Higher magnification of a Purkinje cell perikaryon filled with vesicles (unfilled arrows), lamellar arrays of endoplasmic reticulum (black arrows), and a mitochondrion (black asterisk). Bar = 0.5  $\mu$ m.



**Figure 5.** Transmission electron micrograph. Cerebellum. Heifer 1. Axonal spheroids in the white matter. Note increased axoplasm/myelin thickness ratio in axonal spheroid (black arrow) compared to an adjacent normal axon (unfilled arrow). Bar = 5  $\mu$ m.

Histologic lesions found in experimentally dosed steers were similar to those observed in field cases. Higher dose resulted in increasingly severe cerebellar lesions, that were evident histologically as well as in our estimates of Purkinje-cell populations and degenerative axons (Table 1). The control animal did not have cerebellar lesions.

*Solanum bonariense* L. is an indigenous perennial shrub found in Uruguay, southern Brazil, and northeastern Argentina.<sup>8</sup> It also occurs as a naturalized weed in Europe.<sup>2</sup> Although other similar South American species have been suggested as causative agents of cerebellar degeneration in cattle,<sup>4,6,9,13,15</sup> field cases or experimentally induced cerebellar degeneration from ingestion of *S. bonariense* L. have not been previously demonstrated.

Clinical signs and cerebellar lesions found in cattle in this study were similar to those described in other cases of poisoning in ruminants from other *Solanum* spp.<sup>1,4,6,7,10,11,13,15,16</sup> These findings confirmed that spontaneous and experimentally poisoned cattle developed a degenerative vesicular storage-like disease specific for cerebellar Purkinje cells. The vesicles observed in Purkinje-cell perikarya are most likely lysosomes. In this study, absence of ribosomes associated with endoplasmic reticulum in Purkinje cells confirmed protein synthesis alteration. Similar findings were reported in others neurodegenerative diseases.<sup>4,15,16</sup> Degenerative axons contained not only lysosomes, but also exhibited exocytic or endocytic vesicles accumulated as a result of protein synthesis alteration and consequent cytoskeletal distortion. The vesicle accumulation in Purkinje cell perikarya and axonal spheroids in poisoned cattle suggest that altered axonal transport may play a role in pathogenesis. Further

study is needed to determine if there is plant toxin inhibition of some glycosidase or enzyme of glycoprotein processing.

Similar to toxicity from *Solanum kwebense*<sup>10</sup> and *Solanum fastigiatum*,<sup>13</sup> our findings demonstrate the role of *S. bonariense* in the etiology of the neurological disease syndrome in cattle in Uruguay. Interestingly, as with other plant-induced storage diseases, experimentally poisoned cattle had to consume considerable quantities of the shrub for lengthy periods of time before clinical signs became apparent. *S. bonariense* appear to be toxic in all its growth stages as no seasonal incidence was found in clinical cases. We suspect that *Solanum* is not very palatable, and is only eaten in large quantity when other, more desirable forage is lacking.<sup>13</sup> As many Uruguayan Holstein heifers are placed into *Solanum*-infested pastures after weaning at 4 to 5 months of age, and maintained there until 2 to 3 months before parturition poisoning is likely. As the neurologic lesions are unlikely to resolve, this practice should be modified so that these heifers are not exposed to *S. bonariense*.

*Solanum bonariense* appears to induce only cerebellar lesions without lesions in other tissues. Therefore, it is important to differentiate the toxic syndrome from *Solanum* from other neurological diseases. For example, toxic plants containing the alkaloids swainsonine or calystegines also cause cerebellar degeneration; swainsonine is a potent alpha-mannosidase inhibitor that mimics inherited alpha-mannosidosis, and the calystegines play a similar role as beta-glycosidase inhibitors.<sup>3</sup> Of course the history, clinical signs, and histopathology would be essential in differentiating the sequelae of poisoning from other forms of cerebellar degeneration caused in utero by bovine viral diarrhea (BVD) virus,<sup>17</sup> cerebellar abiotrophy,<sup>14</sup> or congenital cerebellar cortical degeneration.<sup>14</sup> Further studies using *S. bonariense* and cattle are needed to further define the enzyme inhibited, the cytoskeletal alteration, and the storage contents in vesicles of Purkinje cells and axonal spheroids of affected cattle.

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### References

1. Bourke CA: 1997, Cerebellar degeneration in goats grazing *Solanum cinereum* (Narrawa burr). *Aust Vet J* 75:363–365.
2. Bruneton J: 2001, Solanaceae (Solanáceas). *In: Plantas tóxicas: vegetales peligrosos para el hombre y los animales*, pp. 447–481. Editorial Acribia, Zaragoza, España.
3. de Balogh KKIM, Dimande AP, van der Lugt JJ, et al.: 1999, A lysosomal storage disease induced by *Ipomoea carnea* in goats in Mozambique. *J Vet Diagn Invest* 11:266–273.
4. de Barros SS, Riet-Correa F, Andujar MB, et al.: 1987, *Solanum fastigiatum* var. *fastigiatum* and *Solanum* sp. poisoning in cattle: ultrastructural changes in the cerebellum. *Pesq Vet Bras* 7:1–5.
5. Linnaeus C: 1753, *Solanum bonariense*. *In: Sp Pl* 1:185.
6. Medeiros RMT, Guilherme RF, Riet-Correa F, et al.: 2004, Experimental poisoning by *Solanum paniculatum* (jurubeba) in cattle. *Pesq Vet Bras* 24(Suppl):41.
7. Menzies JS, Bridges CH, Bailey EM: 1979, A neurological disease of cattle associated with *Solanum dimidiatum*. *South-western Vet* 32:45–49.
8. Morton CV: 1976, *Solanum bonariense* L. *In: A revision of the Argentine species of Solanum*, pp. 219. Publicaciones de la Academia Nacional de Ciencias, Córdoba, Argentina.
9. Paulovich FB, Portiansky EL, Gimeno EJ, et al.: 2002, Lectin histochemical study of lipopigments present in the cerebellum of *Solanum fastigiatum* var. *fastigiatum* intoxicated cattle. *J Vet Med A* 49:473–479.
10. Pienaar JG, Kellerman TS, Basson PA, et al.: 1976, Maldronksiekte in cattle: a neuronopathy caused by *Solanum kwebense* N. E. Br. *Onderstepoort J Vet Res* 43:67–74.
11. Porter MB, Mac Kay RJ, Uhl E, et al.: 2003, Neurologic diseases putatively associated with ingestion of *Solanum viarium* in goats. *J Am Vet Med Assoc* 223:501–504.
12. Reynolds ES: 1963, The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J Cell Biol* 17:208–212.
13. Riet-Correa F, Mendez MC, Schild AL, et al.: 1983, Intoxication by *Solanum fastigiatum* var. *fastigiatum* as a cause of cerebellar degeneration in cattle. *Cornell Vet* 73: 240–256.
14. Schild AL, Riet-Correa F, Portiansky EL, et al.: 2001, Congenital cerebellar cortical degeneration in Holstein cattle in Southern Brazil. *Vet Res Comm* 25:189–195.
15. Summers BA, Cummings JF, de Lahunta A: 1995, Degenerative diseases of the Central Nervous System. *In: Veterinary neuropathology, Degenerative diseases of the Center Nervous System*, pp. 216, 264–265. Mosby-Year, USA.
16. Van der Lugt JJ: 2002, Cerebellar cortical degeneration in cattle caused by *Solanum kwebense*. *In: The clinicopathology and pathology of selective toxicoses and storage diseases of the nervous system on ruminant in Southern Africa*, PhD Thesis, pp. 49–65. Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.
17. Wilson TM, de Lahunta A, Confer L: 1983, Cerebellar degeneration in dairy calves: clinical, pathologic, and serologic features of an epizootic caused by bovine viral diarrhea virus. *J Am Vet Med Assoc* 183:544–547.