**NOTE** *Clinical Pathology*

# Suspected natural lysosomal storage disease from ingestion of pink morning glory (*Ipomoea carnea*) in goats in northern Argentina

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ABSTRACT. This study describes an occurrence of pink morning glory (*Ipomoea carnea*) intoxication in goats in northern Argentina. The clinical signs displayed by the affected animals were ataxia, lethargy, emaciation, hypertonia of the neck muscles, spastic paresis in the hind legs, abnormal postural reactions and death. The clinico-pathologic examination revealed that the affected animals were anemic and their serum level of aspartate aminotransferase was significantly increased. Cytoplasmic vacuolation in the Purkinje cells and pancreatic acinar cells was observed by histological examination. The neuronal lectin binding pattern showed a strong positive reaction to WGA (*Triticum vulgaris*), sWGA (succinylated *T. vulgaris*) and LCA (*Lens culinaris*). Although *I. carnea* is common in tropical regions, this is the first report of spontaneous poisoning in goats in Argentina.

KEY WORDS: Argentina, goat, *Ipomoea carnea*, neurotoxicity, spontaneous poisoning

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Swainsonine-containing plants, such as *Swainsona galegifolia, Oxytropis* sp.*, Astragalus* sp.*, Ipomoea* sp.*, Turbina cordata* and *Sida carpinifolia*, cause a glycoprotein storage disease in livestock, which has been described in many countries, including China [4], the United States [13], Mozambique [6], Brazil [2, 3, 7, 9] and Argentina [5, 10, 11]. These poisonings were characterized by abnormal behavior of the affected animals, consisting of weakness, loss of appetite, ataxia, recumbence and death [2–4, 6, 7, 9].

*I. carnea* subsp. *fistulosa* naturally grows from Argentina to the southern U.S.A., and it was introduced to the tropics of the Eastern Hemisphere and Hawaii, where it often succeeded in escaping cultivation [6, 15].

*I. carnea* toxicosis was experimentally reproduced in the Corrientes province of northern Argentina [10], because it is the most prevalent toxic plant in the region. However, this is the first report of a spontaneous disease in goats by *I. carnea* in Argentina. The aim of this study is to show the clinical signs, biochemical alterations and histopathological and lectin-histochemical analysis of the spontaneous intoxication by *I. carnea* and to compare both the natural and experimental cases previously reported.

In August 2013, an Anglo-Nubian 4-month-old, 18 kg female goat from Puerto Gonzalez, Corrientes Province, was treated at the Animal Hospital, Faculty of Veterinary Sciences, Northeast National University (UNNE), Argentina. This goat was clinically evaluated and showed suggestive signs of *I. carnea* (Table 1). The farmer reported that the animal first showed the clinical signs a month before. A few days later, our group visited the farm where the disease was occurring. A clinical examination was performed on 7 goats (including the goat examined in the Hospital) and consisted of evaluation of the posture, the behavior and spontaneous and induced movements. Venous blood was collected from the 7 goats, and the following parameters were measured by routine methods: hemoglobin, hematocrit, total erythrocyte and leukocyte counts and the activities of aspartate transaminase (AST) and lactate dehydrogenase (LDH). Two animals (including the goat examined in the Hospital) were sacrificed after staying recumbent for a few days; this occurred after 2 months of intoxication. No gross findings were observed in the different organs. Fragments of several organs, including the central nervous system, were fixed in 10% formalin for 24 hr, routinely processed and stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS). Cerebellum samples from the goat were submitted to lectin histochemical procedures using biotinylated lectins (Vector Laboratories, Burlingame, CA, U.S.A.), as previously described [5] (Table 2). The values are expressed as the mean ± standard deviation (SD). The statistical significance was determined by the *t*-test. Statistical analysis was performed using Infostat software. The level of significance was set at $P \leq 0.05$.

The intoxication occurred on a small farm in Puerto Gonzalez, 50 km from the city of Corrientes. The farm was native field with little forage available for goats and the presence of potentially toxic plants, which were collected and submitted for botanical identification at the Institute of Botany, Faculty of Agricultural Sciences (UNNE-CONICET) in Corrientes,

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Table1. Clinical signs of goats spontaneously intoxicated with *Ipomoea carnea*

| Clinical signs | Case number of goats (n=7) |
|---|---|
| wide-based stance | 1, 2, 3 and 4 |
| ataxia | 1, 2 and 3 |
| lethargy | 1 |
| loss of equilibrium | 1 and 2* |
| Intention tremors | 1 and 2 |
| head and neck tremors | 3 and 4** |
| abnormal postural reaction | 1 and 2 |
| spastic paresis mainly in the hind legs | 1 |
| emaciation | 1, 2, 3, 4, 5, 6 and 7*** |
| palpebral mucous pale | 1, 2, 3, 4, 5, 6 and 7 |

*Euthanized after two months of intoxication. ** Death after disease advanced by three months. *** Goats Nos. 5, 6 and 7 were supplemented, and they recovered.

Table 3. Hematological and biochemical parameters in *Ipomoea carnea*-intoxicated goats

| Parameters | Case number of goats (n=7) 1, 2, 3 and 4[a)] | 5, 6 and 7[b)] |
|---|---|---|
| PCV (%) | 21.00 ± 3.06* | 30.00 ± 1.00 |
| Hb (g/d*l*) | 8.33 ± 1.84 | 10.93 ± 0.62 |
| RBCs (million/$\mu l$) | 5.33 ± 1.41* | 7.50 ± 0.26 |
| WBCs (thousands/$\mu l$) | 12.80 ± 2.21 | 12.73 ± 1.81 |
| AST (U/L) | 326.00 ± 70.67* | 51.67 ± 22.55 |
| LDH (U/L) | 103.25 ± 27.43 | 115.33 ± 33.65 |

a) Goats with signs of neurological dysfunction. b) Goats without neurological signs evidence. *Values ± SD (standard deviation) are significantly different ($P \leq 0.05$) compared with other goats (5, 6 and 7). PCV, packed cell volume; Hb, hemoglobin; RBCs, red blood cells; WBCs, white blood cells; AST, aspartate transaminase; LDH, lactate dehydrogenase.

Table 2. Lectins used in the histochemical study and their major specificities

| Acronym | Source | Major specificity |
|---|---|---|
| WGA | *Triticum vulgaris,* Wheatgerm | D-N-acetyl chitobiose, N-acetyl lactosamine and some sialyl residues |
| sWGA | Succinyl-WGA | β-(1-4)-D-N-acetyl-glucosamine |
| Con-A | *Concanavalina ensiformis* | α-D-glucose and α-D-mannose |
| LCA | *Lens culinaris* | D-mannose and D-glucose |
| BS-I | *Bandeirea simplicifolia*-I | α-D-galactose |

Argentina. Plants, such as *Melia azederach* and *Cestrum strigilatum*, had been ruled out as the cause of the poisoning. However, the plant identified as *I. carnea* subsp. *fistulosa*, known for its toxicity in goats, was considered for further investigation and to elucidate the cause of intoxication in the present research. The toxicological analysis was not determined.

All of the goats lost weight and had pale mucous membranes, but their appetite appeared to be normal. The most important signs are presented in Table 1. Many clinical signs were exacerbated when the goats were disturbed or frightened, often causing the goats to lose equilibrium and fall to their sides or backwards (Fig. 1).

Table 3 shows the hematological and biochemical parameters. Goats 1, 2, 3 and 4 showed an incremental difference in the AST activity level when compared to goats 5, 6 and 7. The results of the hematological parameters revealed that the hematocrit and number of erythrocytes were lower in goats 1, 2, 3 and 4 ($P$=0.04). These animals tended to have lower hemoglobin concentrations than the other group, but no significant difference ($P$=0.07) was seen. The other parameter, white blood cells (WBCs), was similar in all of the animals.

The main lesions were detected in the cerebellum and pancreatic tissue of both killed goats. These lesions were limited to the Purkinje cells in the cerebellum and consisted of a distended perikaryon with fine vacuolization (Fig. 2). The size of the vacuoles in these cells ranged between 0.35–2.60 $\mu$m; severe loss of Purkinje cells and focal gliosis were observed. No vacuoles were observed in the neurons from other regions of the central nervous system (CNS). Occasionally, axonal spheroids and chromatolysis were visualized in the pons and obex. The exocrine pancreas revealed the presence of small cytoplasmic vacuolation of the epithelial cells. However, the content of the vacuoles within the Purkinje cells and acinar pancreatic cells was negative for carbohydrates by PAS stain. Lectin binding demonstrated stored material in numerous cells that appeared normal with the HE stain. The cytoplasm of the Purkinje cells, neurons of the deep cerebellar nuclei, cytoplasm of multiple cells of the cerebellar granular and molecular layers of all affected goats were strongly stained with LCA, WGA and sWGA (Fig. 3). BS-I binding was absent to poor in the studied cases.

Clinical signs and serum enzyme modifications were observed in the current study. These are similar to those previously described in both spontaneous and experimentally induced poisoning with *I. carnea* [2, 10], and these aspects were reported in goats from other swainsonine-containing plants, such as locoweed, other *Ipomoea* sp. and *Sida carpinifolia* [3, 4, 7, 11]. Goats 1, 2, 3 and 4 showed elevation of the serum AST. This enzymatic modification suggested that the nervous signs were a consequence of hepatic damage rather than primary involvement of the brain and spinal cord [6]. In the present study, this suggestion could not be confirmed.

Moreover, according to previous authors, the hematological changes observed in this study indicate the presence of anemia in animals; this is a conspicuous effect of *I. carnea* toxicity in goats and is supported by other authors who study the effects of the plant on both goats and sheep [6, 12]. In our study, the anemia was not characterized.

There were no significant differences in the clinical findings between the spontaneously poisoned goats of our current study and experimentally poisoned animals [2, 10, 14].


Fig. 1. Goat with nervous signs of *Ipomoea carnea* poisoning. This shows abnormal posture and wide–based stances.

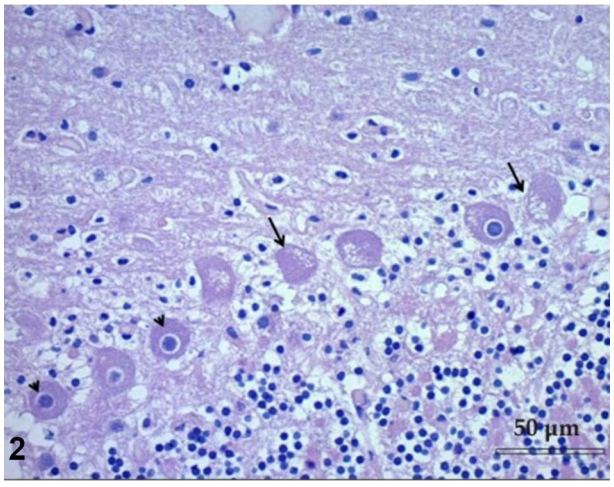


Fig. 2. Cerebellum from a goat with nervous signs of *Ipomoea carnea* poisoning. Fine granular vacuolization in Purkinje perikarya (arrows) and chromatolysis (arrowhead). HE. 50 $\mu$m.

Histopathological evaluation of the intoxicated animals showed a fine vacuolar degeneration of the Purkinje cells in the cerebellum with gliosis. The lesions were restricted to the cerebellum in the CNS. However, histologic lesions in animals poisoned by swainsonine-containing plants have been found all over the CNS, including the cerebral cortex, thalamus, midbrain and spinal cord [4, 9, 10, 13]. In addition, the vacuoles were PAS-negative, similar to previous studies of α-mannosidosis of different genesis and swainsonine poisoning [2, 13]. Previous studies have demonstrated that histological and ultrastructural analyses only allow the identification of a lysosomal storage disease [8]. Additionally, lectin histochemistry contributes to specific identification of the composition of glycoproteins and glycolipids stored in cytoplasmic vacuoles [1–3, 5, 7].

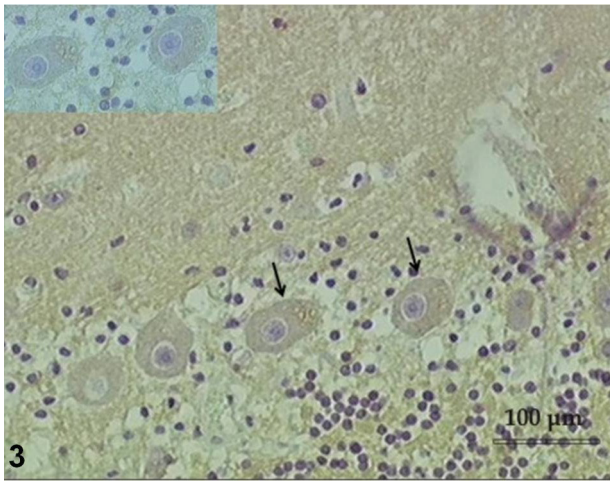


Fig. 3. Cerebellum from a goat with nervous signs of *Ipomoea carnea* poisoning. Strong binding to sWGA in the cytoplasm of Purkinje cells (arrows). An enlarged view is shown on the left. LHQ, Mayer's hematoxylin counterstain, 100 $\mu$m.

In the present study, the accumulated material in the perikarya of the Purkinje cells had marked binding affinities for sWGA, WGA and LCA. Based on these clinical and biochemical studies, we can conclude that acquired intoxication by *I. carnea* in goats resembles in severity that of experimentally intoxicated goats, but the histopathological lesion in our naturally intoxicated animals was less extensive, despite the severity of the clinical signs. However, the intoxication was confirmed by a pattern of lectin binding, which is similar to that observed in naturally occurring or experimental reproduction by *I. carnea*.

Finally, the economic impact on livestock production due to swainsonine-containing plants warrants further investigation.

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