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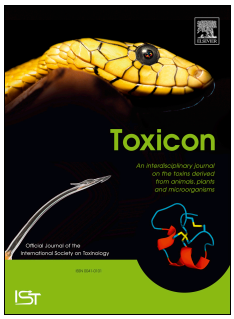

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Case Report

SUSPECTED POISONING IN BEEF CATTLE FROM INGESTION OF *Prosopis nigra* PODS IN NORTH-WESTERN ARGENTINA

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Abstract

The aim of this paper was to present the first report of *Prosopis nigra* poisoning of cattle in Argentina. Outbreaks occurred in five farms located in Salta and Santiago del Estero provinces. All animals were examined, euthanized and necropsied. Clinical signs included tongue protrusion, twitches and tremors of muscles of mastication, weight loss and lethargy. Severe atrophy of the masseter, buccinator and lingual muscles was observed, along with neuronal vacuolation in the nuclei of the trigeminal, facial, and hypoglossus nerves. These findings and the clinical signs are consistent with results obtained in animals, spontaneously and experimentally intoxicated with *Prosopis juliflora* in previous studies. Several species of this genus are native to Argentina. Farmers should be warned about the suspected toxicity by *Prosopis nigra*, since this species has wide geographical distribution in the country.

Keywords: cattle; mesquite pods; neuronal damage; nervous signs; *Prosopis*-poisoning.

1. Introduction

The genus *Prosopis*, family *Leguminosae* (Fabaceae), subfamily *Mimosoideae*, is native to the Americas, Africa and Asia, and comprises a great number of species (Pasiecznik et al., 2001). In Argentina, the species of this genus are known as "Algarrobos" (Verga et al., 2009). Its pods are a source of animal feed in many regions of the world due to their nutritional value (Silva et al., 1981; Silva et al., 1990; Riet-Correa et al., 2012). Despite this, spontaneous cases of intoxication have been observed in animals that remain in areas occupied by "algarrobos" for more than a year or longer than a fruiting period (Lima et al., 2004, Assis et al., 2009).

To date, all reports of toxicity by the genus *Prosopis* in animals have been associated with consumption of *P. juliflora* pods (Lima et al., 2004; Tabosa et al., 2004; Silva et al., 2006; Assis et al., 2009; Câmara et al., 2009). Spontaneous intoxication by *P. glandulosa* in goats was also reported (Washburn et al., 2002). Moreover, a spontaneous poisoning of goats by *Prosopis* sp. pods in Argentina has been recently reported (Micheloud et al., 2018).

The animals poisoned by *P. juliflora* pods exhibited oral dysphagia, masseter atrophy, tongue protrusion, mandible slackening, and progressive weight loss, leading to death (Câmara et al., 2009; Almeida et al., 2017). Experimental studies concluded that lesion in cranial nervous nuclei was the primary damage and that it induced the clinical signs (Tabosa et al., 2000; Almeida et al., 2017).

This paper describes five outbreaks of nervous disease in cattle associated with consumption of *Prosopis nigra* pods, including clinical and epidemiological aspects of the disease, and the gross and microscopic lesions identified.

2. Case report

2.1 *Clinical and epidemiological findings*

A diagnostic visit was made to five farms due to suspected signs of *P. nigra* poisoning reported by the owners. Three of the affected herds were in common grazing areas, located in the region of San Carlos in Salta province. The other two outbreaks occurred in commercial breeding herds located in Tolombón (Salta province) and Frías (Santiago del Estero province). The five holdings were dedicated to beef cattle production based on grazing of natural pastures and fruits of native trees (Fig 1 A-D).

2.2 *Botanical identification*

In the grazing areas the species most closely associated with poisoning was *Prosopis nigra*. The estimated consumption time, and incidence, mortality and lethality data are shown in Table 1. Part of the plant material from grazing areas was collected and submitted to herborization. All samples were identified as *Prosopis nigra* and recorded as specimen MCNS 12880 at the MCNS Herbarium of National University of Salta. Data about sampling sites, *P. nigra* tree/ha and relative abundance are presented in Table 2. Abundance was calculated by counting the percentage of adult trees (*P. nigra*) per unit area and is expressed as the average of the count of 10 randomly selected areas.

2.3 *Clinical findings*

Animals showing symptoms of intoxication were subjected to clinical examination, which included evaluation of the posture, behaviour and spontaneous and induced movements. The salient clinical signs consisted of weight loss and head tilting during chewing; some of these animals had twitches and tremors of the masticatory muscles. Decreased lingual tone was observed, and even 20-25% of the animals exhibited tongue protrusion of about 5 and 10 cm (Fig. 2A, B). Abnormal movements of mastication and

notorious masseter atrophy were observed. Finally, the most severely affected animals had total paralysis of the lower jaw and inability to close the mouth. Thus, the jaw was loose and showed pendulous movements.

2.2. *Pathological findings*

Four of these animals were euthanized and subsequently necropsied during the visit. At necropsy, general poor condition, serous fat atrophy in the omentum, and perirenal and pericardial fat were observed, In addition, varying degrees of atrophy of the muscles of mastication (masseter and temporal muscles) were identified (Table 3). In all cases, the presence of *Prosopis* sp. seed in the rumen content was abundant. Later, fragments of tissue, including the central nervous system and muscles of mastication, were collected in 10% buffered formalin, processed by routine histological techniques and stained with hematoxylin and eosin (HE). Degenerative changes were observed, with cytoplasmic hypereosinophilia, loss of striations and fragmentation of muscle fibres. In some muscle fascicles, degeneration was so severe that muscle tissue was replaced with abundant connective tissue and hyaline floccules. All cases had focal or diffuse mononuclear infiltration of varying degrees. In the processed brain samples, focal gliosis was observed with diffuse vacuolization and swelling of some neuronal bodies in the trigeminal, facial and hypoglossus motor nuclei (Fig. 2.C, D).

3. Discussion

Diagnosis of intoxication by *Prosopis* sp. is based on epidemiological data, clinical signs and pathological findings. The average time of consumption of *Prosopis nigra* pods in the five outbreaks was estimated in 92 days. In bovines experimentally fed with *Prosopis juliflora* pods, clinical signs were observed between days 45 and 75 after the beginning of

the experiment (Tabosa et al., 2006). Goats seem to be more resistant than cattle, since the signs were observed after 240 days of exposure to diets with 60% of *P. juliflora* pods (Tabosa et al., 2000). However, Almeida et al. (2017) showed that sheep became intoxicated after consuming a diet composed of about 80% of *Prosopis* for 21 months (> 600 days), suggesting that sheep are much more resistant to poisoning than cattle and goats. The epidemiological data (incidence, mortality and lethality) were similar to those reported elsewhere for a natural outbreak of *P. juliflora* poisoning in cattle (Câmara et al., 2009, Kingsbury 1964).

All the observed outbreaks occurred at sites where *P. nigra* was the most abundant species (90% approximately), with a very high density (106.8 adult trees/ha). This plant density ensures a good supply of pods for the animals. This assumption agrees with findings obtained by experimentally poisoning bovines with *P. juliflora* pods (Tabosa et al., 2006); in that study, the disease was evident when the supply of pod exceeded 60% for a prolonged period.

In the present study, one of the early clinical signs was faint tremors of the lower jaw, which progressed to severe disorders characterized by rapid mastication and alterations in chewing movements. In more advanced stages of intoxication, evidence of masseter muscle atrophy, weight loss and lethargy was observed. Tongue protrusion was observed in 20% of the animals, which may have been due to paralysis of the lower jaw. All the clinical signs described are in agreement with information obtained from cattle naturally and experimentally intoxicated with pods of *P. juliflora* (Figueiredo et al. 1995; Tabosa et al., 2006; Câmara et al., 2009).

Histopathological studies of the masseter, buccinator and lingual muscles revealed different atrophy degrees. These lesions can explain the disorders in chewing and

swallowing in affected animals; and muscle atrophy is, most probably, due to disorders of innervation, which were confirmed through lesions found in nuclei of the trigeminal, hypoglossal and facial nerves. These lesions were described in cattle (Tabosa et al., 2006; Câmara et al., 2009), sheep (Almeida et al., 2017) and goat (Tabosa et al., 2000) intoxicated with *Prosopis juliflora.*

Neuropathic muscular atrophy has been reported as one of the most common causes of muscle atrophy in animals (Jubb et al., 2007; Valberg 2010). Muscles fibres require a neural stimulation of low tone for maintenance; when there is no neural stimulation, the fibres get retracted and can suffer degeneration and pyknosis (Valberg, 2010). All the findings reported here are consistent with those mentioned by Tabosa et al. (2006) in experimental poisoning with *Prosopis juliflora* in Brazil.

P. juliflora contains piperidine alkaloids, such as juliflorine, julifloricine, julifloridine, juliprosinene, juliflorinine and juliprosopine (Ahmad et al., 1986, Ahmad et al., 1989). According to those authors, these alkaloids are responsible for producing the damage of the mitochondria in neurons of central nuclei (Maioli et al., 2012; Silva et al., 2013). It seems reasonable to speculate that these alkaloids are also the toxic compounds in *P. nigra*. However, the toxic components were not isolated from the plant in this study. Thus, further studies are needed to confirm this hypothesis and to identify the specific toxin and its concentration in the plant. Despite the positive effects of pods as source of food in animals (Riet-Correa et al., 2012), these results suggest that consumption, in large quantities and over a long period can be dangerous. The findings described suggest that the consumption of pods of native *Prosopis* species (*P. nigra*) produces the nervous damage and clinical signs, as observed in cattle poisoning by *Prosopis juliflora* in others countries.

To date, the only *Prosopis* species reported as toxic have been *P. juliflora* (Tabosa et al., 2006, Câmara et al., 2009) and *P. glandulosa* (Washburn et al., 2002). In Argentina, there are 47 native species of this genus (Flora Argentina, 2017). Farmers should be warned about the suspicion of the toxicity of this species, since *P. nigra* has wide geographical distribution in the country.

CONFLICT OF INTEREST

No competing financial interest exists for any of the coauthors of this manuscript.

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229

Figure legends

Fig. 1. A- Grazing areas, locally characterized as "algarrobales", where the dominant species was *P. nigra*. B- Detail of an adult specimen of *P. nigra*. C and D- Inflorescence and fruits of the plant.

Fig. 2. A- Bovine affected by the disease showing partial protrusion of tongue. B- Adult cow tilting the head to prehend the forage. Microscopic image: C- Fibers of masseter muscles, showing degeneration and fragmentation. Abundant mononuclear infiltration is also observed between fibers (H&E 20X).). D- Vacuolation and degenerative changes in neurons (insert) of the trigeminal nucleus (H&E 20X).

Table 1. Epidemiological data (mortality, incidence and lethality) of outbreaks.

Outbreak	Estimated time of P. nigra pod consumption (days)	Incidence (%)	Mortality (%)	Lethality (%)
1	100	15% (9/60)	6.6 % (4/60)	44% (4/9)
2	100	21% (8/38)	13% (5/38)	62% (5/8)
3	100	12.5% (1/8)	12.5% (1/8)	100% (1/1)
4	75	40 % (60/150)	40% (60/150)	100 % (60/60)
5	85	20 (74/300)	20 (70/300)	100% (70/74)
average	**92**	**21.7**	**18.42**	**81.2**

Table 2. Outbreak sites, *P. nigra* density (adult trees /ha) and relative abundance.

Outbreak	Location	GPS	*P. nigra* trees /ha	Adult (*P. nigra*) trees per unit area (%)
1	San Carlos (SAL)*	25°57'15.49"S 65°56'51.82"W	80	88%
2	San Carlos (SAL)	25°55'44.26"S 65°57'20.82"W	75	92%
3	San Carlos (SAL)	25°56'7.71"S 65°57'10.96"W	150	90%
4	Tolombón (SAL)	26°19'10.51"S 65°57'27.57"W	135	99%
5	Frías (SDE)*	28°42'2.59"S 65° 3'46.38"W	94	85%
average			106.8	90.8%

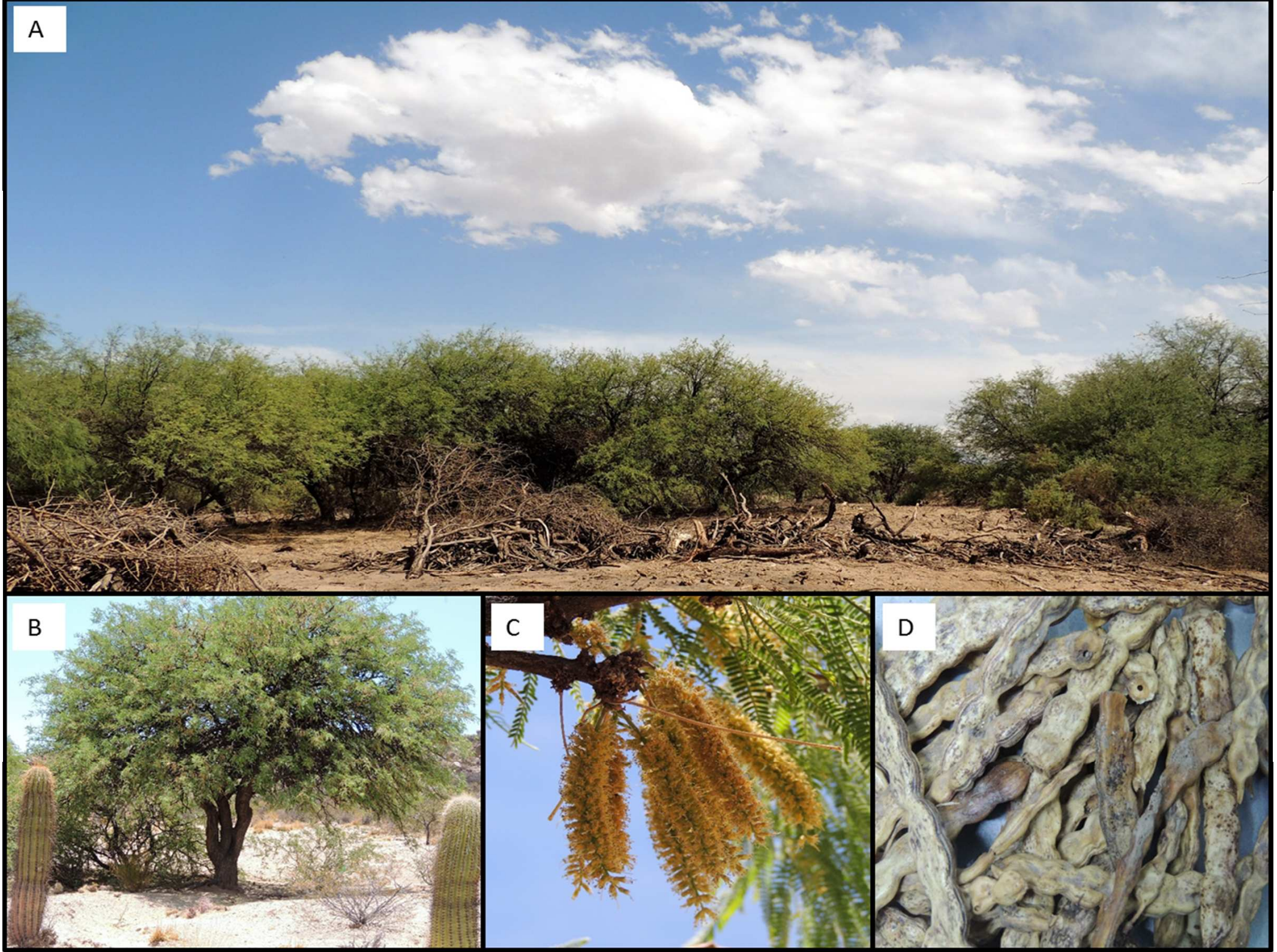
*Salta province (SAL)

*Santiago del Estero province (SDE)

Table 3. Degrees of muscle atrophy in HE-stained tissue sections from cattle spontaneously poisoned with *Prosopis nigra*

Necropsies	Levels of atrophy		
	Masseter muscle	Buccinators muscle	Extrinsic tongue muscles
1	++++	+++	++
2	+++	+	´+
3	++++	++	+++
4	++++	++	+

- = No lesion + = Minors lesions ++= moderate lesions +++ = severe lesions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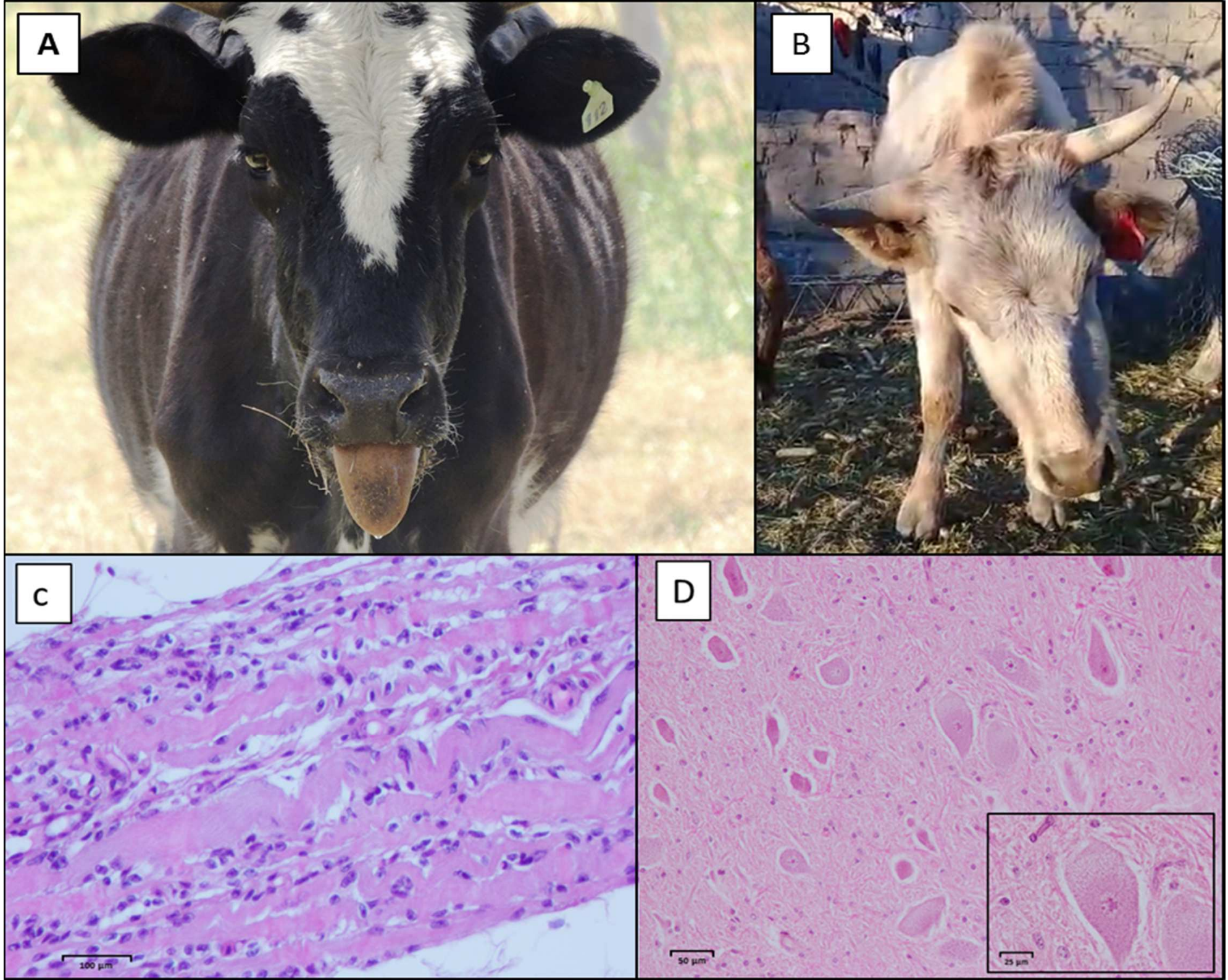
C
D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B
C
100 µm
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50 µm
25 µm

Highlights

- First report providing local information on outbreaks of poisoning by *P. nigra* pods in cattle.
- The macroscopic and microscopic findings were consistent with those observed in poisoning by other *Prosopis* species.
- *P. nigra* has wide geographical distribution in the Argentina, Paraguay, Brazil and Uruguay region.