Toxicon 157 (2019) 84–86

Contents lists available at ScienceDirect

Toxicon

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

Short communication

Spontaneous outbreak of *Astragalus pehuenches* (Fabaceae) poisoning in cattle in Argentina

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ARTICLE INFO

Keywords:
Astragalus pehuenches
Poisoning
Cattle
Swainsonine
Patagonia

ABSTRACT

This is the first report of a spontaneous outbreak of *Astragalus pehuenches* poisoning on a farm in Argentine Patagonia, where 63 out of 70 cattle died. The main clinical signs of affected animals were ataxia, balance loss and progressive emaciation. Purkinje cells presented vacuolation and marginalization of the nucleus. *Astragalus pehuenches* was detected in the paddock as well as in the ruminal content and fecal matter samples of the affected animals. Swainsonine concentrations in *Astragalus* specimens were found to be as high as 0.096%.

In Argentine Patagonia, spontaneous poisoning by *Astragalus pehuenches* Niederl (Fabaceae), commonly known as "Garbancillo" or "Yerba Loca", has been historically reported in horses and sheep (Kauffer and Heinken, 1984; Robles et al., 2000), but never in cattle or goats. Thus, the aim of this paper is to communicate the epidemiological, clinical and pathological findings of a spontaneous outbreak of *A. pehuenches* poisoning recently observed in cattle of this region.

A farm near the locality of Maquinchao (41.61158 S, 68.83936 W), located in Río Negro province, Argentine Patagonia, which breeds Merino sheep and Hereford cattle under an extensive pastoral system, was visited to investigate a nervous disease outbreak in cattle reported by its owner. The farm is located in an ecological area characterized by hills and basaltic plateaus, with a semiarid climate and an annual average rainfall of 200 mm. The landscape is composed of large steppes covered by shrubs and perennial grasses and small meadows covered by annual and perennial grasses (Bran et al., 2000).

The epidemiological data provided by the owner indicated that 63 out of 70 (90%) adult cattle, with neurological clinical signs and progressive emaciation, had died in the field or were euthanized *in extremis* by the owner between February and July 2016. The herd had been grazing a paddock of approximately 2700 hectares. The field, with clear signs of overgrazing, had three patches of *A. pehuenches* of approximately 15 hectares each (Fig. 1). The cattle had started to eat flowered plants in November 2015.

Six of the affected cattle were clinically evaluated. One of them, the most severely affected, was chosen for postmortem examination. Central nervous system samples were fixed in buffered 10% formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin for histopathological evaluation. A sample of ruminal content from the necropsied animal and fecal matter samples from the five remaining clinically affected animals were collected for microhistological analysis (Sparks and Malechek, 1968). A specimen of the plant was collected, pressed, dried and identified as *Astragalus pehuenches* Niederl. by curator Dr. Donaldo E. Bran from CRP Herbarium, INTA Bariloche (CRP #5972). Lastly, ten specimens of *A. pehuenches* were collected from the paddock, dried, mixed and analyzed for swainsonine content by liquid chromatography-mass spectrometry (Gardner and Cook, 2011).

The six cows had a poor body condition, ranging from 1.5 to 2 in a scale from 1 to 5. At clinical examination, the animals showed progressive weight loss, ataxia, dysmetria of the hind limbs, wide-based stance, lateral walking, head tremors, recumbency and difficulties in rising. When the animals were disturbed, they tended to lose equilibrium and an exacerbation of the clinical signs was observed.

Macroscopically, the rumen of the necropsied animal showed undigested *A. pehuenches* stems and seeds (Fig. 2). The microhistological analysis of the ruminal content showed that 48% of the diet of this animal was composed of *A. pehuenches*, 30% of grasses, 19% of several shrubs and 3% of other herbs. The analysis of the feces of the five remaining clinically affected animals showed that their diet was composed of 22% *A. pehuenches*, 48% grasses, 10% shrubs and 20% of other herbs.

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https://doi.org/10.1016/j.toxicon.2018.11.303
Received 24 September 2018; Received in revised form 8 November 2018; Accepted 19 November 2018
Available online 20 November 2018

Fig. 1. Paddock populated by *Astragalus pehuenches* (arrows). Inset: Characteristic leaves and pods of *Astragalus pehuenches*.

Fig. 2. Ruminal content with stems (yellow arrows) and seeds (red arrows) of *Astragalus pehuenches*.

The chemical analysis of the ten plants of *A. pehuenches* showed a swainsonine concentration of 0.096% on a dry weight basis.

The histological examination of the central nervous system samples showed neurons with severe chromatolysis, microvacuolation and marginalization of their nuclei. The main cells affected were the Purkinje cells of the cerebellum and neurons of the hypoglossal, dorsal vagal and trigeminal nuclei from the medulla oblongata. A small number of axonal spheroids were also observed in the medulla oblongata (Fig. 3).

The epidemiological data, clinical signs and microhistological analysis of the fecal matter and ruminal content led to a presumptive diagnosis of *A. pehuenches* poisoning. The lesions observed in neurons from the cerebellum and basal ganglia and the detection of toxic levels of swainsonine in *Astragalus* specimens confirmed the diagnosis.

The morbidity of 100% and the mortality of 90% observed allowed us to characterize this outbreak as severe. These rates are higher than previous cases of *Astragalus* sp. and *Oxytropis* sp. poisoning reported in Argentina (Micheloud et al., 2017) and in other parts of the world like Canada (Harries et al., 1972) and China (Takeda et al., 2014). A previous outbreak of *A. pehuenches* poisoning reported in sheep in the same region was also characterized as severe, with 73% morbidity and 100% mortality (Robles et al., 2000).

The neurological signs observed in this case, like ataxia and incoordination, were consistent with previous reports of spontaneous poisoning by other swainsonine-containing species such as *Sida carpinifolia* (Malvaceae) in cattle in Brazil (Furlan et al., 2009), as well in sheep and goats intoxicated with *Sida carpinifolia*, *Oxytropis glabra* (Fabaceae) and *Astragalus garbancillo* (Fabaceae) (Driemeier et al., 2000; Takeda et al., 2014; Micheloud et al., 2017).

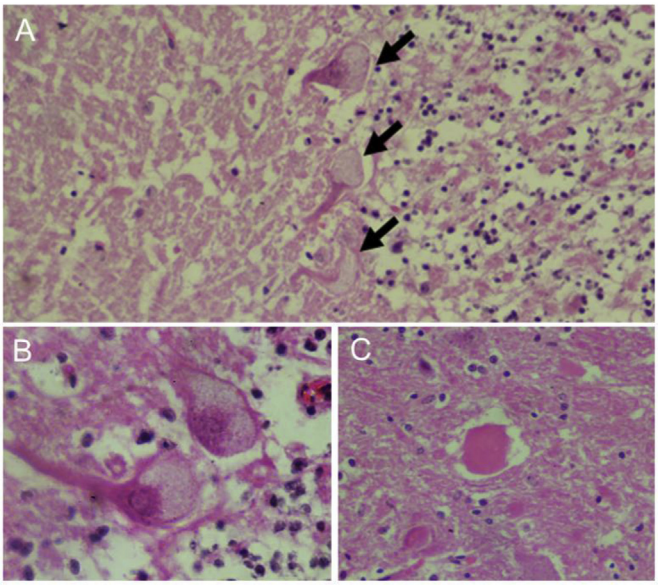

Fig. 3. A) Cerebellum with vacuolated Purkinje cells (arrows). B) Detail of Purkinje cell with marginated nucleus and microvacuoles. C) An axonal spheroid in the medulla oblongata.

The vacuolation observed in Purkinje cells was also similar to cases of intoxication by swainsonine-containing species throughout the world (Driemeier et al., 2000; Furlan et al., 2009; Takeda et al., 2014; Micheloud et al., 2017), and consistent with reports in sheep in Patagonia (Robles et al., 2000). These results support the proposal that vacuolation of Purkinje cells may be considered of high diagnostic value for poisoning by *Astragalus* sp. in Patagonia (Robles et al., 2000). This proposal is supported by the absence of vacuolation in other neurological diseases diagnosed in the region, the absence of other swainsonine-containing plants in Patagonia, and the fact that Argentina is free of other spongiform diseases such as scrapie in sheep or spongiform encephalopathy in cattle (Schudel et al., 1997). Furthermore, a genetic mannosidosis that causes similar lesions, has been reported in Angus, Galloway and Murray Grey cattle elsewhere (Jolly and Walkley, 1997), however these breeds are scarce or not present in Patagonia. Considering that *A. pehuenches* also affects horses and sheep (Kauffer and Heinken, 1984; Robles et al., 2000) this species should be considered an important toxic plant in Patagonia and the poisoning should be included in the differential diagnosis from other diseases affecting the central nervous system of livestock in the region.

The swainsonine concentration in *A. pehuenches* plants found in this outbreak (0.096%) was 96 times above the threshold of toxicity defined as toxic for livestock (Molyneux et al., 1994). This value is similar to the ones found in a study carried out by our group during 2012 (0.07%) and 2013 (0.097%) to determine the swainsonine concentration in *A. pehuenches* populations in Río Negro province (Martinez et al., 2018).

The microhistological analysis of fecal matter is a technique used to identify the diet of herbivores grazing on natural pastures (Sparks and Malechek, 1968). As demonstrated herein, this technique was useful as an auxiliary method for both *post-mortem* and *ante-mortem* diagnosis of intoxication by *A. pehuenches* and may also be useful to diagnose intoxications by other chronic toxic plants.

To our knowledge, this constitutes the first report of *Astragalus* sp. intoxication in cattle in Patagonia, in Argentina and in South America. Taking into account the high levels of swainsonine found in *A. pehuenches*, the wide distribution of this species in the province and the poor condition of the natural pastures that leads the animals to consume *Astragalus*, we conclude that *Astragalus pehuenches* should be considered an important toxic plant for livestock with high mortality rates in Patagonia.

Acknowledgments

We thank Mr. Nelson Botte for allowing us to work in his farms. This work was supported by the National Institute for Agricultural Technology (INTA), Argentina, with funds from the Regional Animal Health System -SIRSA- INTA Bariloche; Regional Project 1281102 “Development of the semiarid region of Río Negro province” and National Project 1115054 “Diagnosis and surveillance of complex and emerging diseases”.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.toxicon.2018.11.303.

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