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Hybanthus calceolaria poisoning in cattle

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Abstract. *Hybanthus calceolaria*, also known as “papaconha” or “ipepacuanha,” is a herbaceous plant found in northeastern Brazil, which is often implicated by farmers as the cause of neurological signs in livestock grazing. Several poisoning outbreaks associated with the ingestion of this plant were observed in cattle in the municipalities of Colônia de Gurguéia in the state of Piauí and Sirinhaém in the state of Pernambuco, Brazil. The main clinical signs were ataxia, recumbency, and myokymia. No significant lesions were observed during necropsy or on histological examination. The disease was experimentally reproduced by the administration of 2 daily doses of 40 g/kg/body weight of the fresh green plant containing fruits. The plants without fruits were nontoxic, which is in accordance with the farmers’ information, as it was stated that the poisoning only occurs when the plant is fruiting.

Key words: Ataxia; cattle; myokymia; papaconha; toxic plants.

Hybanthus calceolaria (L.) Baill. (Fig. 1A–1D), commonly known as “papaconha” and “ipepacuanha,” is an herbaceous plant of the *Violaceae* family that is found in northeastern Brazil, mainly in the states of Ceará, Rio Grande do Norte, Bahia, and Piauí. In Piauí, farmers often mention *H. calceolaria* as a cause of neurological signs and diarrhea in cattle, sheep, and goats^{3,5}; however, spontaneous cases of poisoning by this plant have not been reported. The toxicity of *H. calceolaria* has been experimentally tested in cattle on several occasions using different doses. However, in only 1 of the experiments, the administration of single doses of 10, 20, or 30 g/kg/body weight (g/kg) of fresh plants to calves in 1993, caused digestive signs and lesions of edema and necrosis in the rumen epithelium.⁵ In experiments performed by the current authors, the plant was administered to goats and sheep at daily doses of 40 g/kg for periods of 4–30 days, and no clinical signs were observed (unpublished data). The aim of the current report is to describe the epidemiological, clinical, and pathological aspects of spontaneous *H. calceolaria* poisoning in cattle and report the experimental reproduction of the disease.

Farms in the municipalities of Colônia de Gurguéia in the state of Piauí and Sirinhaém in the state of Pernambuco were visited in March and August 2013, respectively, to collect epidemiological data and observe the clinical signs in cattle reported to be poisoned by *H. calceolaria*. Specimens of the plant collected on these farms were identified by Professor Odaci de Oliveira at the Federal Rural University of the Semiárido (Mossoró, Rio Grande do Norte, Brazil). A voucher specimen of the plant was authenticated (HCSTR3988) and deposited in the Center for Health and Rural Technology

herbarium of the Federal University of Campina Grande (Campina Grande, Paraíba, Brazil).

In March 2013, in the municipality of Colônia de Gurguéia, the disease occurred on several farms. In the 8 farms visited, a total of 616 cattle were at risk, 25 (4%) were affected, and 17 (2.7%) died. The farmers reported that the disease had occurred in previous years, always when the plant was fruiting, and to control the disease they had removed the herds from the paddocks when the plant was fruiting. In the outbreak observed in August 2013 in the municipality of Sirinhaém, which is in the coastal region of Pernambuco, the cattle were reared in a semiextensive system. The cattle were fed twice a day with elephant grass (*Pennisetum purpureum*) in a corral where they also stayed at night, but the cattle spent most of the day in a native pasture. The first signs were observed 2–3 days after the animals were fed elephant grass mixed with *H. calceolaria* that had been cut in an area invaded by this plant. In a cattle herd containing 21 animals, approximately 1 year of age, 14 animals were affected, and 3 died. According to the farmer, the

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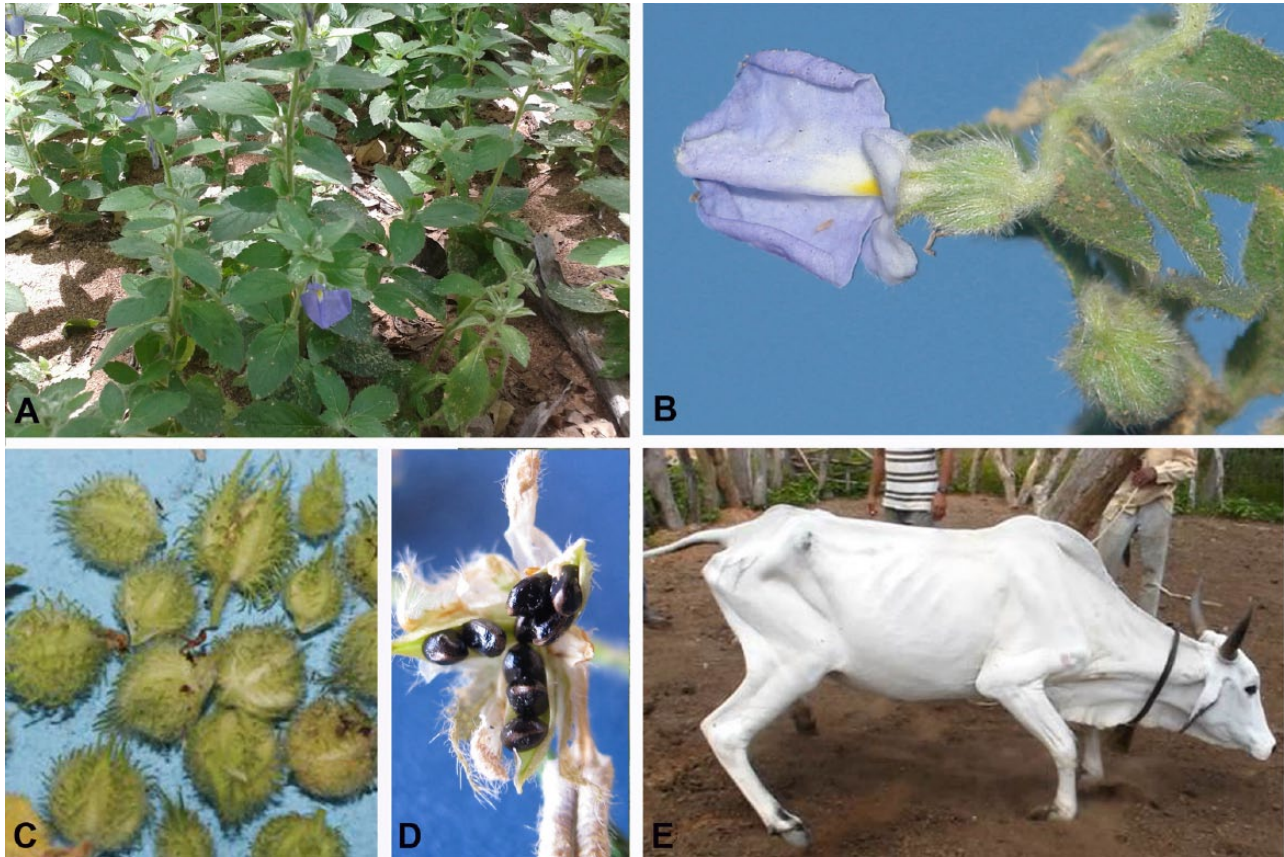


Figure 1. *Hybanthus calceolaria* (A) in the municipality of Colônia de Gurguéia, state of Piauí, Brazil, with flower (B), fruits (C), and fruit with seeds (D). Bovine spontaneous poisoning by *H. calceolaria* in the municipality of Colônia de Gurguéia exhibiting difficulty rising (E).

cattle usually do not eat the plant, unless there is a shortage of forage, and the plant is only toxic at the fruiting stage.

The main clinical signs were ataxia, difficulty rising (Fig. 1E), and muscle tremors, mainly during exercise. At rest or in recumbency, the animals showed myokymia, mainly in limb muscles and the masseter muscle. Anorexia, ruminal hypotony, and soft feces containing the fruits of the plant were also observed. The clinical course varied from 7 to 15 days until death occurred. However, animals that stopped ingesting the plant before recumbency occurred recovered after a few days.

One heifer that remained recumbent for 5 days was euthanized and necropsied. At necropsy, no lesions were observed. However, there was a moderate amount of *H. calceolaria* fruit containing seeds present within the rumen. Samples of the organs from the thoracic and abdominal cavities, oral mucosa, tongue, esophagus, skeletal muscles, thyroid glands, skin, hypophysis, and the central nervous system were collected, fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin wax. Sections were stained with hematoxylin and eosin. Sections of the central nervous system included the frontal, parietal, temporal, and occipital

cortices, corpus striatum, thalamus, rostral colliculi, cerebellar peduncles, cerebellum, pons, obex, and spinal cord (cervical, thoracic, and lumbar segments). Later, serial transverse sections of the brain stem were performed for the histological study of the cranial nerves nuclei. Sections of the trigeminal and root ganglia and the trigeminal and spinal nerves were also studied histologically. No significant changes were observed on histological examination.

For the experimental reproduction of the disease, two 10–12-month-old steers, both weighing 110 kg, were fed the aerial parts of *H. calceolaria* that were collected during the month of March 2013 in the municipality of Colônia de Gurguéia, from properties in which the disease had occurred. Before the experimental period, the animals were subjected to an adaptation period of 20 days, during which the animals ingested Tifton hay (*Cynodon dactylon*) and commercial concentrated feed at amounts equivalent to 1% of their body weight. The fresh plant was orally administered to the 2 steers by repeatedly putting small amounts into their mouths. Bovine 1 consumed 2 daily doses of 40 g/kg of the aerial part of the plant including fruits, whereas bovine 2 ingested 8 daily doses of 20 g/kg without the fruits. Before the start of

the experiment and after the observation of clinical signs, blood samples were collected in Vacutainer^a tubes with and without anticoagulant. Hemograms and the determination of the serum activities of creatinine phosphokinase (CPK), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT), and the serum levels of total protein, albumin, urea, and creatinine were performed in accordance with a previous study.⁴ The experiment was approved by the ethical committee on animal experimentation at the Federal University of Campina Grande (activity CEP 69-2013).

On the third day of the experiment, after ingesting 2 doses of 40 g/kg, bovine 1 was found in the bay in sternal recumbency with myokymia in the muscles of the limbs and face, marked depression, anorexia, congested mucous membranes, engorged episcleral vessels, dehydration, salivation, and presence of mucoid secretion in the nostrils. The animal rose with difficulty, exhibiting ataxia and postural alterations. The corneal, facial, and swallowing reflexes were depressed. There was a marked reduction in tongue tone, which remained outside of the mouth after being pulled out. The heart rate (approximately 160 beats per minute) and respiratory rate (approximately 60 breaths per minute) were increased, and there was an absence of ruminal and intestinal movements. The next day, the animal had mild diarrhea. Due to the anorexia and dehydration, the steer was orally rehydrated. The clinical signs began to decline 3 days after the observation of the first signs, and the steer recovered after a clinical manifestation period of 8 days. Serum activities of CPK, AST, and GGT remained within their normal ranges. No alterations were observed in the hemogram during the experiment.

Bovine 2 showed no clinical signs after ingesting daily doses of 20 g/kg of the plant without fruits, for 8 days. Serum activities of CPK, AST, and GGT, as well as the hemogram, remained within normal values until day 8 after which feeding and monitoring of this animal ceased because no disease had become apparent.

The information obtained in the current study revealed important data on the poisonous nature of *H. calceolaria*. The observation of several outbreaks in 2 different regions, all associated with the presence of the plant, and the experimental reproduction of the disease in a bovine, confirmed the toxicity of *H. calceolaria* and its participation in the observed outbreaks. In the municipality of Sirinhaém, the fact that the disease occurred only when the animals were fed elephant grass mixed with *H. calceolaria* also indicates that this plant was the cause of the disease.

Hybanthus calceolaria poisoning present in the southern part of the state of Piauí is of great importance and has been well known in the community, as numerous farmers have observed the disease for many years. In October 2013, during a visit to the municipality of Conceição do Canindé in southern Piauí, farmers and practitioners claimed that nearly 1,500 out of 6,500 bovines died on 30 farms since March 2013 and that the disease also occurred in other municipalities

of the region, including Simplicio Mendes, Isaías Coelho, Vera Mendes, Campinas do Piauí, Floresta do Piauí, Bela Vista do Piauí, Patos do Piauí, São Raimundo Nonato, João Costa, and Paes Landim in the state of Piauí, and São Francisco de Assis and Nova Santa Rita in the state of Rio Grande do Sul, affecting cattle, sheep, and goats (unpublished data). The years 2012 and 2013 were extremely dry in the Brazilian semiarid region; therefore, the high frequency of the disease may be associated with the abundance of *H. calceolaria* and the lack of other forages.

In the experimental reproduction of the disease, bovine 1, which ingested the plant and fruits, showed clinical signs, whereas bovine 2, which received the plant without fruits, showed no clinical signs, suggesting that *H. calceolaria* is toxic only in the fruiting stage. Although both dose and dosing regimen were different in bovine 2, absence of the fruits seems to be the most likely reason for failure of reproduction of the clinical signs, given information collected from farmers of affected animals. Failure to reproduce the disease in previous reports may have been due to the vegetative state of the plant that was used.

The clinical signs observed in both the spontaneous and experimental poisoning suggest that the disease preferentially affects the nervous system; however, bovine 1 also showed digestive signs, including rumen stasis and soft feces. These digestive signs were also observed during the spontaneous outbreaks. A clinical sign observed both in the experimental and spontaneous cases, which seems to be characteristic of the disease, is myokymia, an involuntary muscle hyperactivity with rhythmic brief discharges that is observed as undulating movements of the overlying skin. Myokymia results from a change in terminal axons of the lower motor neurons, may be generalized or focal, and some forms are inherited.² It occurs in association with different diseases, and is generally considered to result from biochemical alterations in the microenvironment of the axon membrane at any level of the motor unit.^{2,6} There is reasonable evidence that an alteration of the axonal voltage-gated potassium channels is the underlying cause of myokymia.^{2,6} In veterinary literature, widespread myokymia and neuromyotonia have been described in young dogs of the Jack Russell Terrier breed and are associated with a neurodegenerative disease called hereditary ataxia.¹ Focal myokymia has been described in individual cases of dogs with underlying brain disease.^{6,7} In the case of *H. calceolaria* poisoning, studies should be conducted in ruminants and laboratory animals to determine the origin of the myokymia.

In conclusion, *H. calceolaria* is a toxic plant for ruminants of economic importance that is mainly found in southern Piauí. The plant is toxic only in some phases of its growth cycle, most likely during fruiting. Further studies should be conducted to determine the epidemiology and pathogenesis of the disease and to confirm the finding herein that only the fruit of the plant is responsible for the poisoning.

Sources and manufacturers

- a. Vacutainer, BD, São Paulo, Brazil.

Declaration of conflicting interests

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