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Tremorgenic syndrome caused by *Ipomoea pes caprae* in cattle[1]

Flávio A.S. Graça[2], Mariana S. d'Avila[3]*, Ticiana N. França[4], Aníbal G. Armién[5], Marcia F. Rolim[6], Saulo A. Caldas[7], André M. Santos[8], Ileana C. Miranda[9] and Paulo V. Peixoto[10]

ABSTRACT.- Graça F.A.S., d'Avila M.S., França T.N., Armién A.G., Rolim M.F., Caldas S.A., Santos A.M., Miranda I.C. & Peixoto P.V. 2020. **Tremorgenic syndrome caused by Ipomoea pes caprae in cattle.** *Pesquisa Veterinária Brasileira 40(6):443-450*. Departamento de Epidemiologia e Saúde Pública, Universidade Federal Rural do Rio de Janeiro, BR-465 Km 7, Seropédica, RJ 23890-000, Brazil. E-mail: mariana_davila@hotmail.com

Poisonous plants are a significant cause of death among adult cattle in Brazil. Plants that affect the central nervous system are widely spread throughout the Brazilian territory and comprise over 30 toxic species, including the genus *Ipomoea*, commonly associated with a lysosomal storage disease and a tremorgenic syndrome in livestock. We describe natural and experimental *Ipomoea pes caprae* poisoning in cattle from a herd in the Northside of Rio de Janeiro, Brazil. Affected cattle presented episodes of severe ataxia, abnormal posture followed by falling, muscular tremor, contraction, and spasticity, more prominent in the limbs, intensified by movement and forthcoming, and recumbence. Grossly, a substantial amount of leaves and petioles were found in the rumen. Histopathological examination showed degenerative neuronal changes, mostly in cerebellar Purkinje cells, which were confirmed with Bielschowsky silver. The characteristic clinical changes and mild histological lesion strongly suggested a tremorgenic syndrome. Lectin- immunohistochemistry evaluation reinforced this hypothesis; all lectins tested failed to react with affect neurons and Purkinje cells, which ruled out an underlying lysosomal storage disease. One calf given *I. pes caprae* leaves experimentally developed clinical signs similar to natural cases. On the 28th day of the experiment, the plant administration was suspended, and the calf recovered within four days. *I. pes caprae's* spontaneous tremorgenic syndrome in cattle is conditioned to exclusive feeding for several months. We were able to experimentally reproduce toxic clinical signs 12 days following the ingestion.

INDEX TERMS: Tremorgenic syndrome, *Ipomoea pes caprae*, cattle.

RESUMO.- [**Síndrome tremorgênica causada pela ingestão de *Ipomoea pes caprae* em bovinos.**] A intoxicação por plantas tóxicas está entre as três causas de morte mais importantes em bovinos adultos no Brasil. O grupo das plantas que causam alterações neurológicas, muito bem representada no país, encerra mais de trinta espécies tóxicas, entre as quais do gênero *Ipomoea*, amplamente distribuídas no território brasileiro. As plantas tóxicas desse gênero podem causar doenças do armazenamento ou síndrome tremorgênica. Descrevem-se a intoxicação natural e reprodução experimental por *Ipomoea*

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[2] Laboratório de Clínicas e Cirurgia Animal, Universidade Estadual do Norte Fluminense (UENF), Av. Alberto Lamego 2000, Parque California, Campos dos Goytacazes, RJ 28013-602, Brazil.

[3] Graduate Program in Veterinary Medicine, Universidade Federal Rural do Rio de Janeiro (UFRRJ), BR-465 Km 7, Seropédica, Rio de Janeiro, RJ 23890-000, Brazil. *Corresponding author: mariana_davila@hotmail.com

[4] Departamento de Epidemiologia e Saúde Pública, Universidade Federal Rural do Rio de Janeiro (UFRRJ), BR-465 Km 7, Seropédica, Rio de Janeiro, RJ 23890-000, Brazil.

[5] Veterinary Diagnostic Laboratory, Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, United States of America.

[6] Defesa Agropecuária, Secretaria de Estado de Agricultura e Pecuária, Rio de Janeiro, RJ, Brazil.

[7] Departamento de Medicina e Cirurgia Veterinária, Universidade Federal Rural do Rio de Janeiro (UFRRJ), BR-465 Km 7, Seropédica, Rio de Janeiro, RJ 23890-000, Brazil.

[8] Professor assistente UniSociesc, Rua Albano Schmidt 3333, Joinville, Santa Catarina, Brazil.

[9] Section of Anatomic Pathology, College of Veterinary Medicine, Cornell University, Itahaca, NY, United States of America.

[10] Graduate Program in Veterinary Medicine, Universidade Federal Rural do Rio de Janeiro (UFRRJ), BR-465 Km 7, Seropédica, Rio de Janeiro, RJ 23890-000, Brazil.

pes caprae em bovinos, verificada no norte do Estado do Rio de Janeiro. Foram observados episódios de intensa ataxia locomotora, postura anormal seguida de queda, incapacidade de levantar-se, tremores, contrações, espasticidades musculares nos membros, intensificados após estimulação ou a simples aproximação e decúbito. Nos bovinos afetados há mais de 6 meses, os sinais clínicos tornavam-se permanentes. À necropsia havia apenas significativa quantidade de folhas e pecíolos da planta no rúmen. O estudo histopatológico evidenciou lesões neuronais degenerativas principalmente nos neurônios de Purkinje. A impregnação argêntica pela técnica de Bielschowsky ratificou esses achados microscópicos. As lesões histológicas sutis associadas ao quadro clínico indicam que trata-se de intoxicação tremorgênica. O fato de não haver nenhum armazenamento intracitoplasmático, confirmado pelo resultado do estudo lectino-histoquímico (não houve afinidade das lectinas Con-A, WGA e sWGA e de outras lectinas empregadas aos neurônios de Purkinje e outros neurônios afetados), é suficiente para descartar a possibilidade de tratar-se de doença do armazenamento. No bezerro intoxicado experimentalmente verificaram-se sinais clínicos semelhantes, entretanto, com a interrupção do fornecimento da planta no 28º dia, os sinais clínicos desapareceram após quatro dias. *I. pes caprae* causa síndrome tremorgênica espontânea em bovinos, quando ingerida como alimentação exclusiva durante períodos prolongados (muitos meses). Experimentalmente, os primeiros sinais clínicos da intoxicação foram reproduzidos após 12 dias de ingestão da planta.

TERMOS DE INDEXAÇÃO: Síndrome tremorgênica, *Ipomea pes caprae*, bovinos.

INTRODUCTION

According to data from diagnostic laboratories in different regions, between 7.4% and 15.83% of cattle deaths in Brazil are caused by toxic plants (Riet-Correa & Medeiros 2001, Pedroso et al. 2007, Rissi et al. 2007, Casagrande et al. 2008, Assis et al. 2010). Toxic plants of agricultural interest that cause neurological changes can be divided into five distinct groups: plants that cause storage disease; plants that cause tremorgenic syndrome; plants that cause lesions located in the central nervous system (CNS); plants that contain thiaminase and with other neurological actions (Tokarnia et al. 2012). This last group includes more than thirty species of toxic plants - including the genus *Ipomoea*, widely distributed in the Brazilian territory. Some of them are capable of inducing storage diseases, while others determine tremorgenic syndrome (Tokarnia et al. 2012).

This study aimed to describe the epidemiological and clinical-pathological aspects of spontaneous poisoning by *Ipomoea pes caprae*, a plant widely distributed on the Brazilian coast (Tokarnia et al. 2012), in cattle in the north of the State of Rio de Janeiro.

MATERIALS AND METHODS

Natural poisoning. We made six visits (4 visits in 2013 and 2 visits in 2014) to the property where cattle with neurological signs were observed. The property is located on one of the islands of the delta (Ilha da Convivência, located in the municipality of São Francisco do Itabapoana/RJ) at the meeting of the Paraíba do Sul river with the sea, latitude 21°36'0'' South and longitude 41°1'60'' West, northern Fluminense mesoregion.

Clinical follow-up. The clinical follow-up of cattle spontaneously poisoned by *Ipomoea pes caprae* occurred at the property of outbreak, except for Bovine 1, referred to the Veterinary Hospital of UENF. During the visits, we observed 12 cattle (Nelore crossbred) raised at the property. As the animals were raised on pasture, the evaluation was carried out in part by analyzing their behavior in the field, mainly the gait pattern and the presence of tremors.

The complete neurological exam (Dirksen et al. 1993), was only performed in three cattle with more evident signs: a heifer (Bovine 2), a bull (Bovine 3), and a wither (Bovine 4) crossbred Nelore. These were examined and monitored periodically, more carefully, from the first visit to the property until death. The neurological clinical examination protocol included assessment of the level of consciousness, behavior, posture, cranial nerve pairs, fundus examination, locomotion pattern, cervical-facial reflex and cutaneous muscle, muscle mass, sweating, evaluation of the forelimbs and hindquarters (hooves, proprioception, tone, sensitivity, reflexes), tail and anus (tone, reflexes, and sensitivity).

Necropsy and histopathology. Necropsy of Bovine 2, 3, and 4 was performed at the property immediately after death. Fragments of liver, gallbladder, kidneys, spleen, skin, superficial lymph nodes, in addition to salivary glands, pancreas, adrenal, bladder, small and large intestines, rumen, reticulum, omasum, abomasum, lungs, heart, testis or ovary, brain, spinal cord, costochondral junction, thyroid, pituitary, eyeball, and muscles were collected. Two of the animals (Bovine 2 and 4) were euthanized by sedation with 2% xylazine hydrochloride intramuscularly at a dose of 0.2mg/kg, followed by 50ml of 2% lidocaine hydrochloride in the atlantooccipital space. The bull (Bovine 3) drowned after a fall (due to the tremorgenic syndrome) near a stream. The necropsy of this bovine was also performed immediately after death. Bovine 1 was not necropsied, the owner chose to take it back to the property to try to recover it, but the animal died.

The collected fragments were fixed in 10% buffered formalin, except for the CNS, which was fixed in 20% buffered formalin. The samples were fixed immediately, except for muscle fragments, fixed three hours after the bovine's death. The fragments were processed routinely, the sections were stained with hematoxylin and eosin (HE), and the slides examined under an optical microscope and photographed. Silver impregnation was also performed (Bielschowsky staining).

Lectin-histochemistry. Histological sections of the central nervous system of Bovine 2, 3, and 4 were dewaxed, hydrated, and incubated in 3% hydrogen peroxide in two 15-minute steps (dilution of the peroxide at the time of the procedure) to block endogenous peroxidases. After washing the slides for two minutes with phosphate buffer (PBS), they were submerged in citrate buffer (pH 6.0) for antigenic recovery for 15 minutes in a water bath at 98 °C. After this procedure, they were cooled for 15 minutes at room temperature and washed with distilled water. The blocking of non-specific reactions was performed with 5% skimmed milk (Molico® - Indústria Brasileira). The sections were incubated "overnight" with lectins (Vector Laboratories, Burlingame, California, 94010, USA) at a dilution of 5µg/mL (except Con-A lectins with a dilution of 0.5µg/mL and RCA µg/mL with dilution 1.0) in PBS. We used the lectins *Canavalia ensiformis* agglutinin (Con A; ad-Man; ad-Glc-specific), *Dolichos biflorus* agglutinin (DBA; ad-Gal-Nac-specific), *Glycine Max* agglutinin (SBA; ad-GalNac; b-Gal- specific), *Arachis hypogaea* agglutinin (PNA; bd-Gal / (1-3) GalNAc-specific), *Ricinus communis* agglutinin-I (RCA - I; bd-Gal-specific), Ulex europaeus

agglutinin-I (UEA-1; aL-Fuc-specific), *Triticum vulgaris* agglutinin (WGA; ad-GlcNAc/NeuNAc-specific, Succinyl-WGA (sWGA), *Griffonia* (Bandeiraea) *simplicifolia* (GSL), *Sophora japonica* (SJA), *Pisum sativum* (PSA), *Phaseolus vulgaris* (PHA-L and PHA-E), *Lens culinaris* or *L. esculenta* (LCA) (Lectin Kit Biotinylated BK 1000 and 2000, Vector Laboratories Inc., Burlingame/CA, USA), subsequently incubated with the streptavidin-peroxidase complex (red drop only - Vector Laboratories Inc.) for 20 minutes. All sections were counterstained with Harris' hematoxylin and evaluated under an optical microscope. To compare the marking patterns, we used sections of the brain of a sheep affected by glycoproteinosis associated with the ingestion of *Sida carpinifolia* and a bovine poisoned by *Ipomoea asarifolia*, a plant that determines tremorgenic syndrome, and a healthy bovine.

Experimental poisoning. The experiment, submitted and approved by the Animal Ethics and Experimentation Commission (CEUA protocol 222), was conducted at the "Setor de Clínica Médica de Grandes Animais", UENF. In the experimental study, were used a male calf (Bovine 5), crossbred Girolando, weighing 110kg live weight, without alterations to the clinical examination, examined for worms and treated against ectoparasites 15 days before the start of the experiment (ivermectin at a dose of 0.2mg/kg). At first, their food consisted of roughage at will, *Pennisetum purpureum* (elephant grass) chopped, *ad libitum*, and 1kg of concentrate divided into two portions a day. The supply of *I. pes caprae* was initiated in the amount of 20 to 30g per kg/PV day, and supplementation with concentrate was maintained. The plant was collected at the site of the poisoning outbreak every four days and kept under refrigeration at 2 to 8 °C. Bovine 5 spontaneously ingested the plant in the lame.

Bovine 5 was observed every six hours, and the general physical and neurological examination performed every 12 hours. After the onset of neurological signs, the administration of *I. pes caprae* was suspended to verify the possibility of eventual recovery. The complete neurological examination was also performed (Dirksen et al. 1993). Blood samples were collected by puncture of the jugular vein and, at the "Laboratório de Patologia Clínica" of UENF, a complete blood count (12th and 20th days after the onset of clinical signs), platelet counts, biochemical profile (glucose, urea, creatinine, GGT evaluation), ALT, AST) and feces were also collected for coproparasitological examination.

Botanical identification of the plant. Samples of the plant (Fig.1) collected at the property were placed between paper sheets and pressed for 24 hours, with a subsequent change of papers and repetition of the process for 14 days. The obtained exsiccates were sent to the "Departamento de Botânica" of the "Instituto de Biologia", UENF, for taxonomic identification.

RESULTS

Botanical identification of the plant

The samples were identified as *Ipomoea pes caprae*.

Clinical signs of animals naturally poisoned

Bovine 1 was taken to the veterinary hospital at UENF in January 2013, presenting tremors, inability to stay in season, marked decrease in muscle tone, marked thirst, and lack of appetite. The response to stimuli was verified, although the ability to react was greatly diminished.

During the first visit to the island (January 10, 2013), Bovine 2 showed clinical signs characterized by head tremors, uncoordinated walking, decubitus, and paralysis. Tremors and incoordination were exacerbated when approaching and threatening movements were carried out.

In the following three visits (June 5, July 3 and September 17, 2013), Bovine 2 showed worsening of clinical signs and Bovine 3 showed slight tremors in the head region but did not present the tremorgenic crisis when stimulated. In one of the crises, Bovine 3 was unable to get up (Fig.2) and ended up drowning; necropsy was performed immediately.

In two visits (November 27 and December 11, 2014), Bovine 4 was identified with discrete neurological signs; according to the owner, he would have stayed 15 days on the island with only *I. pes caprae* as a food source. Also, Bovine 4 initially would have been without food for three days, before starting to ingest *I. pes caprae*.

In addition to those mentioned so far, other sick cattle also showed tremorgenic signs, some with episodes lasting an average of 20 minutes and subsequent partial recovery with no new stimulus. At this stage, the main findings were acute locomotor ataxia with shaking of the head, broad base (abduction mainly of the pelvic limbs), tremors in the thoracic and pelvic limbs constantly exacerbated on approach. After the intensification of stress, there were falls, inability to stand, flexed

Fig.1. *Ipomoea pes caprae* flower, Ilha da Convivência, Municipality of São Francisco de Itabapoana/RJ, Brazil.

Fig.2. Natural poisoning by *Ipomoea pes caprae*. Attempt to get up only with thoracic limbs, Bovine 3.

limbs. Atypically, attempts to get up were made on the thoracic limbs (Bovine 3, Fig.2). There were no significant variations in temperature, heart rate, and respiratory rate. The evolution of the disease was chronic, and deaths occurred due to accidents.

Macroscopic and microscopic findings

The necropsy of Bovines 2, 3, and 4 revealed only a significant amount of *I. pes caprae* leaves and petioles in the rumen content.

The histopathological study of the CNS showed chromatolysis of Purkinje cells in the cerebellum (Fig.3) with axonal spheroids in the granular layer, degenerative changes in Golgi neurons (Fig.4), proliferation and astrocytic edema in white matter. Additionally, in some neurons of the gray matter of the cerebral cortex, slight regressive neuronal changes, in the form of swelling, vacuolization, and chromatolysis with evolution to lysis accompanied by astrocyte proliferation, astrocytic edema and perineuronal proliferation of glial cells. Similar, but milder, we observed changes in several areas of the brain's base and the gray matter in the spinal cord. In the white matter, regardless of the location, there was mild astrocytic and interstitial edema. The histochemical technique by silver impregnation using the Bielschowsky method allowed better visualization of axonal spheroids of Purkinje neurons (Fig.5).

Experimental poisoning

Bovine 5 ingested the plant spontaneously in the trough with variations in the daily amount of 30g/kg of live weight and showed a predilection for the leaves, but ingested the stem mixed with them. Appetite and dipsia were maintained throughout the experiment.

The first nervous sign was the constant head tremor, observed from the 12th day after the plant started to be supplied. From the 14th day on, there was an increase in tremor, slight ataxia, and abnormal posture. On the 18th day onwards, there was intense sialorrhea, dehydration, and capillary refill time of three seconds followed by mild enophthalmos. The plant supply was maintained until the 28th day, and the symptoms disappeared four days after the suspension.

Regarding laboratory tests, the only change found was polycythemia characterized by an increase in hematocrit between 42 and 44%. The other indexes evaluated were regular. Bovine 5 recovered and was not necropsied.

Lectin-histochemistry

There was no affinity between Con-A, WGA, and sWGA lectins for Purkinje cells and Golgi neurons with degenerative changes (Fig.6), nor for other neurons, in cattle poisoned by *I. pes caprae* and *I. asarifolia* (control used for comparison). With these same lectins, there was a specific marking of the interior and the edge of the intracytoplasmic vacuoles on the Purkinje and Golgi neurons of the control poisoned by *Sida carpinifolia* (Fig.6). The lectins PSA, LCA, PHA-E, despite having lighter labeling, were not specific for the Purkinje cell vacuoles in the positive control (bovine poisoned by *S. carpinifolia*).

DISCUSSION

The cattle poisoned by *Ipomoea pes caprae* presented tremors and motor incoordination like those observed in tremorgenic poisonings of varied etiology. Considering that on the island where the *I. pes caprae* outbreak occurred was the only food source for the affected animals and that the disease was reproduced experimentally, it can be concluded that the cause of the tremorgenic disease was poisoning by *I. pes caprae*.

Similar clinical signs were also observed, in varying degrees and intensity, in cattle poisoned by different agents, including *Ipomoea asarifolia* (Döbereiner et al. 1960), *Phalaris angusta* (Gava et al. 1999, Sousa & Irigoyen 1999), *Marsdenia* spp. (Riet-Correa et al. 2004, Silva et al. 2006, Pessoa et al. 2011, Neto et al. 2013), *Ipomoea carnea* subsp. *fistulosa* (Balogh et al. 1999, Antoniassi et al. 2007, Armién et al. 2007, Oliveira et al. 2009, Tokarnia et al. 2012), *Sida carpinifolia* (Furlan et al. 2008, Pedroso et al. 2010), *Solanum fastigiatum* var. *fastigiatum* (Medeiros et al. 2004, Guaraná et al. 2011, Rego et al. 2012), *Claviceps paspali* (Riet-Correa et al. 1983a), *Neotyphodium lolii* (Munday & Mason 1967, Odriozola et al. 1993, Miyazaki et al. 2001, 2007), *Cynodon dactylon* (Odriozola et al. 2001, Rivero et al. 2011) and *Aspergillus clavatus* (Loretti et al.

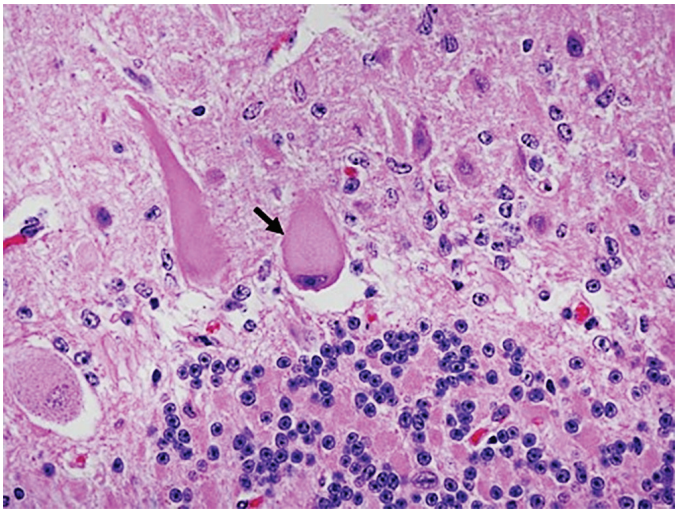

Fig.3. Poisoning by *Ipomoea pes caprae*. Purkinje neuron degeneration (eosinophilic cytoplasm and dissolution of the Nissl substance) (arrow), cerebellum, Bovine 3. HE, obj.63x.

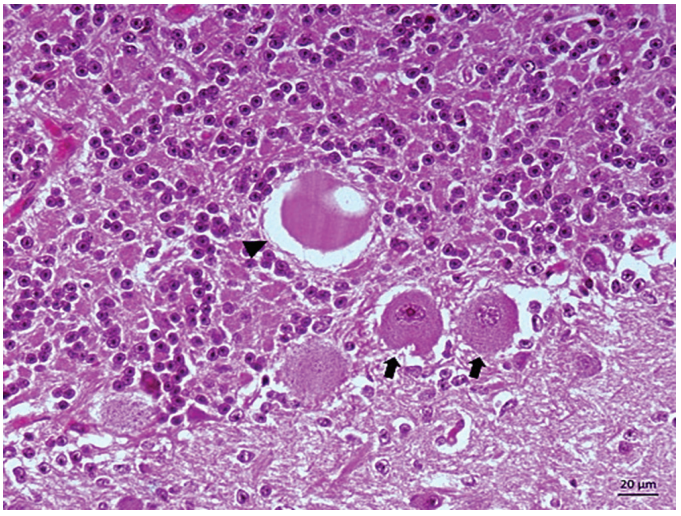

Fig.4. Poisoning by *Ipomoea pes caprae*. Purkinje neurons in chromatolysis (arrows) and Golgi neuron with vacuolated cytoplasm (arrowhead), cerebellum, Bovine 2. HE, obj.40x.

2003, McKenzie et al. 2004, Sabater-Vilar et al. 2004, Bezerra et al. 2009, Oliveira 2016). Tremors have also been described in cases of hepatic encephalopathy (Méndez & Riet-Correa 2001, Karam et al. 2004, Lucena et al. 2010), rabies (Acha & Szyfres 2003), polioencephalomalacia (Lemos & Nakazato 2001, Sant'ana et 2009a, 2009b) and meningoencephalitis due to BoHV-5 (Salvador et al. 1998, Colodel et al. 2002, Elias et al. 2004, Riet-Correa et al. 2006, Rissi et al. 2006). However, there was no sign of the agents mentioned above, nor was there any anatomopathological evidence of indicative or compatible lesions caused by them.

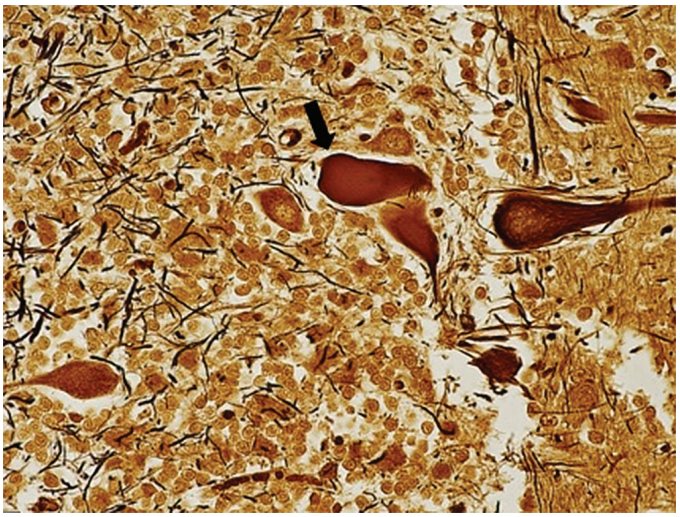

Fig.5. Poisoning by *Ipomoea pes caprae.* Degenerated Purkinje neuron with dilated axon. Axonal spheroid (torpedo), cerebellum, Bovine 3. Bielschowsky, obj.40x.

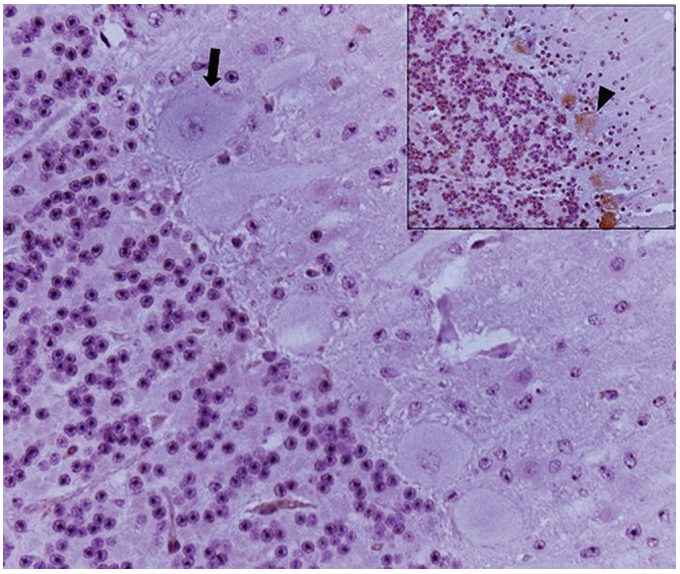

Fig.6. Poisoning by *Ipomoea pes caprae*. Purkinje neurons without lectin-histochemical marking (arrow) using sWGA lectin, cerebellum, Bovine 2. Insert: positive control, bovine poisoned by *Sida carpinifolia* with intracytoplasmic vacuoles with storage and marked edges (arrowhead) in Purkinje neurons. Lectin-histochemistry, obj.40x.

One of the fundamental points to be determined in this report was whether *I. pes caprae* causes storage disease or a condition compatible with that described in so-called tremorgenic syndromes. The absence of storage, confirmed by histopathological analysis and lectin-histochemical evaluation, indicates that *I. pes caprae* is responsible for a tremorgenic picture similar to those described in cases of *Ipomoea asarifolia* (Döbereiner et al. 1960, Riet-Correa et al. 2003, Araújo et al. 2004, Barbosa et al. 2005, Barbosa et al. 2012).

Preliminary studies, probably of short duration and with low doses, carried out in the 1960s (Tokarnia 2013) indicated that *I. pes caprae* was not toxic to cattle. However, this plant, when ingested as an exclusive food for prolonged periods (many months or even a few years), is capable of poisoning cattle, as demonstrated for buffaloes and other ruminants poisoned by *I. asarifolia* (Barbosa et al. 2005). Indole diterpenes were identified in the *I. asarifolia* and *Ipomoea muelleri* species, and associated with the tremorgenic picture in cattle poisoned by these plants (Gardner et al. 2018). This association may be the active ingredient responsible for the symptoms observed in animals poisoned by *I. pes caprae*.

As with poisoning by some other plants that affect the central nervous system (Riet-Correa et al. 1983b, Rivero et al. 2011), deaths from trauma and drowning can occur, as observed in the case of Bovine 3 that occurred during the outbreak of the tremorgenic crisis.

In the experimental case of poisoning by *I. pes caprae*, the onset of clinical signs was later than the two to four days seen in experimental poisoning in cattle by *I. asarifolia* (Barbosa et al. 2005). The daily doses ingested by the bovine submitted to experimental poisoning were like those used by Barbosa et al. (2005) in experiments with *I. asarifolia*.

The silver impregnation by the Bielschowsky method is widely used to evidence neuronal processes, including dendrites, axons, and neurofibrils (Minbay et al. 2001). In Bovine 4, this staining allowed the visualization of axonal spheroids of Purkinje cells (Fig.5). This result proves the degenerative lesions of Purkinje cells of animals poisoned by *I. pes caprae*. The Purkinje neuron is considered the "central functional unit" of the cerebellar cortex, as it receives afferent stimuli directly or indirectly and is solely responsible for the efferent stimulus (Mullen et al. 1976, Bauer-Moffett & Altman 1977). It also forms inhibitory synapses with deep cerebellar nuclei and vestibular nuclei (Damiani et al. 2016). Lesions in this cell could justify the signs described in animals poisoned by *I. pes caprae*. Besides, changes in gait have already been described in other conditions that lead to lesions in Purkinje cells (Mullen et al. 1976, Thomas et al. 1998). Some Golgi neurons were also injured, these neurons have an essential inhibitory function (Watanabe et al. 1998), and this alteration may have contributed to the pathogenesis of the tremors.

The lectin-histochemical evaluation was used, above all, to rule out the possibility of oligosaccharide storage being involved in the pathogenesis of poisoning, as occurs in poisoning by other species of *Ipomoea*, except for *I. asarifolia*. The lectins Con-A, WGA and sWGA that, in general, mark the accumulation of oligosaccharides in animals poisoned by *S. carpinifolia* (Driemeier et al. 2000, Colodel et al. 2002, Loretti et al. 2003, Pedroso et al. 2010) and *I. carnea* (Armién et al. 2007), did not mark Purkinje neurons and other neurons affected in the disease caused by *I. pes caprae*.

CONCLUSION

Ipomoea pes caprae causes tremorgenic syndrome in cattle when ingested as exclusive food for prolonged periods.

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Conflict of interest statement.- There are no conflicts of interest.

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