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Case Report

Crotalaria spectabilis poisoning in a horse

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Abstract

Plant poisoning is an important cause of death in horses and cattle in Brazil. *Crotalaria* spp. has stood out in this scenario due to its toxic potential caused by monocrotaline, a pyrrolizidine alkaloid found throughout the plant, mainly in seeds. Here is reported a case of *Crotalaria spectabilis* poisoning a horse. A horse consumed oats contaminated with *Crotalaria spectabilis* seed and presented clinical signs of toxicosis characterized by jaundice, progressive weight loss, hemoglobinuria, subcutaneous edema in the pectoral region and neurological symptoms typical of hepatic encephalopathy. In the serum evaluation, there was an increase in the activity of the enzymes alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and aspartate transaminase (AST), urea, creatinine and creatine phosphokinase (CPK). At necropsy, the main macroscopic findings were opaque and congested liver with capsular irregularity and accentuated the lobular pattern, trachea with foamy and pinkish fluid and congested and edematous pulmonary lobes. The main histopathological findings were hepatic fibrosis, periportal ductal hyperplasia, centrilobular necrosis, megalocytosis and binucleated hepatocytes. The brain parenchyma showed perivascular edema and Alzheimer type II astrocytes. *Crotalaria* spp. is among the main plants that cause acute or chronic mortality after exposure to the toxic compound in horses and farm animals.

Key words: hepatotoxic plants; pyrrolizidine alkaloids; monocrotaline; hepatic encephalopathy.

Introduction

The genus of plants *Crotalaria* spp. belongs to the *Leguminosae* Family and it is responsible for various intoxication cases of animal production Brazil-wide (23). The *Crotalaria* spp. is highly adaptable to different environments and it is normally considered opportunistic and invasive of plantations (5, 23). However, in some conditions, the planting of *Crotalaria* spp. with the cultivar is carried out to promote nitrogen supplementation in depleted soil or to aid the control of invasive pests (4, 7, 21). In this situation, its seeds may accidentally mix with the grains used for feed formulation (21, 25).

Crotalaria spectabilis is the most toxic species in Brazil (7). Some species of *Crotalaria* are composed of a toxic pyrrolizidine alkaloid (PAs), being responsible for the cases of acute and chronic intoxications reported in horses. Monocrotaline is the main PA found in the plant (7, 21), especially in seeds that have the highest concentration (1, 9). Intoxication can be acute, when the amount of monocrotaline ingested is high or when several portions are ingested over a short period; or chronic, when low doses of the toxic compound are ingested and last for a long period since monocrotaline has a cumulative effect on the animal's body (9).

Clinically, the animals have anorexia, progressive weight loss, jaundice and neurological changes resulted from hepatic encephalopathy such as ataxia, apathy, compression of the head against objects, uncontrolled gallop, protrusion and lingual hypotonia followed by difficulty in swallowing (9, 12, 21). The diagnostic of intoxication by *Crotalaria* spp. is performed correlating the clinical history and signs manifested by the animal with post-mortem examinations

(9, 12, 15). Also, it is important to carry out a local investigation of the animal's pasture, seeking evidence of the plant (16) or casual contamination of other food sources by parts of it (2,1). This study aimed to report clinical, anatomopathological and hematological findings of a horse poisoning by *Crotalaria spectabilis* that has occurred in a property of the Distrito Federal.

Case Report

An 8-year-old equine male, Quarter Mile, raised in a stall was accidental intoxicated in a property located in the Federal District from December 2018 to August 2019. The diet was based on oat grains, balanced feed and supplementation with mineral salt. After nine months of contaminated oats consumption with seeds of *Crotalaria* spp., the horse initially presented hyporexia, apathy, progressive weight loss and jaundice. As the condition worsened, it showed subcutaneous edema in the pectoral region, anorexia, ataxia, dark reddish urine, head pressing, walking randomly and hypotonia of the tongue. According to the owner, other horses with similar clinical signs died in the property and in other nearby properties. Unfortunately, these animals were not examined. It was not possible to estimate the volume of contaminated oats consumed by the animal and there was no quantification of the toxic compound ingested. Samples of the *Crotalaria* spp. seeds mixed with oat grains (Fig. 1) that were supplied to the animals were sent for botanical identification and tthe phytochemical analysis revealed *Crotalaria spectabilis*. The plant was cultivated, and its registration was deposited at the Herbarium of the Universidade Federal de Minas Gerais (UFMG) under the number BHCB 200358.

Blood sample was taken for hematological and biochemical evaluation. The animal presented normochromic normocytic anemia and increase in the levels of ALP, GGT, AST and CPK levels. In addition to these assessments, a low albumin/globulin ratio was noted. Another test routinely performed was the measurement of blood glucose, which showed low at the terminal moment.

During the follow-up, supportive clinical treatment with liver protectors, fluid therapy and glucose was instituted. However, the animal died, and it was submitted to necropsy. Macroscopic findings included low body score, hyperemic and yellowish mucous membranes (jaundice), trachea filled by foamy and reddish fluid, lungs enlargement with shiny

Figure 1. *Crotalaria spectabilis* seeds (arrows) contaminating harvested oat grains

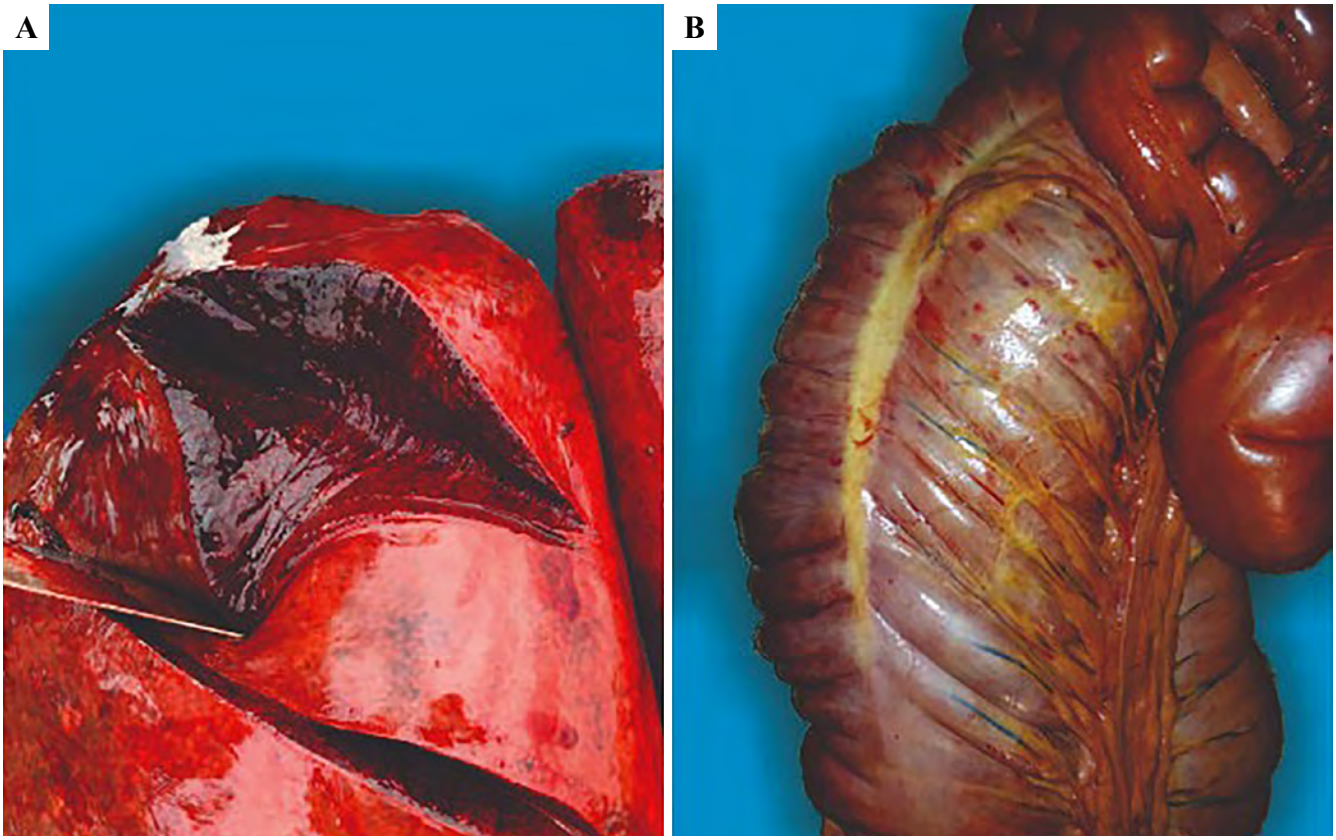

Figure 2. Gross finding observed in a horse intoxicated by Crotalaria spectabilis. **A**. Cut surface of the lung: reddish and consolidated with shiny surface. **B**. Petechiae and ecchymosis in the colon serosa with jaundice.

surface and on cut surface consolidation and congested (Fig. 2A). Petechiae and ecchymosis in the intestinal serosa (Fig. 2B) in addition to mesenteric congestion. The liver presented severe congestion, opacity and capsular irregularity, diffuse accentuated lobular pattern and parenchyma was firm with coppery color. Other less frequent findings were leptomeningeal hyperemia, petechiae, ecchymosis and subcutaneous, epicardial, gastric and intestinal multifocal suffusions, in addition to moderate hydropericardium, dark red kidneys and bladder filled with dark reddish urine.

Samples from all tissues were collected and fixed in 10% neutral buffered formalin, routinely processed with paraffin inclusion, sectioned, stained with Hematoxylin-Eosin (HE) and Masson's Trichrome.

The histopathological findings in liver were regenerative nodules dispersed throughout the parenchyma rounded by fibrosis, moderate periportal ductal hyperplasia, moderate centrilobular necrosis, megalocytosis and binucleated hepatocytes (Fig. 3A, 3B, 3C and 3D). In the kidney there was multifocal and moderate tubular necrosis and some tubules were filled by intensely orange material compatible with hemoglobin cast (Fig. 4A). The lung had mild to moderate multifocal edema and emphysema. The brain parenchyma showed gliosis with mild to moderate multifocal perivascular edema and discrete and multifocal Alzheimer type II astrocytes (Fig. 4B, 4C and 4D).

Discussion

Necropsy and histological findings are the most important data for defining the diagnosis in cases of plant poisoning (23). Besides that, it is important the regional knowledge about toxic herbal hereditary taxonomy and their identification at the animal's environment, to establish a definite diagnosis (7). The diagnostic of this case confirmed an outbreak of intoxication since *Crotalaria* seeds were found in the supplement of the horses in some properties of the Distrito Federal. Moreover, the clinical, anatomopathological and hematological findings confirm the intoxication by PA, a toxic found in various species of *Crotalaria*. The identification of the plant was carried out during investigation of the properties, and the confirmation of the species was carried out through phytochemical analysis (12, 16).

Intoxication of horses by other species of *Crotalaria* spp. have been previously reported (2, 9, 12, 15, 17, 20). On the other hand, natural or experimental intoxication by *C. spectabilis* has only been described in pigs (21, 24, 25). In addition, experimental intoxication of sheep (18) and dogs (3) have also been reported, indicating thatthis may be the first report of natural poisoning by this species in equines.

Probably, the property where the horse remained acquired the oat from a supplier who, when harvesting, accidentally mixed seeds of *Crotalaria* spp. with the oats

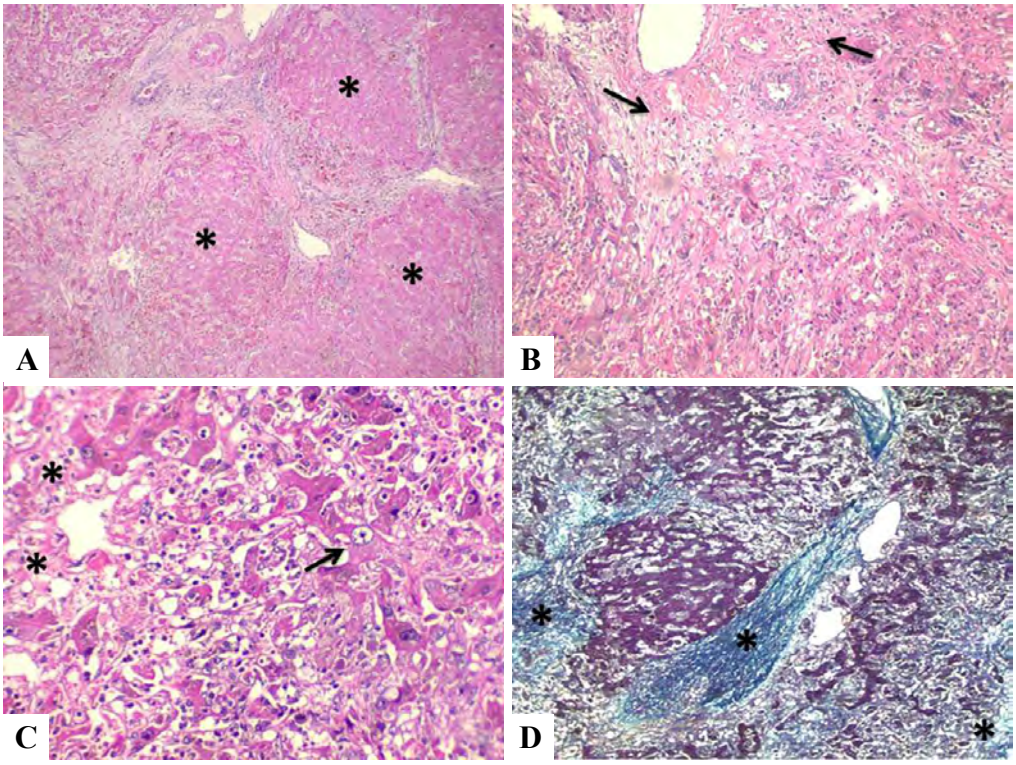

Figure 3. Histological lesions of the liver. **A.** Regenerative nodules dispersed throughout the hepatic parenchyma (asterisks) separated by an abundant connective tissue . HE, 4X. **B.** Severe periportal fibrosis (arrows) substituting the adjacent hepatocytes. HE, 10X. **C.** Hepatocytes with megalocytosis (arrow) and centrilobular necrosis (asterisks). HE, 20X. **D.** Intense proliferation of collagen fibers (asterisks) replacing hepatocytes. Masson's Trichrome stain, 10X.

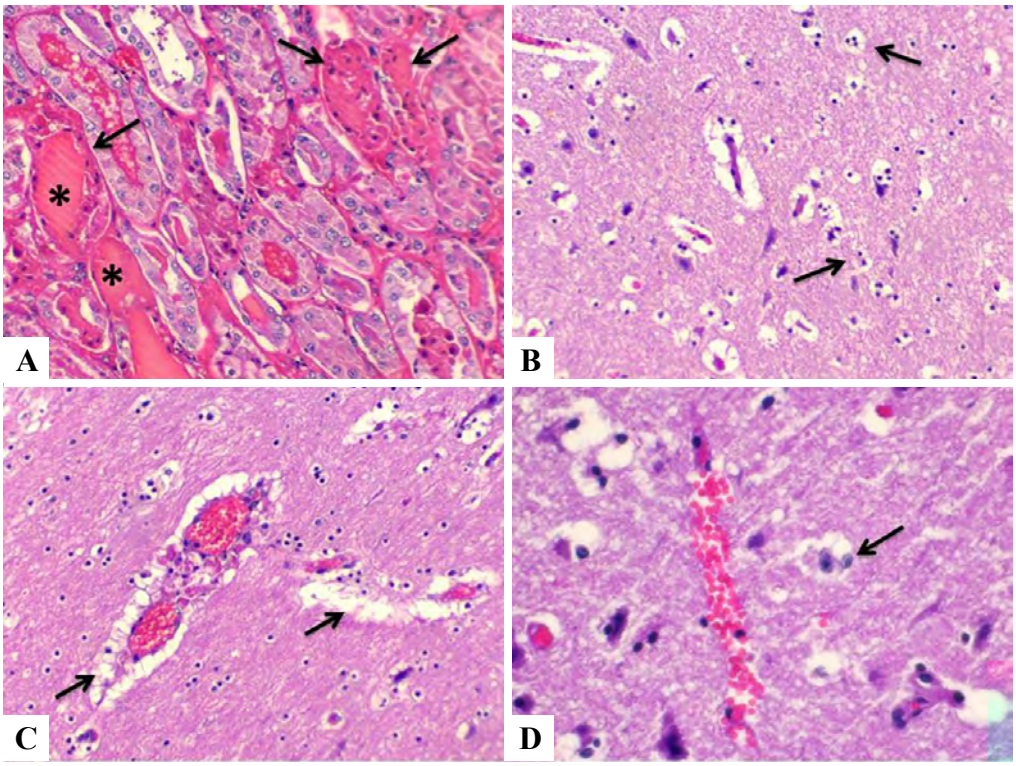

Figure 4. Microscopic findings of the kidney **A** and central nervous system (**B**, **C**, **D**). **A.**Tubular lumen (asterisks) containing orange-reddish casts compatible with hemoglobin and tubular necrosis (arrows). HE, 20X. **B.** Gliosis (arrows) with neuropil pallor. HE, 10X. **C.** Perivascular edema (arrows). HE, 20X. **D.** Alzheimer's type II astrocytes (arrow). HE, 40X.

(personal information from the oat supplier, that had more complains from other horse owners), contaminating it and causing intoxication of the horse. We suggested this hypothesis since other animals from other properties that acquired oats from the same supplier presented similar clinical signs. This mistake in harvesting has already been reported in cases of intoxication by *Crotalaria* spp. in swine (21, 25).

The contaminated oats were supplied for all animals from the property. Few horses presented some clinical signs similar those mentioned before and died, without previous diagnosis. The animal from this report just presented clinical signs nine months after. The toxicosis time is variable as described in a intoxication of horses by *C. retusa* (12). Monocrotaline has a cumulative effect on the animal's organism (9), which may justify the late clinical occurrences in this intoxication.

The animal of this case showed nonspecific, hepatic and extra-hepatic clinical signs. As a nonspecific clinical characteristic, the horse presented hyporexia, progressive weight loss and apathy, as described before (12, 15). Some authors also describe dyspnea (7) and fever (17), but these clinical signs were not observed in the animal of this report. The animal presented tachycardia, which is a sign not yet described in horses, but that has already been reported in donkeys intoxicated by *C. retusa* and *C. juncea* (14).

Other extrahepatic manifestations were related to hepatic encephalopathy and were characterized by: ataxia, wandering, uncontrolled gallop, compression of the head against objects, protrusion and lingual hypotonia, also previouslyreported (9, 12, 15). The lingual hypotonia is a consequence of the damage of cranial nerves nucleuses (12, 15). The horse showed variation regarding the degree and the form of neurological manifestations, evolving according to the progression of liver disease. The clinical signs of hepatic encephalopathy in this species occur in terminal patients (7, 19, 26), reinforcing what happened in the case described.

Initially, the predominant hepatic clinical sign in cases of intoxication by PAs is jaundice (9), as well as observed in this animal. Another sign that could be present is hepatogenous photosensitization (9, 12). However, this alteration was not observed in this animal and in other related cases (15, 17, 20).

Some signs related to hemodynamic disorder secondary to chronic liver disease, such as edema, ascites and hemorrhage, were present among the clinical and necroscopic signs of this horse. The explanation for the edematous and ascitic condition is the decrease in plasma levels of albumin (10), observed in laboratory tests of this animal. Subcutaneous edema in the pectoral region, most seen in horses in this type of intoxication (19).

Another clinical sign observed in the horse in this report was the presence of dark pigmentation in the urine. In urinalysis, it was confirmed that it was hemoglobinuria. This clinical condition has already reported as a poor prognosis, and with nervous disorders, they are considered terminal clinical signs. These authors also describe intravascular hemolysis as a justification, which is present in one third of the animals with liver failure (22).

The hematological exams performed showed normochromic normocytic anemia, but anemic conditions were not described in reports of *Crotalaria* intoxication in horses (2, 9, 12, 15, 17, 20). However, this hematological finding has already been observed in intoxicated by other plants which also have a PAs as toxic principle (7, 13).

In the hepatic profile, an increase in the serum activities of ALP, GGT, AST and bilirubin were noted as previously reported (12). The same was found for CPK. Increase of GGT has also been reported in foals intoxicated by *Senecio* spp. (13). In experimental and natural poisoning in sheep, the activities of GGT and AST were high, but the ALP was not measured (1, 11). In the renal profile, changes in the levels of creatinine and urea were observed, but they were not measured in other reports of intoxication by *Crotalaria* in horses (2, 9, 12, 15, 17, 20).

The anatomopathological findings verified in the animal are consistent with those described by other authors (7, 12, 15, 17). Macroscopically, jaundice, hepatic capsular irregularity, accentuated lobular pattern of the liver and pulmonary congestion with foamy fluid in the trachea and bronchi have already been described (12, 15). Ascites, hydropericardium and congested kidneys have also been observed in other cases (12). Microscopic alterations in a chronic intoxication by PAs also found in our case include hepatic fibrosis predominantly periportal, with proliferation of bile ducts and hepatomegalocytosis (6, 9, 12, 13). Other less frequent findings in this toxicosis are renal medullary hemorrhage, slight degeneration/necrosis of the tubular epithelium and intensely eosinophilic casts (hemoglobinuria) (12).

A very striking finding in this case and observed by other authors, were morphological changes in the cerebral cortex. Alzheimer type II astrocytes are commonly seen in animals intoxicated by PA, resulting from encephalopathic syndrome. In the brain, congestion and hemorrhage, mainly perivascular, can also be observed (6, 8, 9, 12, 13).

Crotalaria spp. is the main plant that causes acute or chronic mortality after exposure in farm animals. Anamnesis, clinical evaluation, local investigation, laboratory and anatomopathological findings were essential to confirm the diagnosis.

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