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Acute intoxication by *Crotalaria retusa* in sheep

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Abstract

Acute intoxication by seeding *Crotalaria retusa* occurred during the dry season, in a flock of 80 sheep, in the semi-arid region of the state of Paraíba, northeastern Brazil. Anorexia, severe depression, mild jaundice, incoordination and recumbence were observed in 16 sheep that died within 12 h. At necropsy the liver had a nutmeg appearance. Seeds of *C. retusa* were found in large amounts in the rumen of the dead animals. Histologic lesions of the liver were characterized by centrilobular necrosis. Seeds of *C. retusa* were given to six sheep at doses of 2.5 (two sheep), 5, 10, 20 and 40 g/kg bw (one sheep for each dose). Sheep ingesting 5–40 g/kg bw were killed when moribund, 38–120 h after the start of the administration. Clinical signs and gross and histologic lesions were similar to those observed in field outbreak. The seeds used in the experiment contained 1.4% of monocrotaline. It is concluded that hungry sheep can be affected spontaneously by acute monocrotaline intoxication when they ingest large amounts of *C. retusa* seeds in a short period of time.

Keywords: Acute pyrrolizidine intoxication; Centrilobular necrosis; *Crotalaria retusa*; Monocrotaline; Sheep

Crotalaria retusa has been reported as a toxic plant affecting horses (Rose et al., 1957; Gardiner et al., 1959; Gibbons et al., 1953; Curran et al., 1996), pigs (Hooper and Scanlan, 1977), chickens (Hooper and Scanlan, 1977; Alfonso et al., 1993), and geese (Alfonso et al., 1993). It is also an important toxic plant for livestock in the semi-arid region of the state of Paraíba affecting mainly horses, but cattle and sheep are occasionally affected (Nobre et al., 2004a,b). In horses the disease is characterized by hepatic encephalopathy, with dullness or hyperexcitability, head pressing, compulsive walking or circling and, occasionally, violent uncontrollable galloping. Decreased cranial nerve reflexes, ataxia and weakness are also observed. Other clinical signs are anorexia, weight loss, photosensitivity and jaundice. Hepatic fibrosis, megalocytosis and bile duct proliferation are the main liver lesions (Nobre et al., 2004a,b).

Monocrotaline (MCT) is a pyrrolizidine alkaloid (PA) plant toxin that produces sinusoidal endothelial cell (SEC) injury, hemorrhage, fibrin deposition, coagulative hepatic parenchymal cell (HPC) oncosis in centrilobular regions of rat livers, and HPC apoptosis in the other regions (Copple et al., 2004). The SEC detachment and loss of endothelium that occurs during MCT hepatotoxicity result of the degradation of basement membrane components with a concurrent increase in the amount and activity of matrix metalloproteinases (MMPs), specifically MMP-9, likely originating from sinusoidal endothelial cells, neutrophils, and probably other cell types (Hanumegowda et al., 2003).

The objective of this paper is to report an outbreak of acute intoxication by *C. retusa* in sheep in Brazil. The experimental reproduction of the acute intoxication by seeds of *C. retusa* is also reported.

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1. Material and methods

The farm where the disease occurred was visited to observe affected sheep and for inspection of pastures. Full necropsies and complete histologic evaluation were performed on three affected sheep. For histologic examination, samples of liver, kidney, heart, lung, lymphnodes, spleen, thyroid, adrenal, forestomachs, abomasum, small and large intestine, skeletal muscles, and the whole central nervous system (CNS) were fixed in 10% buffered neutral formalin, embedded in paraffin and sectioned at 6 μm. Histologic evaluation of the CNS was performed in transverse sections taken from the cervical, thoracic and lumbar spinal cord, medulla oblongata, pons, rostral colliculi, thalamus, basal nuclei and internal capsule, cortex, cerebellar peduncles and cerebellum.

For the experimental intoxication, seeds of *C. retusa* were collected in the municipalities of Pombal, São José do Bomfim and Patos. Voucher specimens of the plants were identified as Brazil, Paraíba, Agra and Góis 3607 (JPB). The seeds were ground, mixed with water, and dosed by stomach tube to a group of six sheep. Another sheep was used as control. The daily and the total dose ingested by each sheep and the period of ingestion are presented in Table 1.

Blood samples were obtained at the start of dosing, and at 48 and 72 h later. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gama glutamiltransferase (GGT), activities and total protein concentrations were determined using standard methods in accordance with the recommendations of the German Society of Clinical Chemistry (Schimidt and von Forstuer, 1986). Analyses were performed within 8 h after collection.

All sheep that showed clinical signs were euthanized when moribund. Complete histologic evaluation was performed with the methods mentioned for the spontaneous cases. Needle liver biopsies were performed in sheep 5 and 6, 4 days after dosing and then every month for 3 and 17 months, respectively. They were euthanized 1 month after the last biopsy.

The concentration of monocrotaline was determined in a sample of the seeds used for the experimental reproduction of intoxication. The ground seeds (640 g) of *C. retusa* were extracted three times with 95% ethanol for 72 h. The ethanolic extracts were evaporated under reduced pressure to yield a brown viscous residue (53.4 g), which was extracted with 3% hydrochloric acid until the washing was negative to Dragendorff's reagent. The acidic aqueous solution was made alkaline (pH 9) with ammonia (25%) and repeatedly extracted with chloroform. The chloroformic solution was dried with Na_2SO_4 anhydrous, filtered, and evaporated until dryness to obtain the total alkaloid fraction (10.48 g). Analytical thin layer chromatography showed the presence of a single alkaloid. The dried residue was recrystallized from ethanol to give colorless prismatic crystals (yield ca. 1.4% based on the dry weight of the seeds material), which melted at 197–198 °C. It was identified as monocrotaline based on ^{1}H NMR, ^{13}C NMR, HMQC and HMBC spectroscopic data and comparison with those reported by Barreiro et al. (1980) and Cheng et al. (1986).

2. Results

The outbreak occurred during the dry season, in a flock of 80 sheep, in the county of Condado, semi-arid region the state of Paraíba, northeastern Brazil. Some sheep of the flock were occasionally introduced in an area severely invaded by *C. retusa* (Fig. 1) in seeding stage and with very little forage available. Sixteen sheep died during August and September 1997. Anorexia, severe depression, mild jaundice, incoordination and recumbence were observed. The animals died within 12 h after the first observation of clinical signs. At necropsies, the liver had a nutmeg appearance (Fig. 2) and fibrin was present on its surface. The gallbladder was enlarged. Hemorrhages were observed in the endocardium and pericardium. Mild jaundice, hydropericardium, hydrothorax and ascites were also present. Seeds and pods of *C. retusa* were found in large

Table 1
Weight of the experimental sheep, daily and total dose of *C. retusa* seeds ingested by each animal, period of ingestion, and onset and duration of the clinical signs

Sheep No	Weight (kg)	Daily dose (g/kg)	Total dose (g/kg)	Days of ingestion	Onset of clinical signs[a] (h)	Duration of clinical signs (h)
1	18.5	20	40	2	6	32
2	17.8	10	20	2	24	48
3	12.6	10	10	1	24	48
4	13.1	5	5	1	48	72
5	13.3	2.5	2.5	1	No clinical signs	
6	12	2.5	2.5	1	No clinical signs	
7	12	Control			No clinical signs	

[a] After the start of administration.