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ENZOOTIC CALCINOSIS IN SHEEP. EXPERIMENTAL REPRODUCTION WITH *Nierembergia veitchii* (Solanaceae)[1]

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SINOPSE.- Riet-Correa F., Schild A.L., Méndez M.C. & Petiz C.A. 1992. [**Calcinose enzoótica em ovinos. Reprodução experimental com Nierembergia veitchii (Solanaceae).**] Enzootic calcinosis in sheep. Experimental reproduction with Nierembergia veitchii (Solanaceae). *Pesquisa Veterinária Brasileira 13(1/2):21–24.* Laboratório Regional de Diagnóstico, Fac. Veterinária, UFPel, Pelotas, RS 96100-000, Brazil.

Calcinose enzoótica foi reproduzida experimentalmente em ovinos mediante a administração de *Nierembergia veitchii* dessecada, misturada a 50% na ração durante 21 dias, e a 10% durante 49 dias. Os ovinos que ingeriram alimentação com 50% da planta perderam peso e os que foram alimentados com 10% da planta tiveram menores ganhos de peso que os controles. Nos 2 grupos tratados houve diminuição do consumo de ração, hipercalcemia e hiperfosfatemia. As alterações patológicas caracterizaram-se por: calcificação dos tecidos moles, mais marcada no grupo que ingeriu *N. veitchii* a 50%; hiperplasia das células parafoliculares da tireóide; atrofia das paratireóides e osteopetrose. *Nierembergia veitchii*, que contém um princípio ativo de ação similar ao calcitriol, é aproximadamente 78 vezes menos tóxica que *Solanum malacoxylon*.

TERMOS DE INDEXAÇÃO: Ovinos, *Nierembergia veitchii*, plantas calcinogênicas, intoxicação por planta, calcinose enzoótica, calcificação dos tecidos moles.

ABSTRACT.- Enzootic calcinosis was reproduced in sheep by the administration of *Nierembergia veitchii* mixed with food at a concentration of 50% for 21 days and at 10% level for 49 days. At a concentration of 50% *N. veitchii* produced loss of body weight, and at 10% lower body weight gains than the control animals. Food consumption was decreased and hypercalcemia and hyperphosphatemia were evident in both groups. Pathological findings included: soft tissue calcification, more severe in those animals that received *N. veitchii* at 50%; hyperplasia of thyroid C cells; atrophy of the parathyroids and osteopetrosis. *Nierembergia veitchii*, which contains a calcitriol-like substance, is approximately 78 times less toxic than *Solanum malacoxylon*.

INDEX TERMS: Sheep, *Nierembergia veitchii*, calcinogenic plant, plant poisoning, enzootic calcinosis, soft tissue calcification.

INTRODUCTION

Nierembergia veitchii has been considered the cause of enzootic calcinosis in sheep in southern Brazil. The disease is characterized by soft tissue calcification, osteopetrosis, osteonecrosis, hypercalcemia, hypercalcitoninism and hypoparathyroidism (Riet-Correa et al. 1987). Similar lesions were reproduced experimentally in rabbits fed with food containing 50% of dry *N. veitchii* (Riet-Correa et al. 1981).

The objective of this study was to reproduce the disease in sheep in order to elucidate some etiologic and epidemiologic aspects of the intoxication.

MATERIAL AND METHODS

Three groups, each with 3 Corriedale wethers 1 to 2 years old, were used in the experiment. The wethers were from an area free of calcinogenic plants and where enzootic calcinosis had not been diagnosed.

N. veitchii was collected weekly, dried in the shade, ground and mixed with a commercial ration for sheep.

All the animals were fed 1.5 kg of food daily. In one group (N. v. 50) the animals were fed with a mixture containing 50% of dry *N. veitchii* and 50% of ration. The second group (N.v. 10) received 10% of dried *N. veitchii* and 90% of ration. The control group (N.v. 0) received only commercial ration.

Blood samples were collected from the jugular vein daily during the first five days of the experiment, and twice a week from the second week until the end of the experiment.

Serum calcium and phosphorus were determined by the methods of Ferro and Ham modified and Basques and Lustosa, respectively, using commercial kits (Labtest(R)). Serum magnesium was calculated colorimetrically with a commercial kit (Merckotest(R)).

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Sheep of the N.v. 50 group were slaughtered by exsanguination at 21 days and those of the N.v. 10 and N.v. 0 groups at 49 days after beginning of the experiment.

Soft tissues were fixed in 10% formalin, embedded in paraffin, sectioned at 6 μm and stained with hematoxilin and eosin (H&E). Two mm sections were excised mid-sagitally from the proximal femur epiphisis-metaphisis and transversely from the mid-diaphisis of these bones. The slices were fixed in 10% formalin and demineralized under vacuum in a solution of formic acid and sodium citrate. Paraffin embedded sections of bones were cut at 6 μm and stained with H&E.

Quantitative morphometry of the parathyroid glands was done by projecting medium-high magnification color slides of the glands on a paper with 50 rows of 40 dots equally spaced. Cytoplasmic and nuclear "hits" were scored for each row and the cytoplasm to nucleus ratio was recorded. The X ± s.e.m. of the 50 rows was then calculated.

RESULTS

Weight and food consumption

Data related to food consumption, *N. veitchii* consumption, and weight variation are shown in Table 1.

Clinical pathology

The serum calcium and phosphorus levels are shown in Fig. 1 and 2. Serum magnesium levels were similar in the 3 groups.

Table 1. *Weight variations, food and* Nierembergia veitchii *consumption in the experimental sheep*

Group	Mean weight (kg)			Mean daily food consumption		Mean *N. veitchii* consumed (g/kg body weight)
	Initial	21 days	49 days	21 days	49 days	
N.v. 50	36	34.467	–	1.333*	–	388.791
N.v. 10	34.567	35.717	39.2	1.219	1.135**	160.890
N.v. 0	34.2	36.9	42.26	1.447	1.447	Nil

* Lower than the control group ($P < 0,01$).
** Lower than the control group ($P < 0,001$).

Macroscopic examinations

Group N.v. 50. The 3 wethers showed mineralization of the aorta. The surface of the intima was rough and covered by mineralized plaques of irregular shape and size. In two animals mineralization was more severe in the abdominal than in the thoracic aorta; in one wether the aortic lesions were homogeneous throughout the whole length (Fig. 3). Mineralization was also observed in carotid, iliac and mesenteric arteries. In the kidneys of one sheep, a white line of mineralization was observed in the medulla. Two wethers showed small whitish areas of mineralization in the bicuspid and left aortic valves. Jugular veins and connective tissue surrounding them were severely calcified in the area where venipuncture was done.

Group N.v. 10. The aorta was mineralized in all 3 animals. The lesions were less severe than in the N.v. 50 group. In two wethers mineralization was localized exclusively in the abdominal aorta. In the same 3 sheep the kidneys and jugular veins showed lesions comparable to the ones described in the N.v. 50 group.

Group N.v. 0. No lesions were observed.

Microscopic examinations

Blood vessels. Arterial lesions were similar in groups N.v. 50 and N.v. 10. Elastic fibers were swollen, eosinophilic and fragmented. Deposits of calcium salts were evident in the degenerated fibers. Severely calcified elastic fibers ended in a completely calcified plaque, where collagenous fibers and ground substance were also calcified (Fig. 4). Proliferation of connective tissue around the calcified areas was observed rarely. Jugular veins and elastic fibers of connective tissue in the area of previous venipuncture were severely calcified.

Heart. The 3 wethers of N.v. 50 group had lesions in the bicuspid and left aortic valves similar to those described in arteries. Coronary arteries had subendothelial proliferation of connective tissue and degeneration and calcification of elastic fibers.

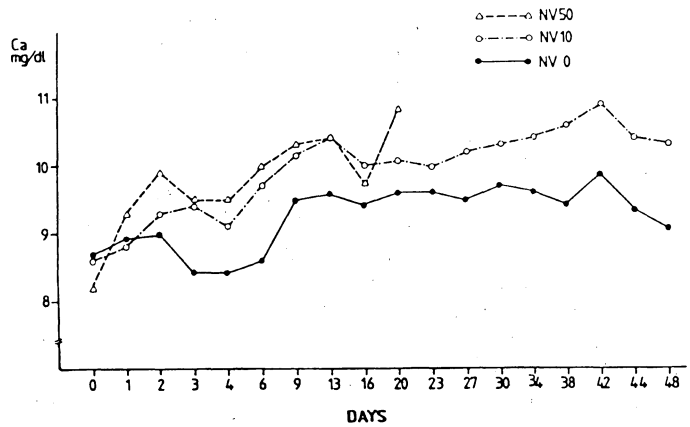

Fig. 1. *Daily means of serum calcium in sheep fed* Nierembergia veitchii *mixed with food at 50% (N.v. 50) and 10% (N.v. 10), and in control sheep (N.v. 0).*

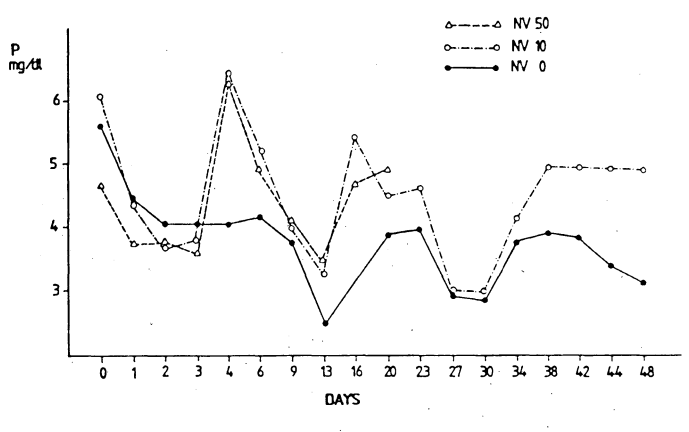

Fig. 2. *Daily means of serum phosphorus in sheep fed* Nierembergia veitchii *mixed with food at 50% (N.v. 50) and 10% (N.v. 10), and in control sheep (N.v. 0).*

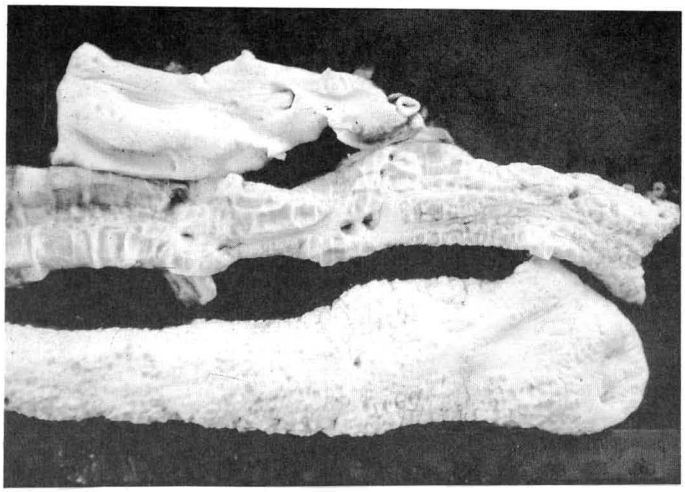

Fig. 3. *Sheep fed with 50%* Nierembergia veitchii *for 21 days. Intima of the aorta covered by mineralized plaques of irregular shape and size throughout the whole length.*

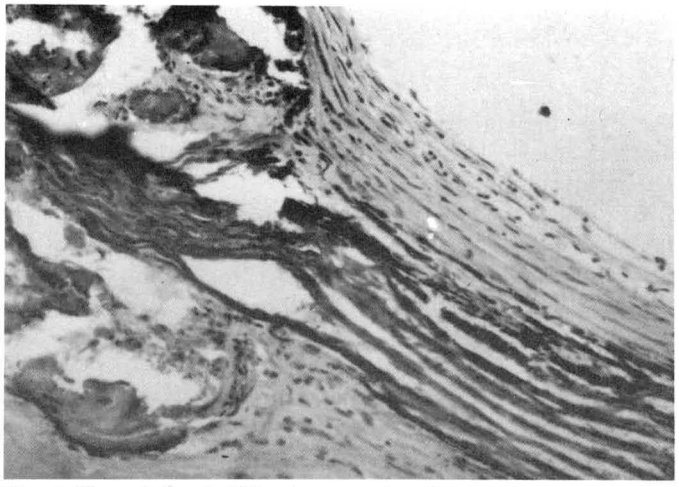

Fig. 4. *Sheep fed with 50%* Nierembergia veitchii *for 21 days. Severe calcification of elastic fibers in the aortic wall ended in a completely calcified plaque. HE, obj. 5.*

Lungs. Only wethers from the N.v. 50 group had lung lesions. Two of three showed degeneration and calcification of the bronchial cartilage and peribronchial muscle.

Kidneys. Degeneration and rupture of the basal membrane, calcification of the insterstitial tissue, calcium deposits in some epithelial cells and calcium casts in the tubules were observed in the medulla of all animals in groups N.v. 50 and N.v. 10.

Thyroids. Two animals from the N.v. 50 group and one of the N.v. 10 group had areas of C cells hyperplasia. The cells appeared as adenomatous proliferation or in multiple layers around the follicules.

Parathyroids. Results of quantitative morphometry from the area of the medial section in parathyroids of sheep from groups N.v. 10 and N.v. 50 are presented in Table 2. These results showed a decrease of the citoplasm/nucleus ratio due to a dark chief cell predominance which represents a parathyroid cell atrophy.

Bones. In wethers of the N.v. 50 and N.V. 10 groups basophilic lines with loss of birefrigency were observed at the surfaces of osteonic and trabecular bone. These lines, probably related to the production of abnormal osteoid tissue by osteoblasts, were more frequent in animals of the N.v. 50 than those of the N.v. 10 group. Wethers from the N.v. 10 group had a discrete osteopetrosis, characterized by enlargement and confluence of trabeculae in the methaphysis and epiphysis. Rare areas of osteonecrosis, characterized by dead osteocytes with empty lacunae, were observed in the center of the enlarged trabeculae.

Table 2. *Citoplasm/nucleus ratio of parathyroids in the experimental sheep*

Group	Wether nº	Cytoplasm/nucleus ratio (X ± Sx)
N.v. 50	1	3.04 ± 0.22
	2	2.41 ± 0.15
	3	2.02 ± 0.11
N.v. 10	4	3.59 ± 0.22
	5	2.18 ± 0.21
	6	ND[a]
N.v. 0	7	5.13 ± 0.46
	8	5.21 ± 0.42
	9	3.35 ± 0.18

[a] Not done; the parathyroid was not found in this animal.

In the articular cartilage in wethers of group N.v. 10 and N.v. 50 there was very poor differentiation into vesicular cartilage and a layer of bone, forming the proximal terminal plate, was attached directly to the cartilage. Such plate was thicker in wethers of the N.v. 10 group than in those of the N.v. 50 group. In the control sheep the articular cartilage showed normal differentiation; a thin zone of vesicular cartilage was present and the vascular penetration of the vesicular cells was active, with normal formation of epiphyseal trabeculae.

In wethers of the N.v. 10 group the epiphyseal cartilage was thinner than in the controls, nearly half of the plate was occupied by resting cartilage, differentiation into vesicular cartilage was poor, penetration of blood vessels into poorly differentiated vesicular cartilage was irregular, and in the secondary spongiosa very thick transverse trabeculae were observed. In wethers of the N.v. 50 group the epiphyseal plate was narrow and constituted mainly by resting cartilage, with a poorly defined zone of columnar cartilage and a very narrow zone of vesicular cartilage. There was virtually no primary spongiosa and the trabeculae of the secondary spongiosa were almost attached directly to the plate. In the control group the epiphyseal plate was normal with a thin layer of resting cartilage and a clear conversion into columnar and vesicular cartilage. Penetration of the vesicular cartilage by blood vessels in the primary

spongiosa and formation of methaphyseal trabeculae were normal.

DISCUSSION

The experimental reproduction of enzootic calcinosis in sheep demonstrated that *N. veitchii* is responsible for the disease in southern Brazil.

Clinical signs observed were loss of body weight in the N.v. 50 group and decreased weight gains in N.v. 10 group, and also decreased feed consumption in both groups. The severe emaciation and stiffness of movements observed in field cases (Riet-Correa et al. 1987) were not evident in the experimental animals. This suggests that in spontaneous outbreaks, in which the incidence of the disease varies between 1% and 80% (Riet-Correa et al. 1987), economic losses related to meat and probably wool production, occur also in animals without detectable clinical signs.

N. veitchii produced a constant hypercalcemia within 48 hours in the N.v. 10 and N.v. 50 groups. Similar hypercalcemia occurred in field cases during the period of *N. veitchii* consumption (Riet-Correa et al. 1987). Phosphorus serum levels were increased after 5 days in both groups that received *N. veitchii*. Hyperphosphatemia was not constant in field cases (Riet-Correa et al. 1987).

Macroscopic and histologic lesions were similar to those described in enzootic calcinosis in sheep in southern Brazil (Riet-Correa et al. 1987), and to the pathology produced by other calcinogenic plants and cholecalciferol intoxication (Carrillo & Worker 1967, Chineme et al. 1976, Krook et al. 1975, Libiseller et al. 1976, Simensen et al. 1978).

Riet-Correa et al. (1987) demonstrated that *N. veitchii* contains vitamin D-like activity since the addition of the plant to a chicken rachinogenic diet stimulated calcium absortion and increased the amount of calcium binding proteins synthesized by the duodenal mucosa. In the same series of experiments a small quantity of calcitriol (1,25 $(OH)_2D_3$) – like substance was demonstrated.

No calcifications were observed in ligaments, tendons or interlobular septae of the lungs, which are common in spontaneous enzootic calcinosis (Riet-Correa et al. 1987). A longer consumption period of *N. veitchii* is probably required to induce these lesions.

Hyperplasia of thyroid C cells observed in 3 sheep, a morphologic evidence of hypercalcitoninism, would be a consequence of hypercalcemia. Atrophy of parathyroid cells, more marked in the N.v. 50 group, is a morphologic evidence of hypoparathyroidism. This could be a consequence of hypercalcemia or due to the direct effect of the *N. veitchii* active principle on the gland.

The amount of *N. veitchii* used to reproduce the disease was higher than the amounts necessary to induce calcinosis with *Solanum malacoxylon* and *Cestrum diurnum*, both plants containing calcitriol-like substances (Wasserman 1978). It is possible to induce severe calcinosis and death in sheep with doses of 5 g per kg body weight of *S. malacoxylon* (Camberos & Davis 1969). This amount is approximately 78 times smaller than that of *N. veitchii* used in this experiment. *Cestrum diurnum* is apparently 10 times less potent than S. *malacoxylon* (Wasserman 1978). *Trisetum flavescens*, a calcinogenic plant with a calcitriol-like substance (Rambeck & Zucker 1982) appears to be less potent than *N. veitchii*. In order to induce severe calcinosis in sheep it was necessary to mix *Trisetum flavescens* in the food to a level of 50% for 154 days (Simon et al. 1978).

The high doses of *N. veitchii* necessary to induce calcinosis are consistent with the fact that the disease occurs spontaneously only in fields where the plant occurs in large quantities (Riet-Correa et al. 1987).

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