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Lingual Calcinosis Circumscripta in a Captive Sitatunga

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ABSTRACT: Calcinosis circumscripta (CC) was found in a 10-yr-old female sitatunga (Bovidae; *Tragelaphus spekei*). At necropsy, there were two white coalescing nodules (3 × 5 × 2 cm, 2 × 2 × 1.5 cm) on the ventral side of the tongue. The cut surface of the nodules had multiple, well-circumscribed loculi with chalky appearance and gritty consistency, separated by thin strands of connective tissue. Histologically, the nodules contained multiple loculi of various sizes and shapes, which consisted of amorphous material that was pale basophilic with H&E stain, surrounded by fibrous connective tissue. Around the loculi were numerous foreign body giant cells and epithelioid macrophages; occasional lymphocyte aggregations also were seen. The amorphous material was positive for PAS and von Kossa's stain. Calcinosis circumscripta is rare in the Bovidae.

Key words: Calcinosis circumscripta, tongue, sitatunga, *Tragelaphus spekei.*

Calcinosis circumscripta (CC) is an occasional form of dystrophic calcification characterized by the formation of hard, well-circumscribed cutaneous nodules most common in dogs and humans (Dodd and Raker, 1970; Legendre and Dade, 1974; Thompson et al., 1959). It is rarely observed in the horse (Goulden and O'Callghan, 1980) and monkey (Nielsen and Cole, 1960). There is one reported case of CC in a cow (Anderson and King, 1988). The pathogenesis of this lesion has not been established (Jones et al., 1997). It has been thought to be associated with cystic apocrine glands of the skin, and some researchers called this lesion "cystic apocrine calcinosis" (Christie and Jabara, 1964). However, CC has been rarely found in the tongue, which lacks apocrine glands (Douglas and Kelly, 1966). In wildlife, to our knowledge, no cases of CC have been reported. Herein, we report CC in the tongue of a captive sitatunga (*Tragelaphus spekei*).

A 10-year-old female sitatunga was submitted to the zoo veterinary hospital (Gunma Safari World, Tomioka, Gunma, Japan) because of an accidental bruise. Her condition did not improve with treatment, and she died of traumatic shock one day after submission. The animal was obtained from a commercial supplier, and maintained for display and breeding purposes. Sitatungas were grazed by day in a field, but were kept together indoors at night. They were provided with a commercial diet for livestock, fresh grass and vegetables, and tap water *ad libitum.*

At necropsy, there were two white coalescing nodules (3 × 5 × 2 cm, 2 × 2 × 1.5 cm) on the ventral side of the tongue. The cut surface of the lingual nodule had well-circumscribed chalky-white lobuli with a gritty consistency. The lobuli varied in size and were separated by thin strands of connective tissue (Fig. 1). The nodules were deep to the muscle, and occupied approximately one fourth of the tongue at the largest diameter. In addition, bone fractures of pelvis and rupture of the urinary bladder were observed.

Organs and tissues were collected and fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). The lingual nodules were sliced at 1 cm, and the sections were processed to H&E sections with or without decalcification. Selected sections of the nodules were stained with periodic acid-Schiff (PAS), von Kossa's, and alizarin red stains (Vacca, 1985). Elemental analysis of the lingual nodules was performed by a Hitachi (Hitachi-naka, Ibaragi, Japan) H-600 electron microscope in scanning mode at an accelerating voltage of 10 kV combined with an energy dispersive X-ray microanalyzer, EDAX (Mahwh, New Jersey, USA)

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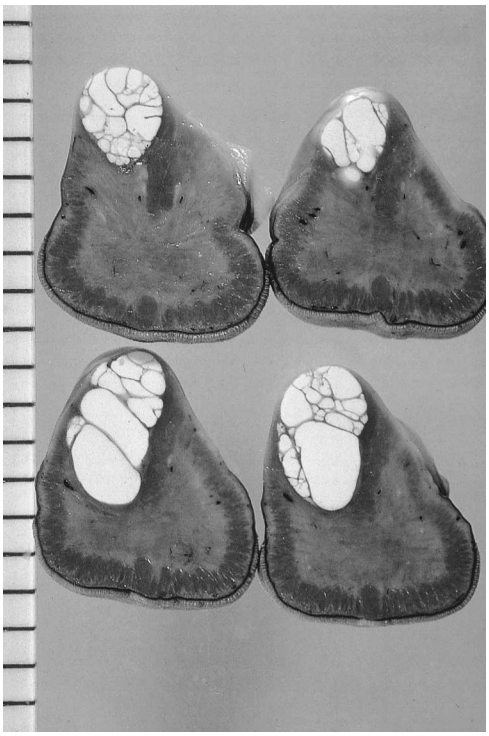


FIGURE 1. Cut section of the lingual nodules from a sitatunga shows multiple loculi with a chalky appearance and a gritty consistency. The loculi are separated by thin fibrous strands. Formalin fixed tissue.

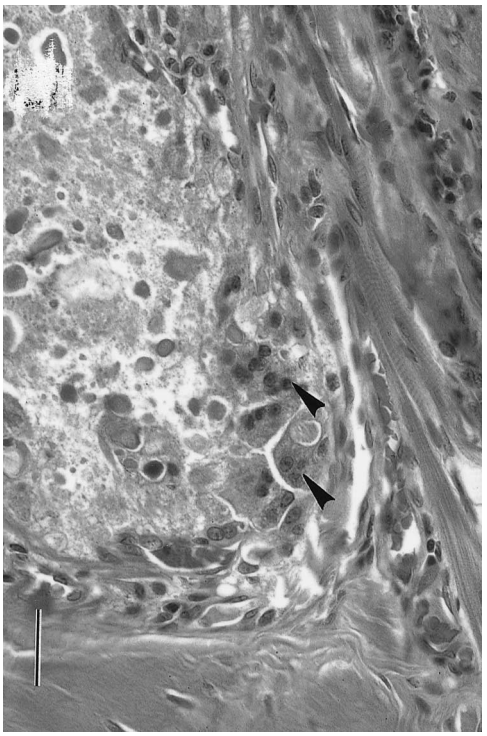


FIGURE 2. At the margin of deposition of calcinosis circumscripta in a sitatunga showing frequent epithelioid and multinucleated macrophages (arrow heads). There are numerous small clumps of basophilic material in the deposition. H&E stain. Bar = 37 μm.

System Model 9100/60, fitted with a microcomputer for spectral processing.

Histopathologically, the lingual nodules consisted of multiple loculi and surrounding fibrous connective tissue. Each loculus contained various amounts of pale to deeply basophilic material, which was acellular, and of homogenous or granular appearance, very hard and easily fragmented in the specimen without decalcification. The size of loculi varied greatly, from a small collection of deeply basophilic calcified foci to a huge pale basophilic homogenous mass. There were numerous small clumps of basophilic material in the deposition, especially near the periphery (Fig. 2). Around the loculi there were various degrees of granulomatous inflammation characterized by the occurrence of many foreign-body giant cells and epithelioid macrophages. There were occasional aggregations of lymphocytes. The material in the depositions was intensely positive for PAS, von Kossa's, and alizarin red stains.

Scanning electron microscopy of the fracture plane of the depositions was relatively rough and granular with a non-organic appearance. X-ray microanalysis on the surface revealed prominent peaks of calcium and phosphorus, with a trace amount of sodium. In addition to the tongue lesions there was moderate centrilobular fatty change in the liver, moderate atrophy of lymphoid tissue in the spleen, and mild anthracosis in the lung.

The morphological features of lingual calcinosis in a sitatunga closely resembled those of CC in dogs and humans (Jones et al., 1997; Legendre and Dade, 1974; Thompson et al., 1959). The term "tumor calcinosis" is used synonymously for this le-

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sion. Whether it be called "calcinosis circumscripta" or "tumor calcinosis" seemed to be a matter of personal preference, for either name is equally applicable (Dodd and Raker, 1970). Calcinosis circumscripta has been occasionally observed in dogs, and humans, but extremely rare in the Bovidae including cow (Anderson and King, 1988). The present case seems to be the second reported in bovids. It is uncertain why CC has been rarely seen in the Bovidae.

In dogs, several hypotheses for the pathogenesis of CC have been proposed. Breed and familial prevalence has been observed in CC in dogs; it is fairly common in young dogs of the larger breeds such as German shepherds (Cordy, 1967).

Dystrophic calcification in cystic and disorganizing apocrine glands is suspected to evolve to CC. However, a relationship to apocrine glands was not easy to trace and did not account for lingual CC (Christie and Jabara, 1964). The onset of CC following subcutaneous administration of a commercial medraxyprogesterone (MPA) preparation has been described recently in a few poodle bitches (Ginel et al., 1995). Nielsen and Cole (1960) proposed that foci of CC were totally mineralized calcifying epitheliomas.

Douglas and Kelly (1966) noted that since there are no apocrine glands in the tongue, glandular hyperplasia is not an essential prelude to a focus of CC. Howell and Ishmael (1968) reported six cases of lingual CC in dogs in which the site of occurrence varied in the tongue, such as on the tip or ventral surface. They also examined the normal tongues of six dogs in the area where the lesion was expected to have developed.

The deposit in the present CC was intensely positive for PAS mucopolysaccaride, and von Kossa's and alizarin red stain for calcium as seen in CC in dogs (Howell and Ishmael, 1968). Examination with the energy-dispersive elemental X-ray microanalyzer (EDAX) revealed prominent peaks of calcium and phosphorus in the deposits. The deposits appeared to contain calcium and phosphorus embedded within a mucopolysaccharide matrix. The pathophysiology of lingual CC seemed to be similar to that seen in dogs. The etiology of this disease remains unclear.

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