CALCINOSIS CIRCUMSCRIPTA IN LIONS (PANTHERA LEO)


From the Smithsonian Institution National Zoological Park, Smithsonian Conservation Biology Institute, Wildlife Health Sciences, 3001 Connecticut Avenue, Washington, DC 20008, USA (Bauer, Sander, Steeil, Walsh, Neiffer). Present address (Sander): The Maryland Zoo in Baltimore, 1876 Mansion House Drive, Baltimore, MD 21217, USA.

Editorial correspondence should be directed to Dr. Bauer, Smithsonian Institution National Zoological Park, Smithsonian Conservation Biology Institute, Wildlife Health Sciences, 3001 Connecticut Avenue, Washington, DC 20008, USA, 812-606-1724, bauer.kendra@gmail.com.
Abstract: Three juvenile, genetically related African lions (*Panthera leo*) were evaluated for discrete dome-shaped subcutaneous masses present over the proximal lateral metatarsal/tarsal area. The lesions measured 3–8 cm diameter, were fluctuant to firm, non-ulcerated, and attached to underlying structures. On radiographic evaluation, the lesions were characterized by well-circumscribed punctate mineralizations in the soft tissue surrounded by soft tissue swelling without evidence of adjacent bony involvement. On cut surface, the lesions were made of numerous loculi containing 2-5 mm round to ovoid, white to gray, firm structures interspersed with fibrous tissue and pockets of serosanguinous fluid. Hematology, serum biochemistry, serum thyroid screening (including total thyroxine, total triiodothyronine, free thyroxine, and free triiodothyronine), and serum vitamin D panels (including parathyroid hormone, ionized calcium, and 25-hydroxyvitamin D) were unremarkable. Histopathological evaluation of the lesions was consistent with calcinosi circumcision with fibroplasia, chronic inflammation, and seroma formation. An additional two genetically related lions were considered suspect for calcinosi circumcision based on presentation, exam findings, and similarity to the confirmed cases. All masses self-regressed and were not associated with additional clinical signs other than initial lameness in two cases.

Key words: Calcinosi circumcision, lion, *Panthera leo*, skin, tumoral calcinosi.
INTRODUCTION

Calcinosis circumscripta describes a syndrome of ectopic deposition of calcium salts in soft tissue structures. The syndrome has uncommonly been reported in both humans and animals.\textsuperscript{2,12} In animals, these lesions occur most commonly on the hind feet or on the tongue.\textsuperscript{12} Large breed dogs less than two years of age are most commonly affected with calcinosis circumscripta.\textsuperscript{12} Etiology of this condition is poorly understood, though different types have been classified into dystrophic, metastatic, iatrogenic, and idiopathic.\textsuperscript{12} The etiology of dystrophic calcinosis circumscripta occurs in the presence of normal calcium and phosphorus levels and is thought to be a response to localized tissue damage.\textsuperscript{12} Tissue damage may come from varied sources; choke chains have been implicated in four cases of calcinosis circumscripta in young large breed dogs.\textsuperscript{4} Metastatic calcification occurs more frequently in humans and may be the result of renal failure or vitamin D toxicosis. These cases are often associated with hypercalcemia and/or hyperphosphatemia.\textsuperscript{12} Cases of iatrogenic calcinosis circumscripta have been associated with subcutaneous medroxyprogesterone and polydioxanone use in dogs.\textsuperscript{5,7} Cases are classified as idiopathic if no tissue damage or metabolic defect is associated with the occurrence of a calcinosis circumscripta lesion.\textsuperscript{12}

While rare, calcinosis circumscripta has also been documented in non-domestic animals, including buffalo (Bos bubalis), cynomolgus macaque (Macaca fascicularis), sitatunga (Tragelaphus spekei), African cheetah (Acinonyx jubatus), and red-bellied short-necked turtles (Emydura subglobosa).\textsuperscript{1,6,10,13} To the authors’ knowledge, this is the first report of calcinosis circumscripta in an African lion (Panthera leo).

CASE REPORTS
A 9-month-old female African lion was reported to have a grade 3/5 lameness of the right hind limb. On visual examination, a mild diffuse swelling was noted along the right tarsal joint and a focal swelling was noted along the lateral aspect of the right fifth metatarsal bone (Figure 1). Treatment was initiated with meloxicam (Yung Shin Pharmaceutical Ind. Co. Ltd., Taichia, Taichung 43769, Taiwan; 0.1 mg/kg p.o., s.i.d. for 3 days). Improvement in the lameness was initially reported, but the lameness recurred and no reduction in the swellings was observed. Twenty-five days after the original presentation, the animal was anesthetized for further evaluation of the observed clinical signs. At that time, firm encapsulated masses (2 x 5 cm) were present bilaterally on the lateral aspects of the leg at the level of the tarsus and metatarsus. Radiographic evaluation of the swellings revealed multifocal mineralized structures within the masses (Figure 2). Fine needle aspiration and 5 mm punch biopsy samples were collected from both lesions. After sampling, 1-2 ml of clear serous fluid and several (approximately 20) dark gray to white, round to ovoid, 2-5 mm, firm structures were expressed from the biopsy openings during palpation of the masses (Figure 3). The biopsy sites were sutured closed, and amoxicillin was prescribed (DAVA Pharmaceuticals, Inc., Fort Lee, New Jersey 07024, USA; 16 mg/kg p.o. b.i.d. for 5 days). Histopathologic evaluation of the biopsies revealed mineralization, fibrosis, and granulation tissue within the subcutis, consistent with a diagnosis of calcinosis circumscripta (Figure 4). Fluid surrounding the mineralized space in an encapsulated mass was consistent with seroma formation. Mild multifocal lymphocytic plasmacytic dermatitis on histopathology was considered secondary to underlying irritation from the mineralized structures. The lameness and swelling resolved following the exam. An additional occurrence was reported 4 months later when the animal was reported to have a 2/5 lameness on the right hind limb and a small raised
mass over the lateral metatarsal area. One dose of meloxicam (0.1 mg/kg p.o.) was administered, and the lameness and swelling resolved within a week without further treatment. No additional reports of lameness or swelling have been reported in this animal.

Case 2

An 11-month-old male African lion was anesthetized for evaluation of masses on the tarsi bilaterally. This animal was a littermate of the animal in Case 1. The masses were originally noted approximately three months prior but were not associated with any lameness and were subjectively considered to have decreased in size over time. On examination, there were subcutaneous masses located bilaterally on the legs at the level of the proximolateral tarsi and metatarsi. The right mass measured 1 x 3 cm and on palpation seemed to consist of multiple firm structures surrounded by a fluctuant material. The mass on the left had a similar appearance but was larger in size (2 x 6 cm) and extended more distally and caudally than the contralateral mass. Radiographic evaluation showed soft tissue swelling containing multiple round mineral opacity structures at the location of the masses bilaterally. A full thickness wedge biopsy was collected for histopathology. Digital manipulation of the mass to express the mineralized material was unsuccessful. The area was sutured closed, and meloxicam (Norbrook Laboratories Limited, Newry, Northern Ireland, BT35 6QQ; 0.1 mg/kg s.c.) was administered. Histopathologic evaluation again showed mineralization, fibrosis, and granulation tissue within the subcutis, consistent with a diagnosis of calcinosis circumscripta. Mild multifocal lymphocytic plasmacytic dermatitis was also present. During an examination for an unrelated condition approximately one year later, fibrotic tissue was palpable at the location of the previous tarsal masses but no masses were visible.
A 13-month-old female African lion was anesthetized for evaluation of a swelling on the right tarsus. This lion shared a sire with the lions in cases 1 and 2 and the dam is a full sister of the dam from cases 1 and 2. This animal also had a history of swelling on the right lateral aspect of the tail base three months previously. On examination, the tail base swelling was no longer apparent. The tarsal mass was located over the proximolateral tarsus and measured 2 x 3 cm. The mass was soft with mild mobility and adhered to underlying structures. Radiographic evaluation of the mass was consistent with a soft tissue swelling containing mineralized structures. An excisional biopsy was attempted to completely remove the mass but was unsuccessful due to its intimate association with underlying structures. At that time, a partial biopsy of the mass was collected and the incision site was sutured closed. Meloxicam (0.1 mg/kg s.c.) and penicillin G benzathine and penicillin G procaine (Bimeda, Inc., Le Sueur, Minnesota 56058, USA; 22,000 IU/kg s.c.) were administered at the time of the examination. Histopathologic evaluation was similar to the previous cases and included mineralization of the subcutis with granulomatous inflammation and fibrosis consistent with calcinosis circumscripta and seroma formation. Amoxicillin (17 mg/kg p.o., b.i.d. for 7 days) was prescribed post-operatively. No lameness or recurrence of swelling was noted in this individual.

A 7-month-old female African lion was anesthetized for evaluation of a one-week history of progressive lameness in the right hind limb that was not responsive to meloxicam treatment (0.1 mg/kg p.o., s.i.d, for 6 days). This lion was a cub from a previous litter of the same sire and dam pairing as the lions in cases 1 and 2. On examination, mild swelling (1 x 3 cm) of the right hock
was detected. Radiographic evaluation revealed multiple 1-2 mm mineralized structures at cranial and caudal aspects of the distal right tibia. No biopsies were taken, but exam and radiographic findings were consistent with calcinosis circumscripta. After the procedure, meloxicam treatment was extended for an additional three days concurrent with amoxicillin (21 mg/kg p.o., b.i.d, for 14 days). The observed lameness resolved within two weeks following the examination. The swelling spontaneously regressed and was no longer apparent at a routine examination under anesthesia 18 months later.

Case 5

An 8-month-old male African lion was anesthetized for evaluation of a 2-month history of left hind limb swelling with no associated lameness or other clinical signs. This lion was a littermate of the lions in case 1 and 2. On examination, lateral swelling (1 x 4 cm) was apparent at the level of the fifth metatarsal bone. Radiographs revealed soft tissue swelling and mineralized structures at the level of the mass. Punch biopsies were collected, and meloxicam (0.1 mg/kg s.c.) and penicillin G benzathine and penicillin G procaine (30,000 IU/kg i.m.) were administered. On histopathologic evaluation, the mass was composed of synovial membrane containing fluid, consistent with synovial outpocketing or an isolated synovial cyst. Of note, the punch biopsy sampling was not deep enough to contain any of the mineralized structures visible on radiographs; however, calcinosis circumscripta was suspected in this case given the radiographic changes consistent with cases 1-4 and similar presentation. The swelling was reported to resolve without additional intervention and was no longer apparent at a routine examination under anesthesia one year later.

DISCUSSION
In domestic species, calcinosis circumscripta lesions vary in severity and in some instances lesions may regress on their own over variable time.\textsuperscript{12} However, surgical removal of calcinosis circumscripta lesions may be required based on the location, size, and associated metabolic abnormalities and clinical signs. The behavior of calcinosis circumscripta lesions has been variable in exotic species. Among carnivores, calcinosis circumscripta has been diagnosed in a cheetah on the lateral aspect of the left femur with the condition being addressed by surgical excision of the lesion.\textsuperscript{8}

In this case series, cases 1, 2, and 3 represent histopathologically confirmed cases of calcinosis circumscripta, while cases 4 and 5 were suspected given physical exam and radiographic characteristics similar to the first three cases. The lesions in case 1 and 4 were initially associated with lameness, but cases 2, 3, and 5 were not associated with additional clinical signs. All masses regressed over time and did not require additional surgery beyond initial diagnostic biopsies. Treatment varied between cases, but all were treated with a non-steroidal anti-inflammatory drug. All animals in this case series maintained normal appetite and behavior and did not develop additional clinical signs that could be attributed to the lesions.

A cause for the lesions described in this series was not identified. Organ dysfunction was ruled out in the three confirmed cases (cases 1-3), as the lions had normal hematology, serum biochemical analysis, vitamin D panel, and thyroid screening.\textsuperscript{3,11} In addition, aerobic bacterial cultures of the lesions in cases 1, 2, 3, and 5 yielded no bacterial growth which, along with histopathologic evaluation, ruled out active bacterial infection as the cause of the masses. All lions were apparently healthy, behaviorally normal, and had no pertinent historical medical concerns.
Of interest, all five lions in this case series are genetically related. The lions in cases 1, 2, and 5 are littermates, while case 4 was a cub from a previous litter of the same sire and dam pairing.

Case 3 shared a sire with the other cases and the dam is a full sister of the dam from cases 1, 2, 4, and 5. Neither of the sires or dams of the affected lions were reported to have similar lesions.

The lions in cases 1, 2, and 5 had one unaffected littermate; the lion in case 3 had one unaffected littermate; and the lion in case 4 had three unaffected littermates. Subclinical unreported cases cannot be ruled out in these cases. The relatedness of the affected lions suggests the potential for a genetic predisposition to calcinosis circumscripta in this group. Genetic relatedness among cases has been noted previously in both domestic and exotic animals, including a group of Irish wolfhounds and a clutch of red-bellied short-necked turtles. In humans, a familial form of calcinosis circumscripta has been shown to be associated with a single autosomal recessive gene.

Age was another common factor in the lions in this case series. All affected lions were under two years old at the time of the original presentation of clinical signs. This is consistent with previous reports of young domestic animals being predisposed to calcinosis circumscripta.

In the lions in this case series, the locations of the lesions are sites susceptible to repetitive trauma through play fighting. Repetitive trauma has been associated with cases of calcinosis circumscripta in domestic animals, and trauma may have also played a role in the cases in this series. Normal calcium and phosphorus levels in these lions combined with suspected inciting tissue damage suggests a diagnosis of dystrophic calcinosis circumscripta in these cases.

Calcinosis circumscripta should be considered as a differential diagnosis for soft tissue swellings in juvenile lions.
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Figure 1: The juvenile female African lion (*Panthera leo*) in case 1 with bilateral swelling present over the lateral aspect of each tarsus, similar to each of the five cases in this series. The lesions measured 4-8 cm diameter each, were fluctuant to firm, non-ulcerated, and attached to underlying structures (white arrows).

Figure 2: Radiographs of the juvenile female African lion (*Panthera leo*) in case 1, including dorsoplantar (A) and lateral (B) projections. The lesions (white arrows) were characterized by well-circumscribed punctate mineralization in the soft tissue lateral to the tarsus with no evidence of bony involvement.

Figure 3: Contents of tarsal swellings in the juvenile female African lion (*Panthera leo*) in case 1. The lesions consisted of numerous loculi consisting of 2-5 mm white to grey, round to ovoid firm structures interspersed with fibrous tissue and pockets of serosanguinous fluid.

Figure 4: Histopathological evaluation of lesions in the juvenile female African lion (*Panthera leo*) in case 1. The lesions from the three confirmed cases were characterized by consistent features of fibroplasia (circle) with fragments of dense mineral (arrow); discrete, large deposits of granular mineral (star); and associated, mild granulomatous inflammation. Empty spaces often contained serosanguinous fluid (diamond). Findings were consistent with a diagnosis of calcinosis circumspecta and associated seroma formation.