

1 **RH: CALCINOSIS CIRCUMSCRIPTA IN LIONS**

2

3 CALCINOSIS CIRCUMSCRIPTA IN A COHORT OF RELATED JUVENILE AFRICAN
4 LIONS (*PANTHERA LEO*)

5

6 Kendra L. Bauer, D.V.M., Samantha J. Sander D.V.M., Dipl. A.C.Z.M., James C. Steeil,
7 D.V.M., Dipl. A.C.Z.M., Timothy F. Walsh, D.V.M. Dipl. A.C.V.P., and Donald L. Neiffer,
8 V.M.D., C.V.A., Dipl. A.C.Z.M.

9

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

14

15 Editorial correspondence should be directed to Dr. Bauer, Smithsonian Institution
16 National Zoological Park, Smithsonian Conservation Biology Institute, Wildlife Health
17 Sciences, 3001 Connecticut Avenue, Washington, DC 20008, USA, 812-606-1724,
18 bauer.kendra@gmail.com.

19

20

21

22

23 Abstract: Three juvenile, genetically related African lions (*Panthera leo*) were evaluated for
24 discrete dome-shaped subcutaneous masses present over the proximal lateral metatarsal/tarsal
25 area. The lesions measured 3–8 cm diameter, were fluctuant to firm, non-ulcerated, and attached
26 to underlying structures. On radiographic evaluation, the lesions were characterized by well-
27 circumscribed punctate mineralizations in the soft tissue surrounded by soft tissue swelling
28 without evidence of adjacent bony involvement. On cut surface, the lesions were made of
29 numerous loculi containing 2-5 mm round to ovoid, white to gray, firm structures interspersed
30 with fibrous tissue and pockets of serosanguinous fluid. Hematology, serum biochemistry, serum
31 thyroid screening (including total thyroxine, total triiodothyronine, free thyroxine, and free
32 triiodothyronine), and serum vitamin D panels (including parathyroid hormone, ionized calcium,
33 and 25-hydroxyvitamin D) were unremarkable. Histopathological evaluation of the lesions was
34 consistent with calcinosis circumscripta with fibroplasia, chronic inflammation, and seroma
35 formation. An additional two genetically related lions were considered suspect for calcinosis
36 circumscripta based on presentation, exam findings, and similarity to the confirmed cases. All
37 masses self-regressed and were not associated with additional clinical signs other than initial
38 lameness in two cases.

39

40 *Key words:* Calcinosis circumscripta, lion, *Panthera leo*, skin, tumoral calcinosis.

41

42

43

44

45

INTRODUCTION

46

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

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

67

CASE REPORTS

68 Case 1

69 A 9-month-old female African lion was reported to have a grade 3/5 lameness of the right
70 hind limb. On visual examination, a mild diffuse swelling was noted along the right tarsal joint
71 and a focal swelling was noted along the lateral aspect of the right fifth metatarsal bone (Figure
72 1). Treatment was initiated with meloxicam (Yung Shin Pharmaceutical Ind. Co. Ltd., Taichia,
73 Taichung 43769, Taiwan; 0.1 mg/kg p.o., s.i.d. for 3 days). Improvement in the lameness was
74 initially reported, but the lameness recurred and no reduction in the swellings was observed.
75 Twenty-five days after the original presentation, the animal was anesthetized for further
76 evaluation of the observed clinical signs. At that time, firm encapsulated masses (2 x 5 cm) were
77 present bilaterally on the lateral aspects of the leg at the level of the tarsus and metatarsus.
78 Radiographic evaluation of the swellings revealed multifocal mineralized structures within the
79 masses (Figure 2). Fine needle aspiration and 5 mm punch biopsy samples were collected from
80 both lesions. After sampling, 1-2 ml of clear serous fluid and several (approximately 20) dark
81 gray to white, round to ovoid, 2-5 mm, firm structures were expressed from the biopsy openings
82 during palpation of the masses (Figure 3). The biopsy sites were sutured closed, and amoxicillin
83 was prescribed (DAVA Pharmaceuticals, Inc., Fort Lee, New Jersey 07024, USA; 16 mg/kg p.o.
84 b.i.d. for 5 days). Histopathologic evaluation of the biopsies revealed mineralization, fibrosis,
85 and granulation tissue within the subcutis, consistent with a diagnosis of calcinosis circumscripta
86 (Figure 4). Fluid surrounding the mineralized space in an encapsulated mass was consistent with
87 seroma formation. Mild multifocal lymphocytic plasmacytic dermatitis on histopathology was
88 considered secondary to underlying irritation from the mineralized structures. The lameness and
89 swelling resolved following the exam. An additional occurrence was reported 4 months later
90 when the animal was reported to have a 2/5 lameness on the right hind limb and a small raised

91 mass over the lateral metatarsal area. One dose of meloxicam (0.1 mg/kg p.o.) was administered,
92 and the lameness and swelling resolved within a week without further treatment. No additional
93 reports of lameness or swelling have been reported in this animal.

94 Case 2

95 An 11-month-old male African lion was anesthetized for evaluation of masses on the tarsi
96 bilaterally. This animal was a littermate of the animal in Case 1. The masses were originally
97 noted approximately three months prior but were not associated with any lameness and were
98 subjectively considered to have decreased in size over time. On examination, there were
99 subcutaneous masses located bilaterally on the legs at the level of the proximolateral tarsi and
100 metatarsi. The right mass measured 1 x 3 cm and on palpation seemed to consist of multiple firm
101 structures surrounded by a fluctuant material. The mass on the left had a similar appearance but
102 was larger in size (2 x 6 cm) and extended more distally and caudally than the contralateral mass.
103 Radiographic evaluation showed soft tissue swelling containing multiple round mineral opacity
104 structures at the location of the masses bilaterally. A full thickness wedge biopsy was collected
105 for histopathology. Digital manipulation of the mass to express the mineralized material was
106 unsuccessful. The area was sutured closed, and meloxicam (Norbrook Laboratories Limited,
107 Newry, Northern Ireland, BT35 6QQ; 0.1 mg/kg s.c.) was administered. Histopathologic
108 evaluation again showed mineralization, fibrosis, and granulation tissue within the subcutis,
109 consistent with a diagnosis of calcinosis circumscripta. Mild multifocal lymphocytic plasmacytic
110 dermatitis was also present. During an examination for an unrelated condition approximately one
111 year later, fibrotic tissue was palpable at the location of the previous tarsal masses but no masses
112 were visible.

113 Case 3

114 A 13-month-old female African lion was anesthetized for evaluation of a swelling on the right
115 tarsus. This lion shared a sire with the lions in cases 1 and 2 and the dam is a full sister of the
116 dam from cases 1 and 2. This animal also had a history of swelling on the right lateral aspect of
117 the tail base three months previously. On examination, the tail base swelling was no longer
118 apparent. The tarsal mass was located over the proximolateral tarsus and measured 2 x 3 cm. The
119 mass was soft with mild mobility and adhered to underlying structures. Radiographic evaluation
120 of the mass was consistent with a soft tissue swelling containing mineralized structures. An
121 excisional biopsy was attempted to completely remove the mass but was unsuccessful due to its
122 intimate association with underlying structures. At that time, a partial biopsy of the mass was
123 collected and the incision site was sutured closed. Meloxicam (0.1 mg/kg s.c.) and penicillin G
124 benzathine and penicillin G procaine (Bimeda, Inc., Le Sueur, Minnesota 56058, USA; 22,000
125 IU/kg s.c.) were administered at the time of the examination. Histopathologic evaluation was
126 similar to the previous cases and included mineralization of the subcutis with granulomatous
127 inflammation and fibrosis consistent with calcinosis circumscripta and seroma formation.
128 Amoxicillin (17 mg/kg p.o., b.i.d. for 7 days) was prescribed post-operatively. No lameness or
129 recurrence of swelling was noted in this individual.

130 Case 4

131 A 7-month-old female African lion was anesthetized for evaluation of a one-week history of
132 progressive lameness in the right hind limb that was not responsive to meloxicam treatment (0.1
133 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
134 pairing as the lions in cases 1 and 2. On examination, mild swelling (1 x 3 cm) of the right hock

135 was detected. Radiographic evaluation revealed multiple 1-2 mm mineralized structures at
136 cranial and caudal aspects of the distal right tibia. No biopsies were taken, but exam and
137 radiographic findings were consistent with calcinosis circumscripta. After the procedure,
138 meloxicam treatment was extended for an additional three days concurrent with amoxicillin (21
139 mg/kg p.o., b.i.d, for 14 days). The observed lameness resolved within two weeks following the
140 examination. The swelling spontaneously regressed and was no longer apparent at a routine
141 examination under anesthesia 18 months later.

142 Case 5

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

156

DISCUSSION

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

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

172 A cause for the lesions described in this series was not identified. Organ dysfunction was
173 ruled out in the three confirmed cases (cases 1-3), as the lions had normal hematology, serum
174 biochemical analysis, vitamin D panel, and thyroid screening.^{3,11} In addition, aerobic bacterial
175 cultures of the lesions in cases 1, 2, 3, and 5 yielded no bacterial growth which, along with
176 histopathologic evaluation, ruled out active bacterial infection as the cause of the masses. All
177 lions were apparently healthy, behaviorally normal, and had no pertinent historical medical
178 concerns.

179 Of interest, all five lions in this case series are genetically related. The lions in cases 1, 2, and
180 5 are littermates, while case 4 was a cub from a previous litter of the same sire and dam pairing.
181 Case 3 shared a sire with the other cases and the dam is a full sister of the dam from cases 1, 2, 4,
182 and 5. Neither of the sires or dams of the affected lions were reported to have similar lesions.
183 The lions in cases 1, 2, and 5 had one unaffected littermate; the lion in case 3 had one unaffected
184 littermate; and the lion in case 4 had three unaffected littermates. Subclinical unreported cases
185 cannot be ruled out in these cases. The relatedness of the affected lions suggests the potential for
186 a genetic predisposition to calcinosis circumscripta in this group. Genetic relatedness among
187 cases has been noted previously in both domestic and exotic animals, including a group of Irish
188 wolfhounds and a clutch of red-bellied short-necked turtles.^{1,9} In humans, a familial form of
189 calcinosis circumscripta has been shown to be associated with a single autosomal recessive
190 gene.²

191 Age was another common factor in the lions in this case series. All affected lions were under
192 two years old at the time of the original presentation of clinical signs. This is consistent with
193 previous reports of young domestic animals being predisposed to calcinosis circumscripta.¹²

194 In the lions in this case series, the locations of the lesions are sites susceptible to repetitive
195 trauma through play fighting. Repetitive trauma has been associated with cases of calcinosis
196 circumscripta in domestic animals, and trauma may have also played a role in the cases in this
197 series. Normal calcium and phosphorus levels in these lions combined with suspected inciting
198 tissue damage suggests a diagnosis of dystrophic calcinosis circumscripta in these cases.
199 Calcinosis circumscripta should be considered as a differential diagnosis for soft tissue swellings
200 in juvenile lions.

201 Acknowledgements: The authors thank Craig Saffoe and the animal care staff at the
202 Smithsonian's National Zoological Park for their diligent work and their unrelenting care for the
203 lions in this case series.

204

205

206

207

208

209

210

211

212

213

214

215

216

217

218

219

220

221

222

223

224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243

LITERATURE CITED

1. Burns RE, Bicknese EJ, Westropp JL, Shiraki R, Stalis IH. Tumoral calcinosis form of hydroxyapatite deposition disease in related red-bellied short-necked turtles, *Emydura subglobosa*. Vet Pathol. 2013;50:443-450.
2. Carmichael KD, Bynum JA, Evans EB. Familial tumoral calcinosis: a forty-year follow-up on one family. J Bone Joint Surg Am, 2009;91:664-671.
3. Crissey SD, Ange KD, Jacobsen KL, Slifka KA, Bowen PE, Stacewicz-Sapuntzakis M, Langman CB, Sadler W, Kahn S, Ward A. Serum concentrations of lipids, vitamin d metabolites, retinol, retinyl esters, tocopherols, and selected carotenoids in twelve captive wild felid species at four zoos. J Nutr, 2003;133:160-166.
4. Gardner DE, Alley MR, Wyburn RS, Goulden BE, Dreadon RG, Kyle MG. Calcinosis circumscripta-like lesions in dogs associated with the use of choke chains. N Z Vet J. 1975;23:95-97.
5. Ginel P, Perez J, Rivas R, Novales M, Rodriguez JL, Mozos E. Calcinosis circumscripta associated with medroxyprogesterone in two poodle bitches. J Am Anim Hosp Assoc. 1992; 28:391-394.

- 244
- 245 6. Ikede BO. Calcinosis circumscripta in the buffalo (*Bos bubalis*). Vet Pathol. 1979;16:260-
246 262.
- 247
- 248 7. Kirby BM, Knoll JS, Manley PA, Miller LM. Calcinosis circumscripta associated with
249 polydioxanone suture in two young dogs. Vet Surg. 1989;18:216-220.
- 250
- 251 8. Mumba C, Squarre D, Mwase M, Yabe J, Shibahara T. Calcinosis circumscripta in a
252 captive African cheetah (*Acinonyx jubatus*). Asian Pac J Trop Biomed. 2014;4:832-834.
- 253
- 254 9. Owen LN. Calcinosis circumscripta (calcium gout) in related Irish wolfhounds. J Small
255 Anim Pract. 1967;8:291-292.
- 256
- 257 10. Radi ZA, Sato K. Bilateral dystrophic calcinosis circumscripta in a cynomolgus macaque
258 (*Macaca fascicularis*). Toxicol Pathol. 2010;38:637-641.
- 259
- 260 11. Rodini DC, Felipe ECG, Oliveira CA. Measurement of thyroid hormones (thyroxine, T4;
261 triiodothyronine, T3) in captive nondomestic felids. J Zoo Wildl Med. 2007;38:125-126.
- 262

263 12. Tafti AK, Hanna P, Bourque AC. Calcinosis circumscripta in the dog: a retrospective
264 pathological study. *J Vet Med A Physiol Pathol Clin Med.* 2005;52:13-17.

265

266 13. Yanai T, Noda A, Kawakami S, Sakai H, Lackner AA, Masegi T. Lingual calcinosis
267 circumscripta in captive sitatunga. *J Wildl Dis.* 2001;37:813-815.

268

269

270

271

272

273

274

275

276

277

278

279

280

281

CAPTIONS

282 Figure 1: The juvenile female African lion (*Panthera leo*) in case 1 with bilateral swelling
283 present over the lateral aspect of each tarsus, similar to each of the five cases in this series. The
284 lesions measured 4-8 cm diameter each, were fluctuant to firm, non-ulcerated, and attached to
285 underlying structures (white arrows).

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

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

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







