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KANGAROO (*MACROPUS RUFUS*)**

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CRYPTOCOCCUS NEOFORMANS VAR. GRUBII-ASSOCIATED RENAL AMYLOIDOSIS CAUSING PROTEIN-LOSING NEPHROPATHY IN A RED KANGAROO (*MACROPUS RUFUS*)

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Abstract: A 10-year-old male castrated red kangaroo (*Macropus rufus*) presented with mandibular swelling. Examination findings included pitting edema with no dental disease evident on examination or radiographs. The results of blood work were moderate azotemia, hypoalbuminemia, and severely elevated urine protein:creatinine ratio (9.9). Radiographs showed an interstitial pattern of the caudal right lung, and an abdominal ultrasound demonstrated scant effusion. Symptomatic and empirical therapy with antibiotics, anti-inflammatory drugs, and an angiotensin-converting enzyme (ACE) inhibitor did not resolve clinical signs. Due to poor prognosis and declining quality of life, euthanasia was elected. Necropsy revealed chronic granulomatous pneumonia of the caudal right lung lobe with intralesional *Cryptococcus*, identified as *C. neoformans* var. *grubii* by DNA sequencing. Severe bilateral glomerular and tubulointerstitial amyloidosis induced protein-losing nephropathy, leading to tri-cavitary effusion, subcutaneous edema, and cachexia. The authors speculate that renal amyloidosis was associated with chronic cryptococcal pneumonia in this red kangaroo.

Key words: *Cryptococcus neoformans* var. *grubii*, Macropod, Protein-losing nephropathy, Red kangaroo (*Macropus rufus*), Renal amyloidosis.

BRIEF COMMUNICATION

A 10-year-old male castrated red kangaroo (*Macropus rufus*) (weight, 42.5 kg) presented for evaluation of mandibular swelling and head shaking with a normal appetite. The animal was immobilized the following day with ketamine (Putney, Portland, Maine 04101, USA; 4.2 mg/kg im) and medetomidine (Zoo Pharm, Windsor, Colorado 80550, USA; 40 µg/kg im) and was maintained on isoflurane gas (Piramal Healthcare Ltd, Mumbai 400063, India) via facemask. Medetomidine was antagonized with atipamezole (Zoetis, Florham Park, New Jersey 07932, USA; 0.21 mg/kg im). Physical examination findings included moderate facial and ventral cervical pitting edema with no evidence of dental disease or trauma and mild muscle atrophy with thin body condition. An echocardiogram showed bradycardia, likely secondary to anesthesia, with no

chamber enlargement or pericardial effusion. Skull radiographs showed no dental disease or fractures. The kangaroo was empirically treated with diphenhydramine (Mylan Institutional LLC, Canonsburg, Pennsylvania 15317, USA; 2 mg/kg im), meloxicam (Putney; 0.2 mg/kg im), and ceftiofur crystalline free acid (Pfizer, New York City, New York 10017, USA; 7.1 mg/kg sc).

Blood work revealed moderate azotemia with elevated blood urea nitrogen (BUN) (48.7 mg/dl; reference interval, 12–42 mg/dl¹³), elevated creatinine (4.8 mg/dL; reference interval, 0.6–2.5 mg/dl¹³), and borderline hypoalbuminemia (2.4 g/dl; reference interval, 2.2–5.4 g/dl¹³). Enrofloxacin (Wedgewood Pharmacy, Swedesboro, New Jersey 08085, USA; 4.8 mg/kg po q. 24 hr for 21 days) and diphenhydramine (Target Corporation, Minneapolis, Minnesota 55403, USA; 1.8 mg/kg po q. 12 hr for 7 days) were prescribed.

Over the next 3 wk, the animal exhibited waxing and waning facial and sternal edema despite treatment and was immobilized with ketamine (2.9 mg/kg im), medetomidine (40 µg/kg im), and midazolam (Akorn, Lake Forest, Illinois 60045, USA; 50 µg/kg im) for reevaluation. Anesthesia was antagonized with atipamezole (0.2 mg/kg) and flumazenil (West-Ward Pharmaceuticals Corporation, Eatontown, New Jersey 07724, USA; 0.01 mg/kg im). The kangaroo had marked pitting edema of the nares, labia, and intermandibular

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Figure 1. Severe facial edema and poor body condition of a red kangaroo with protein-losing nephropathy secondary to renal amyloidosis, likely induced by chronic cryptococcal pneumonia. Photograph courtesy of Christa Klein.

region. The abdomen appeared mildly distended, and thoracic auscultation was normal.

Blood work findings included moderate hypoalbuminemia (1.7 g/dl) and moderate azotemia (BUN, 42 mg/dl; creatinine, 3.9 mg/dl). *Leptospira* spp. polymerase chain reaction (PCR) of blood and urine (Idexx, Westbrook, Maine 04092, USA) was negative. Radiographs showed a mild interstitial pattern of the caudal right lung lobe, consistent with findings from 1 yr prior, and no evidence of uroliths. Abdominal ultrasound revealed subjectively normal renal parenchyma and scant abdominal effusion. Urine obtained via cystocentesis had a urine specific gravity (USG) of 1.008 with moderate proteinuria (>300 mg/dl). Urine protein:creatinine ratio (UPC; Idexx) was considered elevated at 9.9 (normal cat/dog values are less than 1.0⁹). Urine culture (Idexx) was negative. The kangaroo received ivermectin (Norbrook, Corby, Northamptonshire NN18 9EX, United Kingdom; 0.12 mg/kg sc).

Progressive hypoalbuminemia with markedly elevated UPC was suggestive of protein-losing nephropathy (PLN). The kangaroo remained on enrofloxacin and received fenbendazole (100-mg/ml suspension, Intervet Incorporated, Millsboro, Delaware 19966, USA; 9.4 mg/kg po q. 24 hr for 5 days) as empiric treatment for parasitism causing protein loss or chronic inflammation leading to

amyloidosis, despite a negative group fecal analysis. The animal was prescribed enalapril to decrease proteinuria (Wockhardt Pharmaceuticals, Parsippany, New Jersey 07054, USA; 0.5 mg/kg po q. 24 hr) and aspirin for thromboprophylactic treatment (Bayer, Whippany, New Jersey 07981, USA; 1 mg/kg po q 24 hr). Due to historically elevated *Toxoplasma* titers (1:512 the 2 prior years; Idexx), the kangaroo received trimethoprim sulfa (Amneal Pharmaceuticals, Bridgewater, New Jersey 08807, USA; 17.1 mg/kg po q. 12 hr).

Over the next 2 wk, the kangaroo developed intermittent dysphagia and ptyalism secondary to severe facial edema (Fig. 1). Palliative medical management was elected, and the animal was empirically started on prednisone (Roxane Laboratories Incorporated, Columbus, Ohio 43228, USA; 0.5 mg/kg po q. 24 hr) to decrease inflammation and pimobendan (Boehringer Ingelheim, Ridgefield, Connecticut 06877, USA; 0.2 mg/kg po q. 24 hr) to increase cardiac contractility in case the edema was cardiac in origin. Subjectively, the animal had increased appetite and activity for the following 5 days.

Forty-five days after presentation, the animal's condition worsened to include generalized pitting edema with eyelid involvement, lethargy, abdominal distension, and severe muscle wasting. Euthanasia was elected; blood work findings at that time included severe hypoalbuminemia (0.3 g/dl), normal BUN (35 mg/dl), elevated creatinine (3.6 mg/dl), and moderate hyponatremia (120 mEq/L; reference interval, 133–151 mEq/L¹³). The red blood cell count was mildly decreased (3.17×10^6 cells/ μ l; reference interval, $3.35\text{--}6.45 \times 10^6$ cells/ μ l¹³), and the hematocrit was at the low end of normal (33%; reference interval, 31.5–58.7%¹³), with no reticulocytes. Urine obtained by cystocentesis showed a USG of 1.038 with moderate proteinuria (>300 mg/dl), and the UPC had decreased to 3.1. Ultrasound-guided abdominocentesis revealed clear fluid with trace protein (<10 mg/dl) and a specific gravity of 1.004.

Gross necropsy was performed within 4 hr of euthanasia (University of California Davis Anatomic Pathology Service, Davis, California 95616, USA). There was approximately 1 L clear, pale yellow, watery abdominal effusion; 100 ml clear, pale pink, slightly viscous thoracic effusion; and 8 ml clear, light red, nonviscous pericardial effusion. The renal surfaces were mottled pale tan to pink, and the cortices were pale tan with diffuse red pinpoint foci. The lung surface was mottled pink to tan with a pale tan to light green nodular,

firmer region affecting approximately 50% of the right caudal lobe. Histopathologic findings included chronic granulomatous pneumonia with multifocal necrosis and intralesional *Cryptococcus* sp. of the caudal right lung lobe, as well as severe, bilateral, global glomerular, and multifocal tubulointerstitial amyloidosis with mild multifocal tubular atrophy. The spleen had a mild multifocal vascular wall and perivascular amyloidosis.

Special staining with mucicarmine highlighted cryptococcal capsules within lung granulomas (Fig. 2). Acid fast and Gram stains did not identify bacteria, and aerobic and anaerobic lung culture grew contaminants. Congo red staining confirmed amyloid deposition in both kidneys and the spleen (Fig. 2). A fungal culture of the lung was negative, and quantitative PCR was negative for *Cryptococcus neoformans* var. *neoformans*. DNA sequencing of formalin-fixed paraffin-embedded lung granulomas containing cryptococcal organisms had 100% sequence identity with *Cryptococcus neoformans* var. *grubii* (GenBank accession no. KT250732, Washington Animal Disease Diagnostic Laboratory, Pullman, Washington 99164, USA).

The amyloid distribution seen in this kangaroo was consistent with reactive systemic amyloidosis, presumably resulting from chronic granulomatous cryptococcal pneumonia. Significant protein loss occurred via PLN secondary to severe renal amyloidosis, leading to severe hypoalbuminemia, tricavitary effusion, and pitting edema. The improvement in UPC over time may have been a response to enalapril, empiric antibiotic therapy, or anti-inflammatory steroid therapy decreasing PLN severity. Alternatively, the animal may have had severe reduction of protein stores, decreasing potential for protein loss. The mild anemia at the time of death might have been secondary to decreased erythropoietin secretion due to renal failure or secondary to chronic disease.

C. neoformans is separated into *C. neoformans* var. *grubii* (serotype A; genotypes VNI and VNII) and *C. neoformans* var. *neoformans* (serotype D; genotype VNIV).³ *C. neoformans* var. *grubii* is the cause of 95% of human cryptococcosis cases worldwide² and is associated with immunosuppression.¹⁰ An analysis of global cryptococcal infections found a high prevalence of *C. neoformans* var. *grubii* infections in North America, with 33% reported as VNI and 1% as VNII.³ In one study in California, where this kangaroo lived since 2006, 33% of *Cryptococcus* cases in cats and dogs were caused by *C. neoformans* var. *grubii*.¹²

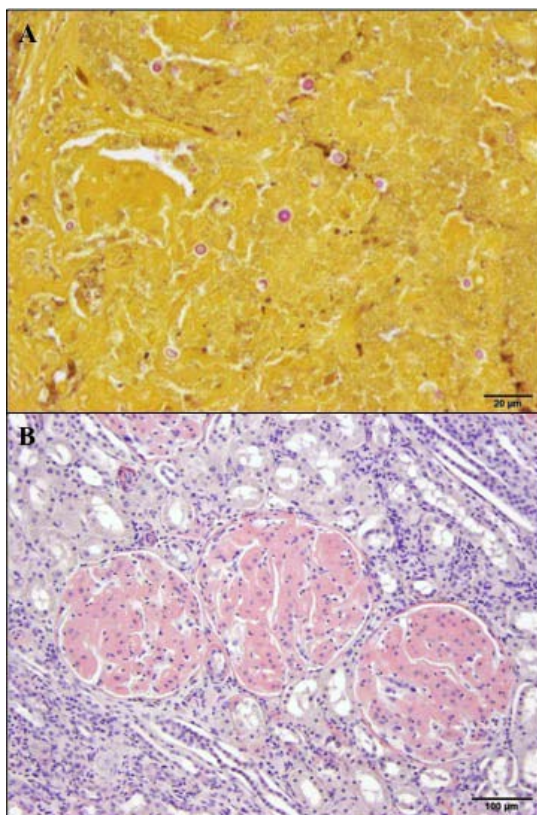


Figure 2. Renal amyloidosis secondary to chronic cryptococcal pneumonia in a red kangaroo. **A.** Mucicarmine staining of cryptococcal capsule (magenta) within necrotic foci in the pulmonary parenchyma at 40× magnification. DNA sequencing identified *C. neoformans* var. *grubii*. **B.** Congo red staining of amyloid deposited in renal glomeruli and a few surrounding tubule basement membranes at 10× magnification. These regions also exhibited apple-green birefringence when viewed with polarized light, consistent with amyloid.

Cryptococcal infections are frequently reported in koalas (*Phascolarctos cinereus*) but infrequently in other marsupial species such as macropods, with cases of cryptococcosis documented in a quokkas (*Setonix brachyurus*) and a red-necked wallaby (*Macropus rufogriseus banksianus*).⁸ *C. neoformans* var. *grubii* can be associated with eucalyptus⁵ and avian droppings,³ both of which may be found near or within this animal's enclosure. It is suspected that this kangaroo inhaled cryptococcal organisms, establishing chronic granulomatous pneumonia of the caudal right lung lobe. It is unclear when this animal was infected, although radiographic changes were present for 1 yr prior to presentation. It is possible that

another underlying condition predisposed this animal to cryptococcal infection.

Systemic reactive amyloidosis can be induced by chronic infectious, inflammatory, or neoplastic disease, leading to extracellular deposition of polymerized serum amyloid A protein (SAA), an acute phase protein produced by the liver.⁹ Reactive amyloidosis can induce PLN due to glomerular deposition of polymerized SAA protein.⁹ Renal disease due to systemic amyloidosis has been reported secondary to chronic infectious respiratory disease in several species, such as with *Mycoplasma pneumonia* in Beira antelope (*Dorcatragus megalotis*),⁶ chronic pneumonia in Dall's sheep (*Ovis dalli dalli*),⁷ and a chronic pulmonary abscess in an Eastern bongo (*Tragelaphus eurycerus isaaci*).¹⁴ Amyloidosis has been reported in an eastern grey kangaroo (*Macropus giganteus*) concurrently infected with *Babesia macropus* and in another with *Fasciola hepatica*.^{4,11} However, amyloidosis appears to be rare in macropods. In a review of captive marsupial mortality, there were no reports of amyloid in 763 macropods.¹ We speculate that renal amyloidosis was associated with chronic cryptococcal pneumonia in this red kangaroo.

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