Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

Conference 14

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National Zoological Park
Washington DC, 20008

CASE I: 18-4856 2 (JPC 4116557).

Signalment: Western Pond Turtle, female adult.

History: The turtle was found on a road in Waterloo campground in Lebanon, Oregon. Eyes were swollen bilaterally. Fed ensure and cat foot but ate very little. Treated with eye ointment and baytril.

Gross Pathology: A 468g male Western Pond Turtle (*Actinemys marmorata*) is necropsied on 10/6/2017 in fair body condition and mild post mortem autolysis. Eyelids are markedly swollen bilaterally with thick, light yellow, caseous material present under the lids. Globes are not appreciated grossly. Approximately 30% of the total lung parenchyma is red and firm and thick, light yellow, caseous material oozes out on cut surfaces.

Laboratory results: N/A.

Microscopic Description:

Cross sections of the skull, brain, and eyes. There is hyperplasia and hyperkeratosis of the eyelids and external ear canal. There is marked squamous metaplasia and layered keratin accumulation within the nasal vestibule, more prominent on one side, with dilation of some sinus mucous glands containing moderate numbers of heterophils. In one of these heterophil-rich foci there is rimming layer of epithelioid macrophages and multinucleate giant cells with small numbers of bacteria visible in the exudate. Other glands in the region (ophthalmic or sinus mucous glands) multifocally show moderate to severe squamous metaplasia and occasional keratin accumulation. There are multifocal, asymmetric areas of marked osteoclast activity.

Head, pond turtle: A transverse section through the head at the level of the globe demonstrates marked expansion of the lacrimal glands. (HE, 7X)

Contributor's Morphologic Diagnosis:

Whole body: Emaciation

Multiple organs: Squamous metaplasia consistent with hypovitaminosis A;

Sinuses: Severe diffuse heterophilic rhinitis with squamous metaplasia and keratin accumulation

Other lesions:

Lungs: severe chronic active heterophilic and proliferative bronchointerstitial pneumonia with bacteria

Eyelids/eye: Hyperplastic keratoconjunctivitis

Ears: Bilateral severe hyperplastic otitis media

Contributor's Comment: Histopathology is consistent with hypovitaminosis A; squamous metaplasia with keratinization was observed in multiple tissues throughout the body. Other disease processes may be occurring simultaneously/secondarily, (opportunistic bacteria) but the most severe lesions were due to the vitamin deficiency.

The cause of the increased bone resorption/osteoclast activity is unknown. As the change is not generalized, a Vitamin D deficiency/hypocalcemia is unlikely. , In addition, clinical reports of hypovitaminosis D in turtles mention paradoxical minera-

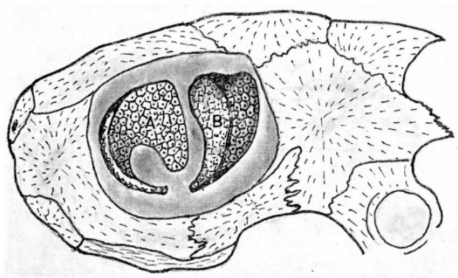


Fig. 1. Topography of the ophthalmic glands in the terrapin (diagrammatic). The Harderian gland (A) is anteromedial or rostral. The lachrymal gland (B) is posterolateral or temporal. The globe fits into the cup formed by the two glands.

Head, pond turtle: Illustration of the position of Harderian and lacrimal glands within the orbit of the turtle. (Photo courtesy of: Elkan E, Zwart P. The ocular disease of young terrapins caused by Vitamin A deficiency. Pathol Vet 1967; 4:201-222.)

lization of soft tissues (including muscle, renal tubules, etc) and such changes are lacking here. In this case, osteoclasis often occurred near areas of squamous metaplasia, so perhaps local tissue swelling/pressure was the force for osseous remodeling.

Contributing Institution:

Oregon Veterinary Diagnostic Laboratory, Carlson College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331.
https://vetmed.oregonstate.edu/diagnostic

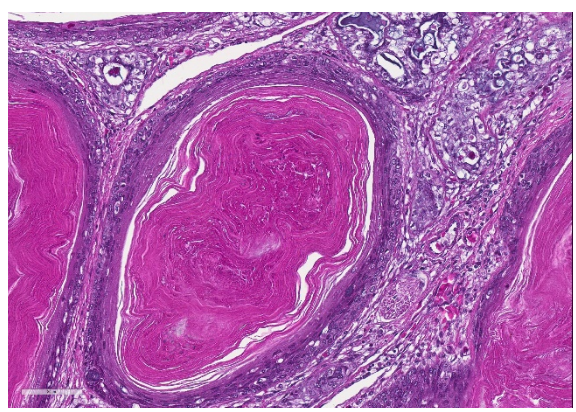
JPC Diagnosis: 1. Lacrimal glands and ducts: Squamous metaplasia, diffuse, severe, with marked hyperkeratosis, and diffuse glandular degeneration, heterophilic adenitis, and dochitis.

2. Skull, vomer and frontal bone: Bone resorption, diffuse, marked.

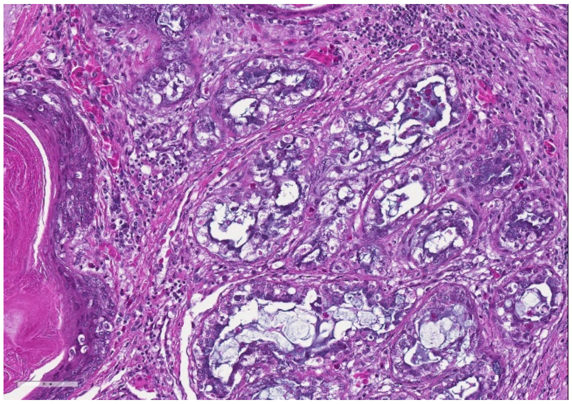
JPC Comment: This slide is an excellent example of squamous metaplasia seen in the lacrimal glands in chelonians as a result of Viamin A deficiency.

The 1967 paper in *Pathologia Veterinaria (* the forerunner of *Veterinary Pathology)* by Elkan and Zwart is an outstanding reference on the systemic effects of Vitamin A deficiency in the "young terrapin", as well as a tremendous example of scientific prose, the like of which we do not see today in modern publications.[3] While a quotation of the entire first page is poor form (and the reader can download it online), a short snippet is illustrative of how far current medical writing has fallen - "In vain do the textbooks on herpetology mention the exacting dietary requirements of juvenile terrapns, in vain their advice on how to meet these requirements…Neither the sellers nor the buyers read these books, presuming that a plastic bowl and some 'ant's eggs' suffice to keep a juvenile terrapin alive. The annual holocaust among the imported terrapins proves the error of this assumption."[3]

As mentioned by the contributor, Vitamin A deficiency in chelonians is a systemic process, rather than just one confined to the structures of the orbit.[3] Squamous metaplasia also occurs in the external ear, where it has been associated with aural "abscessation" in a number of chelonian species in association with a mixed flora of gram-negative bacilli, presumably ascending



Lacrimal gland, pond turtle. Lacrimal glands or ducts are markedly expanded by a wall of multiple layers of stratified squamous epithelium and their lumina are filled with lamellar keratin. (HE, 283X)

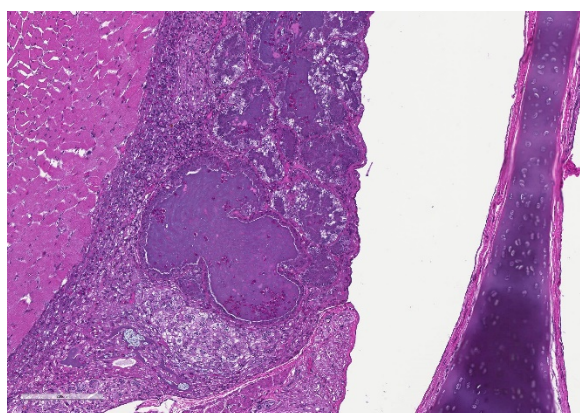


Lacrimal gland, pond turtle. Remaining lacrimal glands are shrunken and cuboidal and infiltrated by low to moderate numbers of heterophils. A low number of epithelial cells are pyknotic or fragmented. The interstitium is infiltrated by low numbers of heterophils and lymphocytes. (HE, 283X)

from the Eustachian tube.[1,7] In addition, squamous metaplasia accompanied by excessive keratinzation occurs in other organs as well. Pancreatic ducts are thickened, hyperkeratotic, and ensheathed in heterophils. In the kidney, collecting ducts are distended and occluded by keratin debris, often resultin in severe nephritis. Blockage by keratin debris may also be seen in the ureters and urinary bladder. Degenerative and inflammatory changes were noted by Elkham in the thyroid and liver, but squamous metaplasia was not documented in these animals.[3]

Some participants had difficulty in orienting themselves on the submitted section, and there was considerable variation between sections (necessitated by the submission requirement of 165 sections.) As illustrated by Elkham and Zwart, the chelonian globe fits into the cup formed by the two glands, the anteromedial Harderian gland, and the posterolateral lacrimal gland.[3] It is preseumed that the lacrimal gland is present in the submitted sections due to its position lateral to the globe. The ear canal is not present on these sections. Moreover, at this stage of lesion development, the participants had great difficulty determining which of the large keratin filled structures might be derived originally from glands or ducts. Isolated mucus cells were present within the walls of several dilated keratin-filled structures, which would not be consistent from ductal derivation, but at this level of change, it is difficult and likely unnecessary to be certain. Some participants had focal granulomas within the sinuses and adjacent skeletal muscle within their sections.

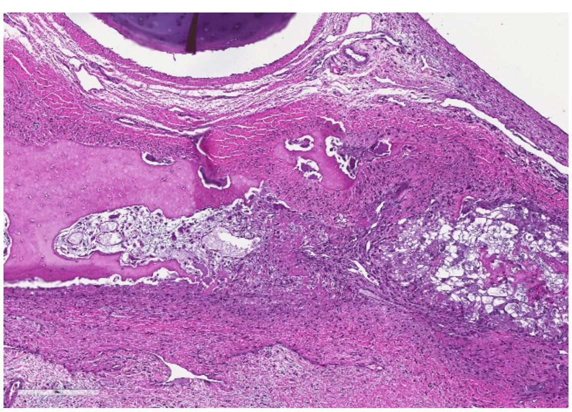
The effects of Vitamin A deficiency have been documented in a wide range of poikilothermic and homothermic species. In crocodiles, squamous metaplasia result in similar changes in the conjunctiva and kidenys, as well as nodular distentions of the ostia of the lingual glands.[2] In captive anurans, squamous metaplasia may be seen in mucus glans of the skin, tongue, oral mucosa, esophagus, cloaca, renal tubules, oviduct, and bladder.[5] Periocular gland squamous metaplasia has been seen in penguins.[8] Squamous metaplasia may be seen in other species of birds, including psittacines in a wide range of tissues including glands of the oral cavity, esophagus, salivary gland and respiratory tract. Protruding keratin masses from



Nasal glands, pond turtle. Unilaterally nasal glands are ectatic and filled with abundant basophilic secretory product and often infiltrated with heterophils, which expand the interstitium as well.

esophageal glands are classsic lesions associated with Vitamin A deficiency in poultry. Squamous metaplasia of Harderian glands, although not to the level seen in chelonians, has also been seen in laboratory rodents as well.[3] In suckling calves, it has been incriminated in a sider range of ocular abnormalitis to include cataract formation, lens luxation, micropthalmia, and reduction in the size of the optic nerve.[9]

While it might be thought that in present days, Vitamin A deficiency and related opthalmic disease would be a rarity, the human species has found new ways to impair its ability to take in this important dietary ingredient. Starvation still rules in many parts of the world, but in developed nations, hypovitaminosis A is still seen, as a result of restrictive and monotonous diets (including vegan diets, "cafeteria" and "junk food" diets, and eating disorders.[4] Malabsorptive syndromes, to include bariatric surgery also accounts for a number of cases each year. While signs of deficiency are system wide, well-documented ocular diseases associated with Vitamin A in humans include night blindness, xeropthalmia ("dry eye") Bitot's spot (a buildup of keratin located superficially in the conjunctiva associated with corneal drying), keratitis and keratomalacia.[4]



Skull, pond turtle. There is marked remodeling of the bones of the skull including the vomer bone (seen here, suggestive of starvation. (HE, 150X)

Several pathologists attending the conference suggested that bacterial infection or chronic irritation should also be considered in the potential differential diagnosis for this particular lesion in light of the lack of information on husbandry of this particular animal. Dr. Andrew Cartoceti of the National Zoo mentioned that samples for Vitamin A should be packaged in a brown container due to the susceptibility of the compound to sunlight. The active resorptionof the bones of the skull in this animal was attributed to protein and caloric malnutrition.

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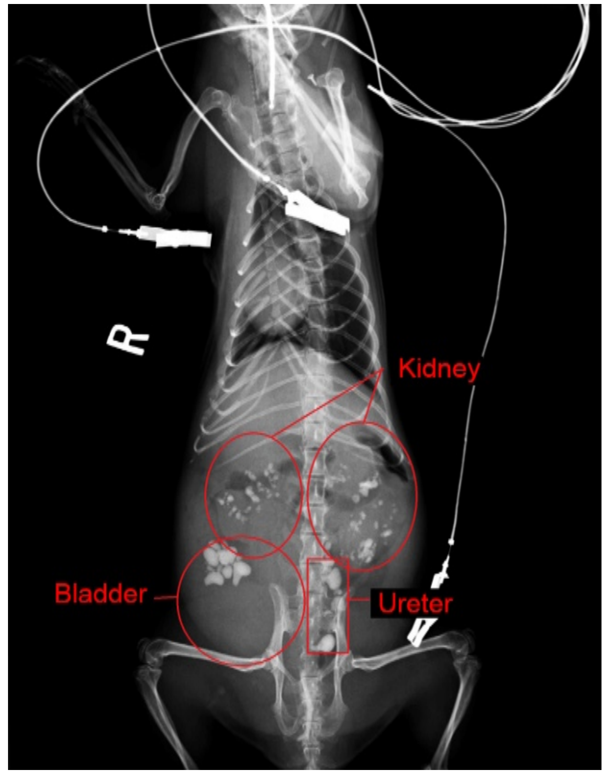
CASE II: 2926 (JPC 4118632).

Signalment: 7-year-old, male, Asian small-clawed otter (*Aonyx cinerea*).

History: This otter was presented to a veterinary hospital with a short history of anorexia. Marked increases of blood urea nitrogen, creatinine, inorganic phosphorus, and ammonia were noted in serum biochemistry. Radiographs indicated numerous (more than 20) calculi in both kidneys, ureters, and urinary bladder. Lithotomy was performed, and approximately 30, up to 10.2 mm in diameter, yellowish-green to white calculi were removed from the ureters and urinary bladder. The otter persistently showed anorexia and died at seventh day after the surgery.



Radiograph, Asian small clawed otter. This radiograph demonstrates numerous radiopaque uroliths within the kidneys, ureter, and bladder. (Photo courtesy of: Laboratory of Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University, https://www.vetmed.hokudai.ac.jp/)

Gross Pathology: The animal showed mild emaciation and dehydration at necropsy. Multiple yellowish-green calculi, maximum 6 mm in diameter, were noted in the renal pelves, ureters, and urinary bladder. Both ureters dilated moderately. Some renal lobes were mildly atrophic with fibrosis.

Laboratory results:

Blood urea nitrogen, 215.2 mg/dl (reference range: 9.2 – 29.2 mg/dl in dogs); creatinine, 6.6 mg/dl (0.4 – 1.4 mg/dl in dogs); inorganic

Kidney, Asian small-clawed otter. The major calyx is markedly dilated and contains a large urolith. Uroliths can also be identified in minor calyces and there is marked atrophy of several lobules. (Photo courtesy of: Laboratory of Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University, https://www.vetmed.hokudai.ac.jp/)

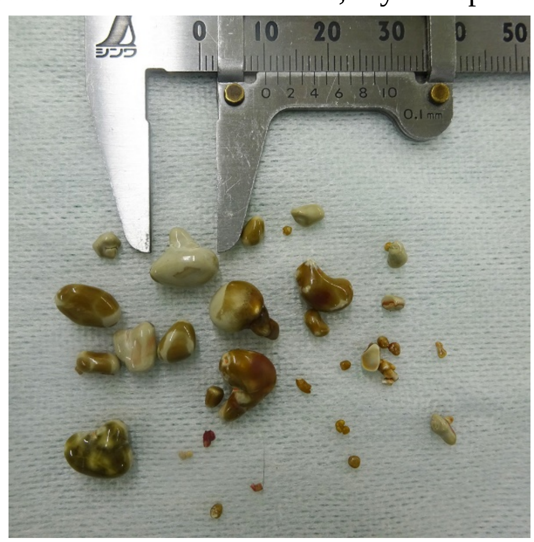
phosphorus, 25.8 mg/dl (1.9 – 5.0 mg/dl in dogs); ammonia, 247 µg/dl (16 – 75 µg/dl in dogs).

Microscopic Description: Kidney: Diffuse moderate interstitial fibrosis is observed. A few lymphocytes infiltrate into the interstitium of the medulla. Prismatic pale yellowish crystals scatter in the lumina of renal tubules, collecting ducts, and dilated renal pelvis. These crystals show birefringence under polarized light. Degeneration and necrosis of the tubular epithelial cells and intraluminal protein cast formation are noted in the medulla.

Contributor's Morphologic Diagnosis: Kidney: Oxalate urolithiasis and nephropathy, chronic, multifocal, moderate.

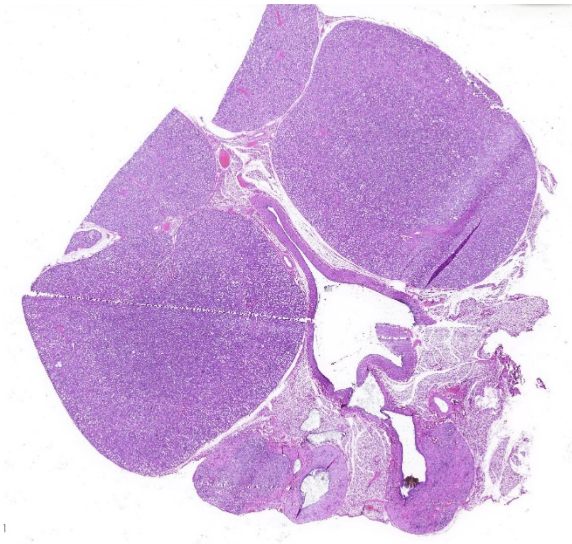
Contributor's Comment: Urolithiasis is frequently observed in otters such as Asian small-clawed otters (*Aonyx cinerea*), North American river otters (*Lontra canadensis*), or Eurasian otters (*Lutra lutra*).[1-3,6,8] It has been reported that 16.7% of free-ranging North American river otters[5] and 23.4% of free-ranging Eurasian otters[1] had uroliths. The prevalence of uroliths appears to increase in captive populations, as urolithiasis has been found in 66.1% of captive Asian small-clawed otters[2] and 64.7% of captive Eurasian otters[1].

In Asian small-clawed otters, the majority of affected individuals had bilateral renal calculi.[2] In addition, about one-thirds of individuals with renal calculi also had cystic calculi.[2] Ureteral obstruction due to uroliths causes dilation of ureters, hydronephrosis,



Uroliths, Asian small-clawed otter. Numerous oxalate crystals were retrieve from the urinary tract of this individual. (Photo courtesy of: Laboratory of Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University, https://www.vetmed.hokudai.ac.jp/)

and pyelonephritis.[4] In the affected animals, renal disease can be the cause of, or a contributing factor in, the deaths.[2] Calculi are



Kidney, Asian small-clawed otter. A section of kidney is presented for examination. Lobules at top have no gross lesions. Two lobules at bottom are markedly shrunken and the calyx contains a brownish occlusive urolith. (HE, 5X)

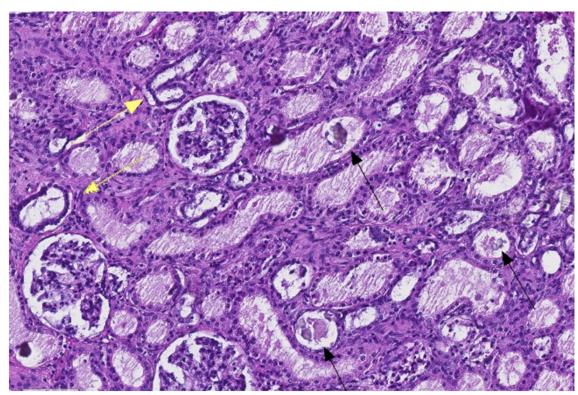
mainly composed of calcium oxalate or ammonium acid urate.[1,2,4]

In North American river otters, bilateral renal calculi are not common, and the majority of otters with calculi are generally healthy without gross renal abnormalities.[5] Only severely affected animals showed bilateral calculi or ureteral obstruction.[3] Although calculi have not been systematically analyzed so far, calcium phosphate and urate have been reported as a primary component of the calculi.[3,6]

In Eurasian otters, only one-thirds of total cases showed bilateral renal calculi and calculi were restricted to within the kidney in the majority of affected animals.[8] Only a few severely affected individuals showed ureteral or cystic calculi.[1,8] The main component of calculi is ammonium acid urate.[1,8]

Gender does not affect the susceptibility to urolithiasis in otters.[1,2,6] Meanwhile, age appears to relate to the prevalence of urolithiasis or disease severity. The positive rate or the number of uroliths increase with age in North American river otters and Eurasian otters.[1,6,8] In Asian small-clawed otters, renal disease was more frequently reported as the cause of death in older individuals.[2]

Although husbandry, diet, genetics, chronic infections, or metabolic disorders have been suggested as contributing factors, the exact mechanisms for the high prevalence of urolithiasis in otters have remained unknown. Since the prevalence of urolithiasis is higher in captive populations, it is possible that the captive diet may contribute to urolithiasis to some extent.[2] Meanwhile, as a significant portion of free-ranging individuals also have uroliths, there might be a predisposing factor to urolithiasis in otters. It is noteworthy that the main components of uroliths differ among species of otters as with the species-dependent difference in stone composition in



Kidney, Asian small-clawed otter. In other, more normal lobules, tubular lumina often contain oxalate crystals (black arrows) or epithelium is attenuated and mineralized (yellow arrows) (HE, 92X)

Mustelid sp.	Captive or Free Ranging	Stone Type	Reported Prevalence	Associated Lesions	Possible Risk Factors
Asian small clawed otter	Captive	Mix of urate and calcium oxalate	66%–89%	Not reported	Older age (>2 years)
Eurasian otter	Free ranging	Ammonium acid urate	10.2%	Nephrolithiasis	Dietary purine intake, protein quality, and digestibility
North American river otter	Free ranging	Calcium phosphate	16.2%	Not reported	Older age, capture location
North American river otter	Free ranging	Uric acid	0.33%	Bilateral nephrolithiasis, expanded renal calyces, marked hydroureter, mild renal medullary loss, and cortical tubular atrophy	Not reported
Wolverine	Free ranging	Ammonium acid urate with magnesium ammonium phosphate and calcium phosphate	8.9%	Nephroliths were unilateral in 87.5% of cases.	Older age (>2 years) males

Table 1. Urolithiasis in Otters and Wolverines. From Terio, McAloose. St Leger eds., *Pathology of Wildlife and Zoo Animals*., 2019.[10]

dogs.[6] In addition, disease severity is also different among species, *i.e.*, Asian small-clawed otters show severer lesions compared with North American river otters or Eurasian otters. Therefore, genetic factors predisposing to urolithiasis might also differ among species. As effective, preventive measures against urolithiasis in otters have not been established, periodic evaluations of uroliths would be important in captive populations such as routine abdominal radiographs, evaluation of serum creatinine and blood urea nitrogen, and urinalysis.[2]

Contributing Institution:

Laboratory of Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University. https://www.vetmed.hokudai.ac.jp/

JPC Diagnosis: Kidney, pelvis: Oxalate urolithiasis, diffuse, severe, intratubular oxalate crystals, and diffuse moderate chronic interstitial fibrosis.

JPC Comment: The contributor provides an excellent review of urolithiasis in captive otters, which can be a significant management problem in zoological collections. Table 1 is an recapitulation of the various types of uroliths and prevalence in otter and wolverines.

Table 1. Urolithiasis in Otters and Wolverines. From Terio, McAloose. St Leger eds., *Pathology of Wildlife and Zoo Animals*., 2019.[10]

Other mustelids also develop uroliths on a fairly frequent basis, which may result in obstruction. Struvite urolithiasis is well-documented in ferrets and farmed mink. In pet ferrets, protein composition (with higher amounts of plant proteins than a wild mustelid would normally take in) are thought to be one of several causative factors. In farmed mink, urolithiasis is likely multifactorial, as the sexes have distinct seasonal predisposition to development of uroliths, with pregnant females developing stones in the spring, and male kits in the fall. Concurrent bacterial infection with *Staphylococcus intermedius* is common in mink, but bacterial infection is uncommon in the affected ferret.[10]

Oxalate nephrosis/nepholithiasis/urolithiasis is well known in a number of other species. Cheetahs, and less commonly cougars, jaguards and leopards may develop oxalate nephrosis with crystal development in tubules, acute renal failure, azotemia and death. Toxicosis, such as may be seen with ethylene glycol ingestion has been ruled out in most cases, but the pathogenesis of this disease remains unclear.[9]

Marsupials also commonly suffer from oxalate nephrosis, which is best known in the koala, but may also be seen in wallabies, possums, and potoroos. Mild oxalate neprhosis is a common finding in koalas in collections around the world, so dietary contributions are unlikely. Once again, the pathogenesis of oxalate nephrosis in these species is unknown.[5]

In domestic species, oxalate uroliths are the second most common type of calculus with small breeds such as miniature poodles, miniature schnauzers, Bichons, Lhasa Apsos and Shih-Tzus being predisposed. They may be encountered in a numbr of conditions other than ethylene glycol toxicosis, including hyperparathyroidism, hypercalcemia, and increased levels of endogenous and exogenous steroids. In ruminants (and in koalas), oxalate-degrading bacteria are a common flora of the rumen and lower tract, likely lessening the effect of a high-oxalate diet in certain locales.

Participants identified a focal area of extreme cortical and medullary atrophy of one of the lobules within the section of kidney adjacent to a large oxalate crystal. The area was interpreted as an area of obstruction if not actual pressure necrosis and focal hydronephrosis.

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CASE III: 71618 (JPC 4136426).

Signalment: Adult male big brown bat (*Eptesicus fuscus*); 24 g, wet fixed.

Wild caught, Maryland, USA. (Submitted partially fixed in 10% neutral buffered Formalin)

History: This adult male big brown bat (*Eptesicus fuscus*) was found dead two months after being captured in Maryland for use in a research colony. It had been

Kidney, big brown bat. Multiple sections of kidney are submitted for examination. One section contains multiple 2mm renal cysts, including one collapsed subcapsular cyst. (HE, 5X).

vaccinated for rabies and treated for mites, but no experimental manipulation had been performed.

Gross Pathology: The right kidney has 4 bulging, rounded, pale tan structures, 2-5 mm diameter. On cut section these are restricted to the cortex and contain pale tan, turbid fluid.

Laboratory results:

- Blood examination: increased CK 1199U/L (99-436), increased GOT/GPT and bile acids (no value available), thrombocytopenia 23 K/µL (148-484) and increased PT 18s (11-17) and aPTT 144s (72-102).

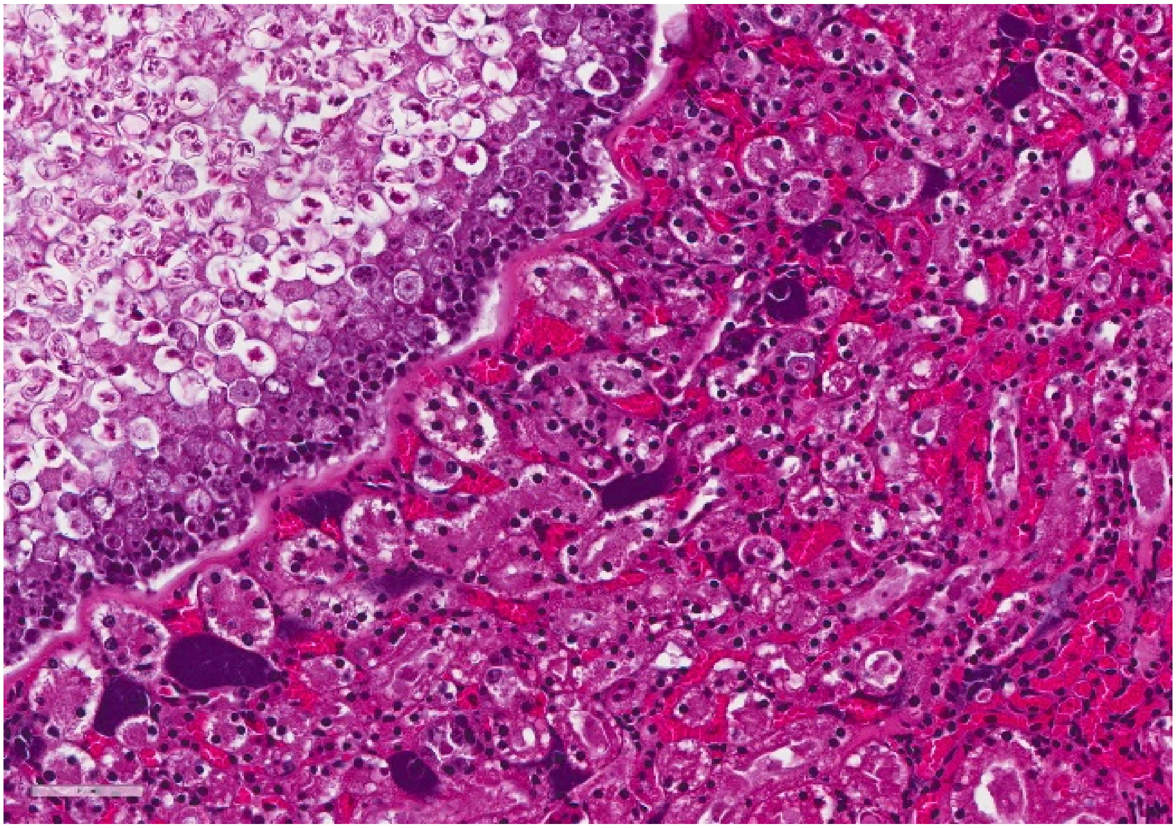
-Toxoplasmosis and Neosporosis were excluded (IgG/M-determination.)

- Parvovirosis and Angiostrongylosis were tested with a negative result.

Microscopic Description:

Expanding and compressing the renal cortex are multifocal cystic structures, which vary in size from 0.5 to 2 mm in diameter, and are surrounded by a 1-5 micron hyaline wall. The inner surface of the cyst is lined by 2-3 layers of hypertrophied and hyperplastic renal tubular epithelial cells. Epithelial cells contain numerous meronts and macrogametocytes, and rare microgametocytes. Within the cyst lumen

Kidney, big brown bat. Higher magnification of the renal cysts. Each cyst is lined with epithelium and surrounded by a thin eosinophilic capsule, and contain eosinophilic flocculent material. (HE, 26X).



Kidney, big brown bat. Dilated tubule at upper left. The cyst is lined by hyperplasia epithelium which often contain coccidial gamonts. The lumen is filled with gametocytes and sporulating oocysts. At the periphery, there is marked congestion and capillaries often contain bacterial colonies (post-mortem overgrowth). (HE, 400X).

are numerous round-to-oval, ~15-micron diameter oocysts with a thin, delicate eosinophilic capsule, which contain 2 sporocysts each containing 4 sickle-shaped sporozoites. Within the cyst lumen are occasional erythrocytes and abundant eosinophilic debris. Scattered throughout the cortex and medulla of both kidneys are multiple rafts of deeply basophilic bacteria, with no tissue response.

Contributor's Morphologic Diagnosis:

Kidney: coccidiosis, chronic, multifocal, moderate, with cystic renal tubules

Contributor's Comment: Renal coccidiosis has recently been described as an incidental postmortem lesion in wild big brown bats, caused by the coccidian parasite *Nephroisospora eptesici.*[5] The organism was first described in 2010, after being identified in the kidneys of 29 wild big brown bats in Minnesota.[5] Although renal coccidiosis had been previously reported in other species of bats, the causative organisms had not been fully characterized.[1,2,3]

Nephroisospora eptesici is a member of the family Sarcocystidae, most closely related to *Besnoitia* spp. Unlike related apicomplexans, *Nephroisospora eptesici* has a single-host life cycle, with the complete life cycle occurring in the kidney.[5] Unlike other coccidians with a single-host life cycle, such as *Eimeria* spp., *Nephroisospora eptesici* is believed to have evolved from an ancestor which required two hosts.[5] Thus, the single-host life cycle is a derived trait. The biology and host specificity of this organism remain to be elucidated. However, reports of morphologically similar coccidian parasites in other bats and the recent identification of *Nephroisospora* genetic material contaminating a published bat genome suggest that related organisms exist, although they have not been fully described.[1,2,3,4]

The associated gross lesions are white-to-beige spherical, well-demarcated cystic structures within the renal cortex.[5] Histologically, these structures represent cystically dilated renal tubules enclosed by a PAS-positive hyaline membrane, which contain numerous intracellular and extracellular coccidian organisms representing all life stages.[5] Oocysts within the lumen of the cystic tubules contain 2 sporocysts, which each contain 4 sporozoites.[5] Periodic acid-Schiff (PAS) and Feulgen special stains can be used to better demonstrate protozoal structures.

Renal coccidiosis due to *Nephroisospora eptesici* is rarely associated with inflammation, and is not believed to be responsible for clinical disease.[5] The primary significance of renal coccidiosis in bats is as an obfuscating factor in research. Genetic material of an undescribed *Nephroisospora* species has recently been identified as a contaminant in the published genome of David's myotis (*Myotis davidii*).[4]

Abundant intravascular bacteria, without associated inflammatory response, were noted in the kidneys and other tissues. These were considered to represent postmortem overgrowth, as no wounds or other nidus of bacterial infection were identified. Another bat autopsied from this colony had similar cystic structures in the kidneys. In that case, the structures had central mineralization, indiscernible organisms, and associated lymphoplasmacytic inflammation. These were considered to represent degenerating parasitic cysts.

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JPC Diagnosis:

1. Kidney, tubules: Cysts, focally extensive, with numerous intraepithelial microgamonts and schizonts and sporulating intraluminal oocytes.

2. Kidney, tubules: Intraluminal bacterial colonies, numerous.

JPC Comment: The contributor has provided an excellent review of the species-specific renal coccidian *Nephroisospora eptesici.*

Coccidia that parasitize the kidney in their natural hosts are uncommon; the vast majority infect the gastrointestinal tract in natural hosts. Renal apicomplexans typically infect the renal epithelium, with a number of species undergoing schizogony in more proximal segments of the tubular epithelium and gametogony in more distal aspects. Bradyzoite-laden cysts of a number of apicomplexan may be found within the kidneys, such as *Neospora* or *Besnotia*, but these cysts are usually found within inflammatory or mesenchymal cells and do not infect renal epithelium.

Klossella is an apicomplexan that infects renal tubules of horses (*K. equi*), and guinea pigs (*K cobayae*), as well as mice (*K. muris and mabokensis*), opossum (*K. tejara*), Australian water rats (*K. hydromyos*), snakes (*K. boyae*), and a number of unidentified species in other rodents and marsupials. *Klossiella sp.* characteristically undergo the first wave of schizogony in glomerular epithelial cells, with second waves of schizogony in proximal convoluted tubles and gametogony in the loop of Henle and beyond. Like most renal coccidians, clinical disease is rare; tubular nephrosis and interstitial nephritis is generally subclinical. [6]

Eimeria also has several species that infect the kidney, with *E. truncata* of geese the most well known, but also *E. boschai* and *somatarie* in ducks, and *E. christianseni* in swans and several unnamed species in owls. . These apicomplexans also rarely cause clinical disease except in young and stsress birds, which may manifest with emaciation. Severe outbreaks may result in mortality.[7]

Not all participants had seen the concentration of bacilli within the renal vessels as a postmortem findings, and their identity generated enthusiastic discussion. A Von Kossa Stain was negative, and the bacilli stained with a Brown-Brenn Gram stain.

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CASE IV: 016-045846 26 (JPC 4101490).

Signalment: A 10 year-old, female intact, Red Kangaroo (*Macropus rufus)*

History: A ten year old, intact, female Red Kangaroo *(Macropus rufus)* was submitted for necropsy from a local zoo. The animal had presented acute dyspnea two days prior to death, and radiography was performed revealing extensive mixed pulmonary pattern. A large mass in the right inguinal area was noted, and the mammary gland was slightly enlarged. The animal was unresponsive to supportive care, including fluid therapy, antibiotics and analgesics, among others. Three years before, the animal had an arthrodesis and stem cell therapy in the right hock due to severe traumatic injury. Also, a wallaby peer from the same enclosure had died from gastrointestinal histoplasmosis recently.

Gross Pathology: The right axillary lymph node was moderately enlarged with a large amount of surrounding fat. On cut section, it was cavitated and contained serosanguinous fluid. The subcutis in the left inguinal area had a focally extensive area of hemorrhage, measuring 2 x 4 x 5 cm (biopsy-induced, as per the history in the submission form) with an underlying, thickly encapsulated, 2 cm in diameter, round mass with a solid, dark brown, necrotic center. The right inguinal lymph node was markedly enlarged, with a cavitated, straw-colored fluid-filled center. All other lymph nodes examined were dark-red, cavitated and fluid-filled. Bilaterally, the caudal nipples were diffusely enlarged and blue (cyanotic). The left caudal mammary gland was diffusely and markedly enlarged and firm, measuring 9 x 5 x 3 cm 2 x 0.7 x 3 cm, with two additional lobules measuring 4.5 x 3.5 x 2 cm and 2 x 1.5 x 1 cm. On cut section, they were cavitated, filled with serosanguinous fluid and a solid, tan area that contained a focal, necrotic center.

The thoracic cavity contained 130 mL of straw-colored, cloudy fluid. Affecting about 75 % of the pulmonary parenchyma, there were multifocal to coalescing, variably sized, dark red to tan, firm nodules, some of them with an umbilicated center. The pleura was diffusely thickened and cloudy. Adjacent to the right ovary and corresponding with the right lumbar lymph node, there was an approximately 6.5 x 3.5 x 3 cm, multilobulated, firm, tan mass firmly adhered to the caudal vena cava at the iliac bifurcation. The endothelium of the caudal vena cava at this site had multifocal 1 mm in diameter, firm nodules. On cut section, the mass was solid tan, with a brown, necrotic center. The pelvis of the right kidney was markedly distended with moderate atrophy of the renal medulla. Along the urinary bladder, following the ureteral insertion, there were multifocal, 4 mm in diameter, firm, tan nodules situated in a linear pattern.

Laboratory results: N/A.

Microscopic Description:

Lungs: Affecting approximately 70% of the sections examined, the parenchyma is effaced, replaced and compressed by a well-demarcated, unencapsulated, moderately cellular neoplasm arranged in acini, nests and tubules supported by a thick fibrovascular stroma, and intermixed with variably sized areas of hemorrhage and congestion. The neoplastic cells are polygonal, with variably distinct cell borders, a scant, finely vacuolated, eosinophilic cytoplasm, and a single, round to oval, vesicular nuclei with up to two

Lung, red kangaroo. Numerous 1mm noduless expand the pulmonary parenchyma. (Photo courtesy of: Kansas State University-Veterinary Diagnostic Laboratory and the Department of Diagnostic Medicine/Pathobiology, Manhattan, KS 66506. http://www.ksvdl.org/)

distinct basophilic nucleoli. There are marked features of anisocytosis and anisokaryosis with occasional binucleated cells, and there are seven mitotic figures per ten 600X fields examined. The mitotic figures are occasionally multipolar and bizarre. Frequently, the ductules formed by the neoplastic cells are filled with red blood cells, and foamy macrophages that often contain intracytoplasmic, brown, granular pigment (hemosiderin). The remaining alveolar spaces are either markedly compressed, congested or filled with an extracellular, eosinophilic fibrillar material (fibrin), hemorrhage and increased numbers of alveolar macrophages.

Liver: Affecting around 30% of the sections examined, the hepatic parenchyma is effaced, replaced and compressed by the same neoplastic population as described for the lungs. Occasionally in the portal triads, there are neoplastic cell emboli within the lumen of the vessels and there is a mild to moderate, biliary duct hyperplasia. The remaining hepatocytes, especially those in proximity of the neoplasm, contain a golden brown, granular pigment (suspected bilirubin) and several intracytoplasmic, clear vacuoles that occasionally push the nuclei eccentrically (vacuolar degeneration). The sinusoidal spaces are diffusely and mildly congested.

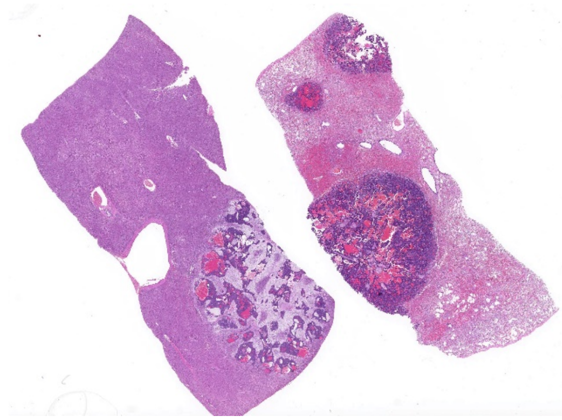
Contributor's Morphologic Diagnosis:

Lungs and Liver: Mammary carcinoma, metastatic.

Contributor's Comment: Other tissues affected that were histologically evaluated include right and left mammary glands, inguinal and lumbar lymph nodes, liver, lung and adrenals. Many sections had neoplastic cells as neoplastic emboli or closely associated with the vasculature. The lumbar lymph node was markedly enlarged, compressing the adjacent ureter causing unilateral hydroureter and hydronephrosis.

Immunohistochemistry for vimentin, cytokeratin (AE1/AE3) and chromogranin, as well as Grimelius staining for argyrophilic granules were performed to rule-out a neoplasm of non-mammary origin, such as neuroendocrine cells as suggested by occasional "pseudorosette-like" structures within the neoplasm. The neoplastic cells were strongly positive for cytokeratin, and the surrounding scirrhous reaction was vimentin positive. All other immuno- and histochemical-staining performed were negative (no internal control nor validation for a red kangaroo was performed for chromogranin immunohistochemical staining).

The mammary glands are classified as mammalian-specific modified subcutaneous apocrine sweat glands of ectodermal and mesodermal origin with a function of new-born nourishment and passive immunity. [2] Neoplasms are infrequently reported in *Macropodidae*, with little literature available regarding mammary adenocarcinomas in Red Kangaroos (*Macropus rufus)* which according to few studies, is the most common neoplasm along with oral squamous cell carcinomas in this species. [5] However, mammary neoplasms are exceedingly common in dogs, cats and humans. [2] In dogs, which has the highest incidence among domestic species, most mammary tumors are clinically benign regardless of their phenotype. [1, 4] Mammary carcinomas are very heterogeneous and complicated, creating increasingly intricate classification schemes in order to characterize them. Nevertheless, simple, solid and complex carcinomas are the most prevalent type reported in the dog, with variable survival times but low metastasis. [4] It must be pointed that cytology and metastasis potential, most commonly via lymphatics, does not correlate well.[4] Dogs have a higher incidence of mammary neoplasm development in the caudal glands, while cats do not have a predilection site within the mammary chain. [4]



Liver and lung, red kangaroo. Both sections contain one or more well-demaracated neoplastic nodules. (HE, 6X)

Generally, six main features are characterized as prognostically important in mammary tumors, which include metastasis to draining lymph node, intravascular tumor emboli, peripheral invasion, unique histological phenotype, histological grade (dysplasia and mitotic rate), and tumor size. In general, mammary carcinomas in cats with a size more than 3 cm in diameter have poorer prognosis, with shorter survival time.[1,2,4] In addition, less differentiated carcinomas have a greater metastasis chance and therefore, have shorter survival time. Many risk factors are associated in the development of mammary neoplasms in dogs and cats, such as age, reproductive status, exogenous hormone exposure, breed susceptibility, diet, and obesity.

Grading schemes for carcinomas, using an adaptation of the Elson and Ellis/Nottingham method for human breast carcinomas are available, although there are few cases with good follow-up. This scheme is based on the degree of tubular formation, nuclear pleomorphism, and mitotic index. [1,2]

According to the limited literature available, mammary tumors are one of the most common neoplasms in Red Kangaroos *(Macropus rufus)* and exceedingly common in dogs, cats and humans, with a heterogeneous, and complicated classification and characterization, but frequently malignant in the feline and benign in the canine population.

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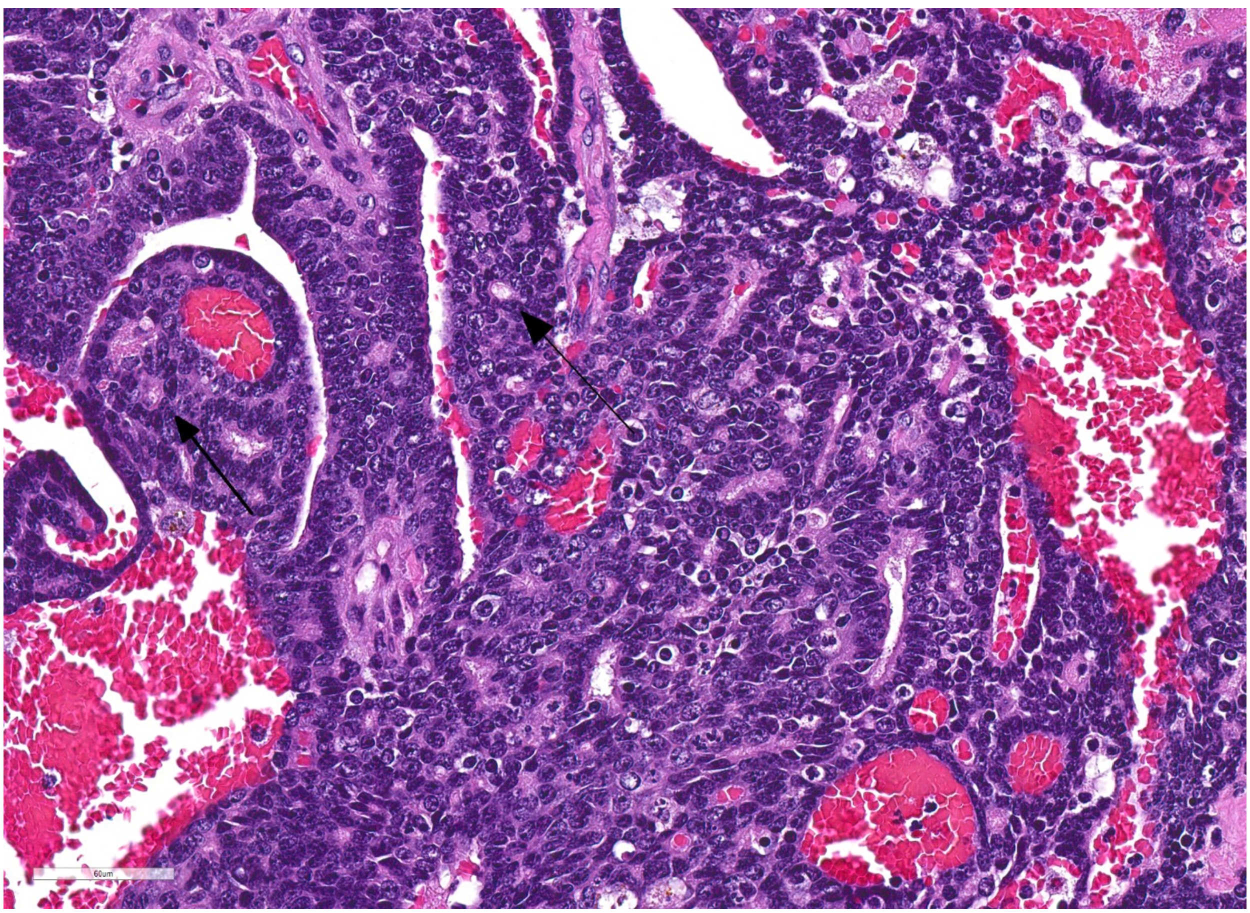
JPC Diagnosis: Liver, lung: Carcinoma, metastatic.

JPC Comment: The attendees agreed that without the history or a mammary mass, it would be difficult to identify the multiple metastatic foci as of mammary origin. We agree with the contributor's differential diagnosis of neuroendocrine carcinoma based on the morphology of the neoplastic cells as well as the tendency to form rosettes or rosette like structures within the submitted sections. In concurrence with the contributor's findings, a



Lung, red kangaroo. The neoplasm is composed of closely packed, nest and cords of epithelial cells which occasionally form tubules (arrows). Areas of necrosis and dropout often contain hemorrhage and there are scattered individual apoptotic cells. (HE, 400X)

JPC-run chromagranin and synaptophysin were both negative in this case.

As mentioned by the contributor, neoplasia in kangaroos is likely underreported, but also appears to be relatively uncommon based on available literature. There are no previous submssions of neoplasia in kargaroos in the WSC, and no mentions of macropod neoplasia in the recently published *Pathology of Wildlife and Zoo Animals*, edited by Terio et al. In a 2007 report on 28 kangaroos autopsied in a major metropolitan zoo in the 1990's, six developed neoplastic disease, two of which were mammary neoplasia (additionally, two developed oral squamous cell carcinoma, with one lymphoma and one gastric lipoma). A review of the available literature at the time, revealed a similar incidence of reports of mammary gland adenocarcinoma (3/17) and oral cavity (3/17).

Using the tammar wallaby as a model, the mammary gland of the marsupial has recently been investigated by Khalil et al. and shows significant difference from other mammals, likely because of the type of young (extremely immature and almost fetal in nature) that it bears following an extremely short gestation (26.5) day gestation.. Pouch young remain attached to the teat for 100 days, where upon they detach and suckle periodically for up to an additional 110 days in the pouch. Marsupials have developed a lactation strategy consistnt with these distinct periods of nursing. In the phase of lactation during permanent attachment of the altricial yound, the milk is high in complex carbohydrates and low in fat and protein. Following detachment from the nipple during a period of rapid growth, eventual exit of the joey from the pouch, and a gradual involution of the mammary gland, the composition of the milk changes to a high protein, low carbohydrate composition, In addition, a number of novel milk proteins inclding SOD3 which protects agains mammary gland toxicity, as well as a number of other proteins which protect the mammary gland from potential infection during a period of milk stasis.

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