Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE
2020-2021

Conference 19

17 February, 2021

Joint Pathology Center
Silver Spring, Maryland

CASE 1: 200720011 (4152712-00)

Signalment:
A 57.4 lb, adult, female, tan and white kangaroo (*Macropus* sp.) in good body condition.

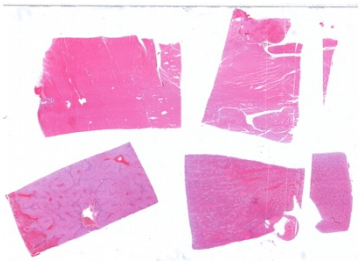
History:
Kangaroos in an exhibit were stung by a swarm of honeybees (*Apis mellifera*). Several kangaroos died over the next 4 days.

Gross Pathology:
At necropsy, there was marked facial swelling, more prominent over the right mandible, and diffuse icterus. The lungs were rubbery and meaty with red to dark red coalescing areas. The liver was diffusely yellow with an accentuated reticular pattern.

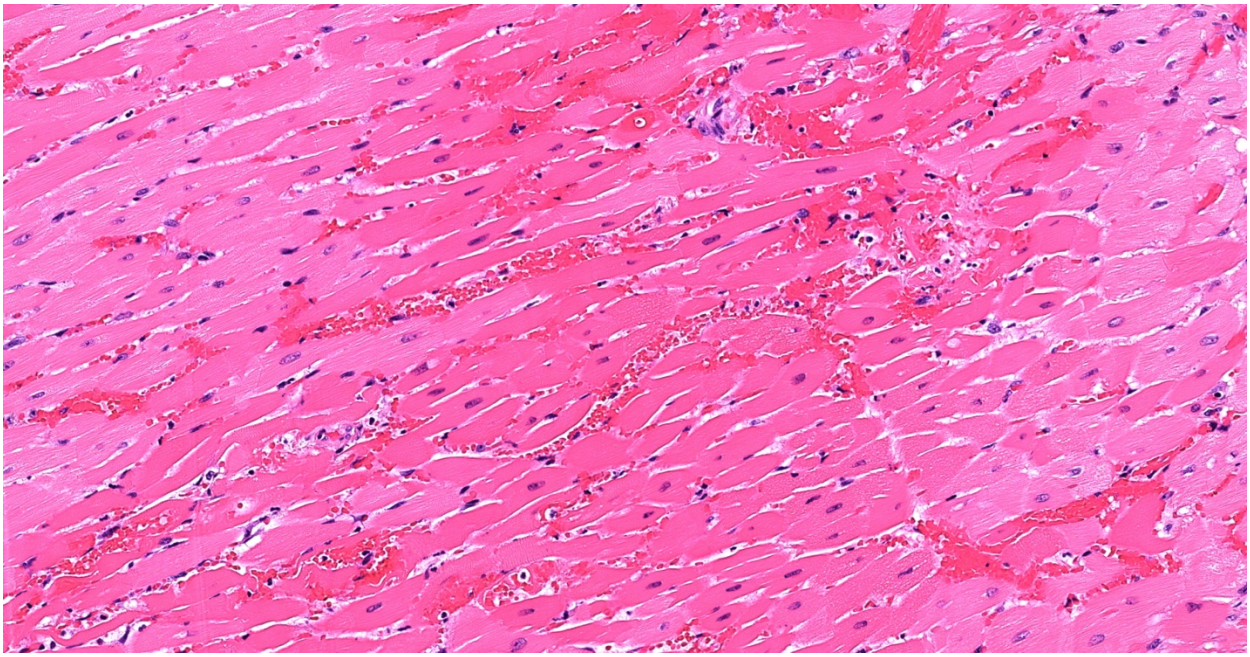
Laboratory results:
N/A

Microscopic description:
Heart (left ventricle papillary muscle and myocardium): The subendocardial myocardium has multifocal areas of acute coagulative necrosis (more prominent in other sections) accompanied by moderate hemorrhage. Within these areas, and to a lesser extent deeper in the myocardium, cardiomyocytes exhibit variable swelling, sarcoplasmic hypereosinophilia, vacuolation, myofibrilysis, fragmentation and/or loss of cross striations. Early neutrophilic infiltration and pyknosis/karyorrhexis/apoptosis of leukocytes, interstitial cells, and cardiomyocytes are noted. In affected areas, capillary walls are disrupted and may have lost their endothelial lining and wall detail. The interstitium is expanded by clear space, light basophilic fluid and eosinophilic globules (serous fluid, myoglobin).

Kidney (cortex and medulla): Despite some postmortem autolysis and decomposition phenomena, diffusely, tubuloepithelial cells of cortical and medullary convoluted tubule segments exhibit acute degenerative and necrotic changes. These include attenuation, swelling, vacuolation, hypereosinophilia, pyknosis/karyorrhexis/apoptosis, partial preservation of cellular outlines and detachment



Multiple tissues, kangaroo. Multiple sections of heart, liver, and kidney are submitted for examination. Areas of hemorrhage may be seen in all three organs at low magnification. (HE, 6X)



Heart, kangaroo. There are coalescing areas of coagulation necrosis of cardiac myocytes, as well as interstitial hemorrhage, edema, and early inflammation. (HE, 188X)

from the underlying tubular basement membrane. Some of these tubules show tubulorrhexis. Affected lumens are often dilated and contain a combination of cellular and proteinaceous casts and red granular material (hemoglobin/myoglobin). Few tubule segments show early regenerative attempts including ample polygonal epithelial cells with large nuclei, prominent nucleoli and rare mitotic figures. Multifocal acute hemorrhage and hypereosinophilic fluid expand the interstitium.

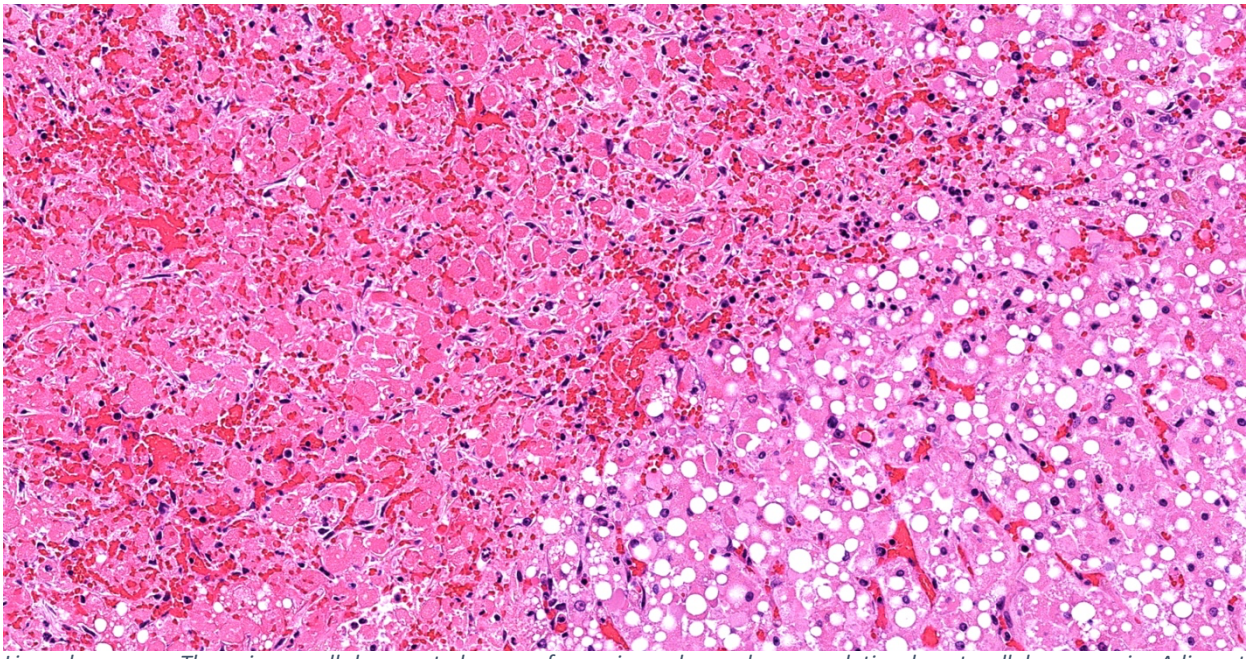
Liver (including Glisson's capsule): Multiple subcapsular hepatic lobules show diffuse acute coagulative necrosis and hemorrhage. In these areas, hepatocytes exhibit cytoplasmic vacuolation, hypereosinophilia, pyknosis/karyorrhexis/apoptosis, cell disruption, dissociation and/or loss. Also, there is early neutrophilic infiltration with fibrin and edema. Few bile duct profiles remain and contain scarce early oval cell hyperplasia/ductular metaplasia are noted. In adjacent lobules, hepatocytes exhibit prominent macro- and microvacuolar change (lipid type), more severe in midzonal to centrilobular areas. Smaller discrete clusters or single hepatocyte necrosis and hemorrhage are noted in these areas. Sinusoids are collapsed and there is loss of the space details. Some terminal plates and subterminal hepatocytes are of Disse and endothelial profile relatively preserved.

Contributor's morphologic diagnosis:

1. Heart: Marked, multifocal, acute myocardial coagulative necrosis with hemorrhage.
2. Kidney: Marked, diffuse, acute tubuloepithelial degeneration and necrosis with tubular proteinosis, hemoglobin/myoglobin casts and hemorrhage.
3. Liver: Marked, multifocal, acute hepatic necrosis with hemorrhage.

Contributor's comment:

Based on the clinical history and the pathologic findings, fatal "bee massive envenomation" (so called "systemic toxic reaction") was determined as the cause of death in this kangaroo.[14] Reasonable differential diagnoses in this case may include: infectious diseases e.g., toxoplasmosis and acute toxicities e.g., mebendazole toxicosis (previously associated with "hemorrhagic septicemic syndrome", anticoagulant poisoning, copper toxicosis, Lantana camara toxicosis, snake envenomation, among others.[20] Visual confirmation of honeybee attack and the cutaneous changes observed over

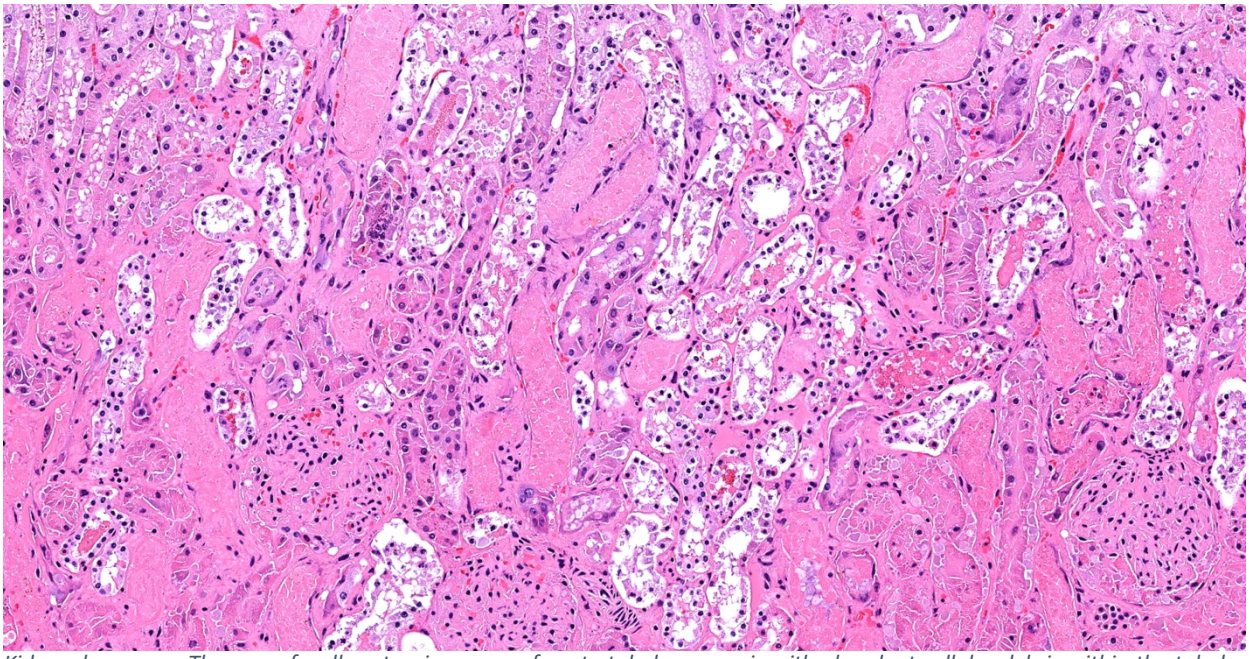


Liver, kangaroo: There is a well-demarcated area of massive subcapsular coagulative hepatocellular necrosis. Adjacent centrilobular and midzonal hepatocytes have large cytoplasmic lipid vacuoles. (HE 203X).

the right mandible were crucial components to reach a final diagnosis.

The Apoidea (bees) and Vespoidea (wasps, hornets, and yellow jackets) are among the most medically relevant hymenoptera worldwide. The incidence of bee stings has increased over the last decades and are regarded a public health concern throughout most of the Americas.[17] Given that the stinging events are rarely witnessed, and the pathologic findings are non-specific,[7] fatal bee envenomation other than in humans and dogs is likely underreported. Few cases involving horses and sheep have been published.[15,18,19] Most hymenopteran stings are self-limiting events albeit in a number of cases, victims may experience immediate life-threatening anaphylactic reactions. In humans, the estimated lethal dose ranges between 2.8 and 3.5 mg of venom per kg of body weight.[12] Noteworthy, anaphylactic reactions to Hymenoptera stings are not volume-dependent (or related to the number of stings). This is opposed to the direct toxic effects (volume-dependent) exerted by some of the venom's components. Indeed, the severity of the envenomation is determined by a combination of the victim's age, body weight, number of stings, and other individual characteristics of the victim such as the immune status, comorbidities, and previous sensitization.[12]

Bee venom is a complex mixture of compounds that includes proteins, peptides, amino acids, phospholipids, sugars, biogenic amines, volatile compounds, pheromones, and water.[4] The main components with clinicopathological relevance are melittin, phospholipase A2 (PLA2), apamin, hyaluronidase, mast cell degranulating peptide (MCD), vasoactive amines (e.g., histamine, dopamine, and norepinephrine), phospholipases B (PLB), serotonin, adolapin, acid phosphatase, and minimine. Briefly, melittin is a lytic peptide that is able to disrupt membrane phospholipids. Phospholipase A2 acts in concert with melittin ("bee hemolytic factor") to cause intravascular hemolysis. Apamin is a neurotoxin that selectively inhibits Ca^{2+}-dependent K^{+} channels, with potent action on the central nervous system. Hyaluronidase ("spreading factor") alters cell permeability and disrupts collagen, thereby, allowing other venom components to penetrate into the victim's tissues. MCD causes mast cells to degranulate, releasing histamine and vasoactive amines; however, in high quantities, it may be anti-inflammatory.[12]



Kidney, kangaroo. There are focally extensive areas of acute tubular necrosis with abundant cellular debris within the tubular lumen. (HE, 150X).

Clinically, bee envenomation may be divided into 1) local inflammatory reactions; 2) allergic manifestations; 3) anaphylactic shock; 4) delayed hypersensitivity reaction, and 5) systemic toxic reactions (so called "massive envenomation" or "envenoming syndrome").[6] Most deaths related to hymenoptera stings are the direct result of the immediate hypersensitivity reaction. In sensitized individuals (previous contact with bee venom), IgE antibodies attach to tissue mast cells and basophils causing their degranulation with subsequent release of vasoactive substances, leukotrienes, histamine, and eosinophil chemotactic factor-A. In anaphylactic reactions, death may ensue within several minutes. Furthermore, some deaths are attributed to severe local reactions involving the upper airways and respiratory obstruction (asphyxia). In those, the respiratory tract may present massive edema, obstructive secretions, and total collapse or severe reduction in functional airway diameter.[16] Massive envenomation from swarm stings can also cause death via non-allergic mechanisms.[3] In the latter scenario, death may result from three major mechanisms: 1) direct venom toxicity, 2) intravascular hemolysis mediated by bee hemolytic factor, and 3) severe hypotension resulting from massive histamine release.[16] Together, these mechanisms have a cumulative, cascading effect, resulting in multiorgan dysfunction syndrome (MODS) characterized by acute renal failure, respiratory distress. rhabdomyolysis, myoglobinemia, myocardial infarction, hepatic necrosis, disseminated intravascular coagulation (DIC), intravascular hemolysis, and hemorrhage.

Histopathologic findings in fatal bee envenomation are not specific. In cases of massive envenomation, acute renal tubular necrosis; fatty degeneration of the kidneys, liver, and myocardium; hyaline membrane disease; and splenic hemorrhage and infarction have been documented. Specifically, renal injury may result from the combination of hemoglobin/myoglobin noxious effect, direct toxicity and hypotension secondary to anaphylactic shock.[6] Melittin and PLA2 may cause endothelial damage (angiopathy) leading to increased vascular permeability, thrombosis and infarction. The histopathological features observed in the presented kangaroo recapitulated these pathological findings documented in human and animal fatalities linked to bee massive envenomation. These lesions were the result of systemic microangiopathy, DIC and hemorrhage leading to and aggravated by shock with eventual death. To the best of our knowledge, this

represents the first description of fatal bee envenomation in macropodids.

The diagnosis of bee massive envenomation is one of exclusion and should stem from a history of suspected or confirmed exposure to numerous bees matched with the onset of appropriate clinical signs and compatible pathologic findings. Furthermore, determination of tryptase levels (a mast cell-specific enzyme released upon mast cell degranulation) and venom-specific IgE are of postmortem diagnostic value in human forensics.[5,10] Its usefulness has not been investigated in veterinary species; further research is warranted

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JPC diagnosis:

1. Heart, myocardium: Coagulative necrosis, multifocal to coalescing, with hemorrhage.
2. Liver, hepatocytes: Coagulative necrosis, subcapsular, massive, with diffuse, hepatocellular lipidosis.
3. Kidney: Acute tubular necrosis, multifocal to coalescing, with cellular and erythrocytic casts.

JPC comment:
The contributor provides a concise summary of this interesting case, and a great deal of the current knowledge regarding bee envenomation. While envenomation from the honeybee is always a concern for some individuals, the Africanized honeybee became a new threat to human and veterinary patients starting in the late 1980's. These bees (*Apis mellifera scutellata*) tend to be more territorial, faster to defend and attack, have slightly different composition of their venom, and have been called "killer bees" colloquially. After a colony of European honeybees is disturbed, it takes the bees approximately 2.9 minutes to "settle", but killer bees take 28.2 minutes to "settle". Killer bee venom contains higher concentrations of phospholipase A2 than European honeybee venom, the most allergenic component of the venom.[13]

While phospholipase A2 (also called Api m 1) is well characterized in bee venom, phospholipase A1 is more abundant in wasps and ants. It has been well characterized in fire ant (*Solenopsis invicta*) venom. The phospholipase A2 in bee venom belongs to a subgroup of small, secreted Ca^{2+}-dependent phospholipase A2s with a highly conserved Ca^{2+} binding site. These molecules are dependent on millimolar concentrations of calcium for their catalytic activity. And while PLA2 is responsible for histamine release from leukocytes, it is also involved in LTC_4 production and upregulation of CD63 in mast cells and basophils. Additionally, PLA2 induces production of IL-4, a critical component of the Th2 response and class switching to IgE in B-cells.[11]

As the contributor stated, intravascular hemolysis may be found clinically in animal affected by bee envenomation. Specifically, bee venom may cause hemolytic anemia, echinocytosis, spherocytosis, thrombocytopenia, hemoglobinemia, and hemoglobinuria. In the past, it had been assumed that in all cases, hemolytic anemia was immune-mediated, but recent cases have illustrated the cause of hemolysis to be destruction of erythrocytes by melittin forming large pores in the cellular membrane. PLA2 also causes echinocytosis, spherocytosis, and mitochondrial degeneration in platelets. However, in delayed reaction, some mechanisms of disease are still likely antibody mediated.[8]

References:

1. Abd El-Wahed AA, Khalifa SAM, Sheikh BY, et al. Bee venom composition: from Chemistry to biological activity. In: Atta-ur-Rahman, ed. *Studies in Natural Products Chemistry*. Oxford:Elsevier; 2017: 459-484.
2. Bonagura JD, Kirk RW. Management of bee and other Hymenoptera stings. In: Bonagura JD. Kirk RW, eds. *Kirks Veterinary Therapy XII: Small Animal Practice.* Philadelphia: WB Saunders; 1995.
3. Castells MC, Hornick JL, Akin C Anaphylaxis after hymenoptera sting: is it venom allergy, a clonal disorder, or both? J

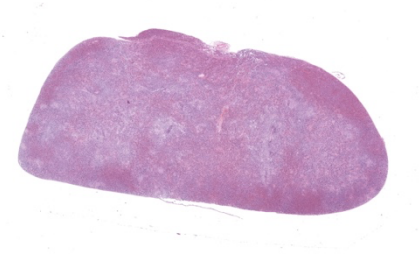
Allergy Clin Immunol Pract. 2015;3:350-355.
4. Danneels EL, Van Vaerenbergh M, Debyser G, Devreese B, de Graaf DC. Honeybee venomproteome profile of queens and winter bees as determined by a mass spectrometric approach. *Toxins* (Basel). 2015;7:4468-4483.
5. Elieh Ali Komi D, Shafaghat F, Zwiener RD. Immunology of Bee Venom. Clin Rev *Allergy Immunol*. 2018;54:386-396.
6. Hughes RL. A Fatal Case of Acute Renal Failure From Envenoming Syndrome After Massive Bee Attack: A Case Report and Literature Review. *Am J Forensic Med Pathol.* 2019;40:52-57.
7. Manoquerra AS. Hymenoptera stings. In: Ling L, Clark R, Erickson T, eds. *Toxicology secrets*. Philadelphia: Hanley and Belfus; 2001.
8. Nair R, Riddle EA, Thrall MA. Hemolytic anemia, spherocytosis, and thrombocytopenia associated with honey bee envenomation in a dog. *Veterinary Clinical Pathology.* 2019;48(4):620-623.
9. Oliveira EC, Pedroso PM, Meirelles AE, et al. Pathological findings in dogs after multiple Africanized bee stings. *Toxicon*. 2007:49:1214-1218.
10. Payne V, Kam PC. Mast cell tryptase: a review of its physiology and clinical significance. *Anaesthesia*. 2004;59:695-703.
11. Perez-Riverol A, Lasa AM, Santos-Pinto JRA, Palma MS. Insect venom phospholipases A1 and A2: Roles in the envenoming process and allergy. *Insect Biochemistry and Molecular Biology*. 2019;105:10-24.
12. Pucca MB, Cerni FA, Oliveira IS, et al. Bee Updated: Current Knowledge on Bee Venom and Bee Envenoming Therapy. *Front Immunol*. 2019;10:2090.
13. Rahimian R, Shirazi FM, Schmidt JO, Klotz SA. Honeybee stings in the era of "killer bees": Anaphylaxis and toxic envenomation. *The American Journal of Medicine*. 2019; 133(5):621-626.
14. Rayamane AP Kumar MP, Kishor DG. Honey bee stings and anaphylaxis. *J Forensic Med Sci Law*. 2014;23:53-60.
15. Ribeiro PR, Bianchi MV, Henker LC, Gonzales F Pavarini SP. Acute renal failure in a horse following bee sting toxicity. *Ciencia Rural*. 2020;50:1-5.
16. Riches KJ, Gillis D, James RA. An autopsy approach to bee sting-related deaths. *Pathology*. 2002;34:257-262.
17. Schumacher MJ, Egen NB. Significance of Africanized bees for public health. A review. *Arch Intern Med*. 1995:155:2038-2043.
18. Staempfli HR, Wollenberg G, Jewell G, McCutcheon LJ. Acute fatal reaction to bee stings in a mare. *Equine Veterinary Education.* 1993;5:250-252.
19. Veado HC, Conceicao RS, Nogueira K, et al. Massive Africanized honeybee stings in two hair sheep and a mare. *Toxicon*. 2020;177:35-40.
20. Vogelnest L, Portas T. *Current Therapy in Medicine of Australian Mammals*. Clayton South, Victoria, Australia: CSIRO Publishing, 2019.

CASE 2: VP18063 (4134595-00)

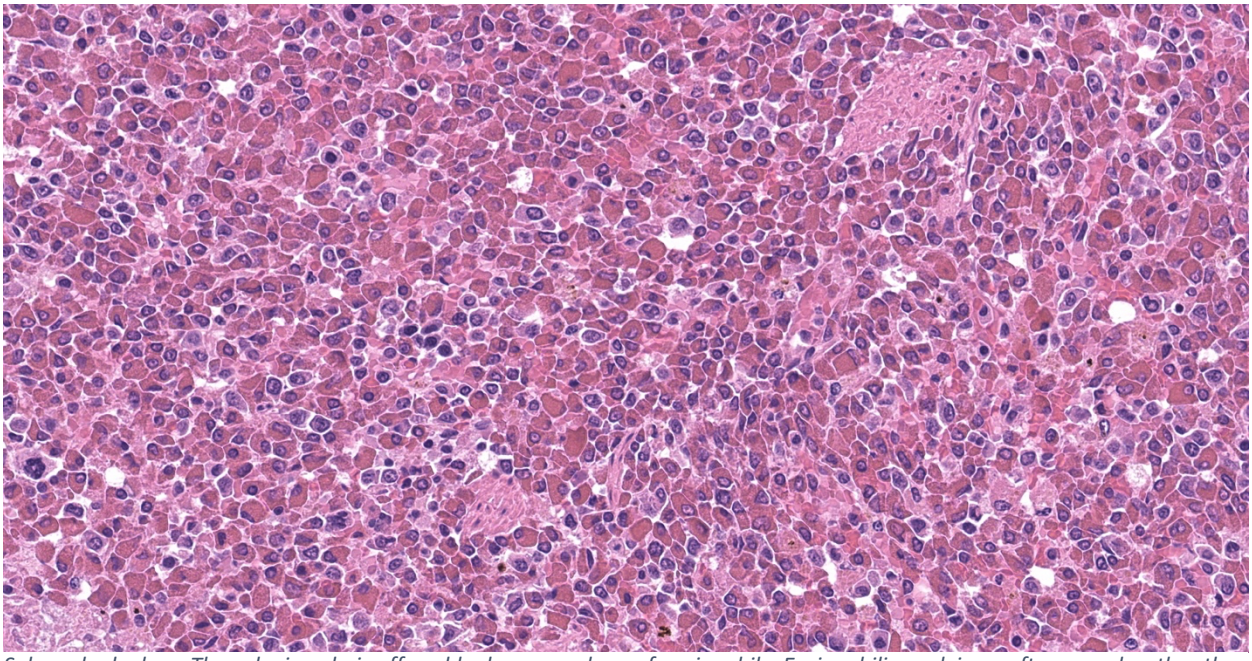
Signalment:
Two-year-old, male, four-toed hedgehog (*Atelerix albiventris*)

History:
He had 10-day history of lethargy and anorexia. Abdominal ultrasonography showed splenomegaly, and chest X-ray imaging revealed decreased permeability in the lung fields. Corticosteroid and antibiotic therapies were continued for 4 days, but he died eight days later.

Gross Pathology:
At necropsy, the hedgehog was thin and weighed 288 g (normal body weight is approximately 400 g). The left hindlimb was markedly swollen and on its distal end was a black scab. The spleen was



Spleen, hedgehog. The spleen is enlarged, and its architecture effaced by a cellular infiltrate with multifocal areas of pallor (necrosis). (HE, 5X).



Spleen, hedgehog. The splenic pulp is effaced by large numbers of eosinophils. Eosinophilic nuclei are often round, rather than bilobed. (HE, 400X).

discolored and markedly enlarged. The spleen, liver, and lung had multifocal, white foci of varying sizes (1 mm-4 mm). The left inguinal lymph node was moderately enlarged.

Laboratory results:

Peripheral blood smear: Peripheral blood smear showed prominent leukocytosis with a ratio of 11% stab cells (bands), 16% segmented neutrophils, 2% lymphocytes, 4% monocytes, and 67% eosinophils. Eosinophils were large (14–18 µm diameter) and round with abundant small, round, eosinophilic cytoplasmic granules. The nuclei were round to reniform and had coarse and aggregated chromatin pattern. Mature segmented eosinophils were sometimes observed.

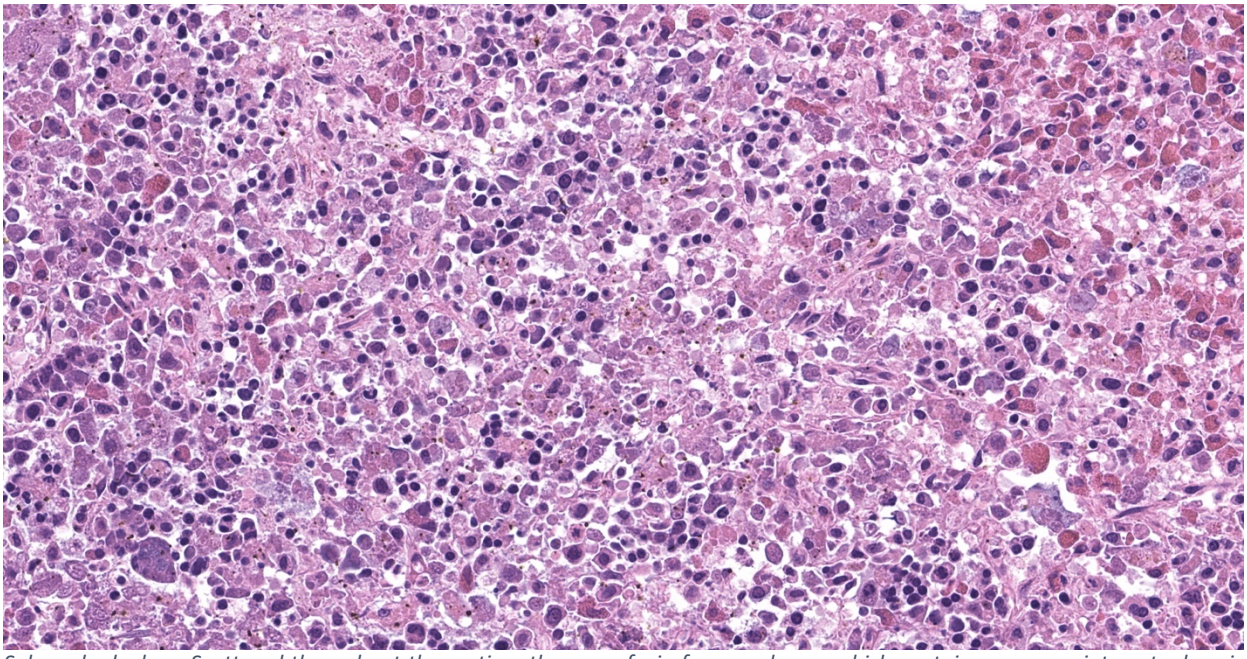
PCR: *Mycobacterium marinum* was confirmed by broad-range PCR amplification of bacterial 16S rDNA and sequencing of paraffin tissues from the lung.

Special stain (Ziehl-Neelsen stain): Spleen, lung, liver, left inguinal lymph node, and distal end of left hindlimb: Moderate to large number of acid-fast bacilli were within the granulomatous lesion.

Immunohistochemistry: Spleen, lung, liver, left inguinal lymph node, and distal end of left hindlimb: Immunohistochemistry using rabbit polyclonal *Mycobacterium bovis* antibody confirmed the presence of intracellular and extracellular bacilli.

Microscopic description:

Spleen: The splenic parenchyma was effaced by diffusely infiltrated round cells and multifocal-to-coalescing necrotizing granulomatous lesions infiltrated with macrophages, neutrophils, and eosinophils. The infiltrated round cells were of intermediate size with distinct cell borders and had moderate amount of cytoplasm composed of abundant eosinophilic granules. The eosinophils had round, hyperchromatic nuclei with diameters ranging from 4 to 6 microns, and sometimes showed reniform or two-lobed nuclei. Mitotic figures were rare. Eosinophils infiltrated the splenic trabeculae and around trabecular arteries, and partially, in the extracapsular region. Rarely, poorly stained bacilli were observed within macrophages and necrotizing area in the granulomatous lesion. Erythroid and myeloid precursors and megakaryocytes were diffusely scattered (extramedullary hematopoiesis).



Spleen, hedgehog. Scattered throughout the section, there are foci of macrophages which contain numerous intracytoplasmic bacilli. (HE, 400X)

Lung, liver, left inguinal lymph node, and distal end of left hindlimb (not submitted): Eosinophil infiltration observed around the vessels and granulomatous lesions of the spleen were similarly observed in the lung, liver, left inguinal lymph node, and distal end of the left hindlimb.

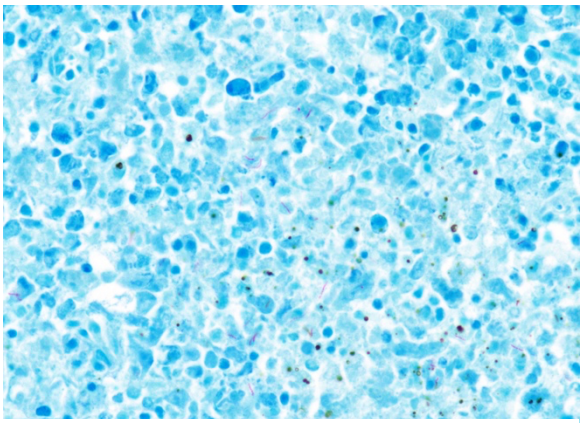
Contributor's morphologic diagnosis:

1. Spleen: Eosinophilic leukemia.
2. Spleen: Splenitis, necrotizing granulomatous, multifocal to coalescing, with intracellular and extracellular bacilli.
3. Spleen: extramedullary hematopoiesis.

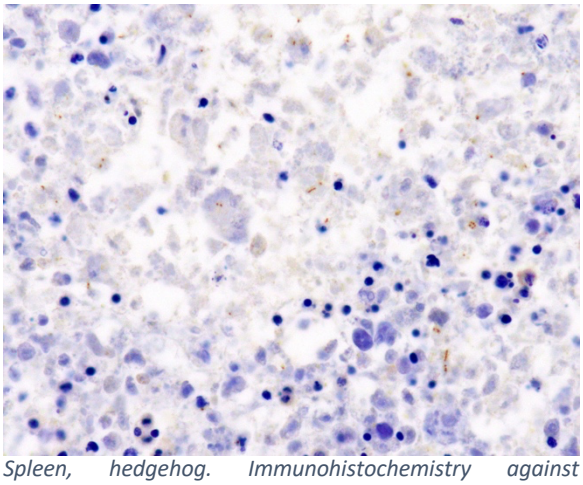
Contributor's comment:

Histopathologic lesions in the spleen showed eosinophilic leukemia and necrotizing granulomatous inflammation, suggesting involvement of a pathogen. *Mycobacterium marinum* was confirmed by PCR, Ziehl-Neelsen stain, and immunohistochemistry.

Eosinophilic leukemia (chronic eosinophilic leukemia), also called myeloproliferative neoplasia, is a subtype of chronic myeloid leukemia that is rare in animals.[3] In four-toed hedgehogs, five cases of eosinophilic leukemia have previously been reported.[4,6,8] Eosinophilic leukemia needs to be differentiated from hypereosinophilic syndrome, as both conditions are characterized by eosinophil infiltration of bone marrow and systemic organs. Eosinophilic leukemia is distinct from hypereosinophilic syndrome in that the eosinophils show a more immature morphology and that there are no factors that increase eosinophils, such as allergic diseases and parasitic infections.[13] Although the bone marrow could not be examined because a cosmetic necropsy was performed, a diagnosis of eosinophilic leukemia was made as the



Spleen, hedgehog. An acid-fast stain demonstrates long filamentous bacilli within macrophage cytoplasm. (Ziehl-Neelsen, 400X). (Photo courtesy of: Laboratory of Pathology, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotohge-cho, Hirakata, Osaka 573-0101, Japan)



Spleen, hedgehog. Immunohistochemistry against Mycobacterium marinum highlights bacilli within macrophage cytoplasm. (anti-M. marum, 400X) (Photo courtesy of: Laboratory of Pathology, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotohge-cho, Hirakata, Osaka 573-0101, Japan)

eosinophilic nuclei were round to uniform in shape, which suggest immature morphology.

Mycobacterium marinum is a causative agent of mycobacteriosis in freshwater and saltwater fishes.[5] This pathogen often causes skin infections via traumatic injuries in humans through contaminated fish and fish tank. Therefore, many human patients are found to be aquarium fish owners, pet shop workers, aquarium workers, and workers in the fish market.[5,11] Generally, *M. marinum* acts as an opportunistic pathogen, and causes localized skin lesions called 'fish tank granuloma' in most human patients.[5] However, immunocompromised or immunosuppressed patients often develop more severe lesions that may progress to disseminated infection.[5] *M. marinum* is ubiquitous in the aquatic environment and is often isolated from reptiles and amphibians, and sometimes causes disease in these animals.[7,14] In mammals except humans, nontuberculous mycobacteriosis caused by *M. marinum* has only been reported in the European hedgehog (*Erinaceus europaeus*).[12]

This was a case of systemic infection caused by *M. marinum*. It is thought that the acid-fast bacilli infected the left hindlimb first, then spread to systemic organs. The four-toed hedgehog was kept in the household as a pet, but the source of infection is unknown. It is possible that the infection worsened due to a reduction in systemic immunity caused by eosinophilic leukemia.

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JPC diagnosis:

1. Spleen: Eosinophilic leukemia (chloroleukemia).
2. Spleen: Splenitis, granulomatous, multifocal, to coalescing, with intrahistiocytic bacilli.

JPC comment:

The contributor summarized this case in an article published in 2020. While considered a rare disease in domestic animals, eight cases have previously been reported prior to this case, suggesting that these species may have a predisposition to this disease. While not available in this case, evaluation of bone marrow would provide more clinical data to definitively classify this case.[9]

The World Health Organization (WHO) currently defines chronic eosinophilic leukemia - not otherwise specified (CEL-NOS) a clonal expansion of eosinophils, with $\geq$ 1.5 x 10^9/L absolute eosinophils in peripheral blood accompanied by either the presence of myeloblast excess (>2% in peripheral blood or 5-19% in bone marrow) or the presence of a clonal cytogenetic abnormality.[2] The CEL-NOS excludes a variety of chronic eosinophilic leukemias that are attributed to known mutations, such as rearrangement of PDGFRA, PDGFRB, or FGFR1, or PCM1-JAK2, ETV6-JAK2, or BCR-JAK2 fusion.[1]

Recent investigation of a human case of chronic eosinophilic leukemia revealed no mutations in PDGFRA, PDGFRB, FGFR1, and several other known myeloproliferative mutations. However, using next-generation sequencing, this patient's leukemia was characterized by an insertion/deletion mutation in exon 13 of JAK2

(*JAK2*^{ex13InDel}).[10] While this mutation is not the basis of every case of human chronic eosinophilic leukemia, and veterinary species may have a different basis of pathogenesis, additional research may reveal a common mutation that could be targeted with therapeutics to improve outcomes.

References:

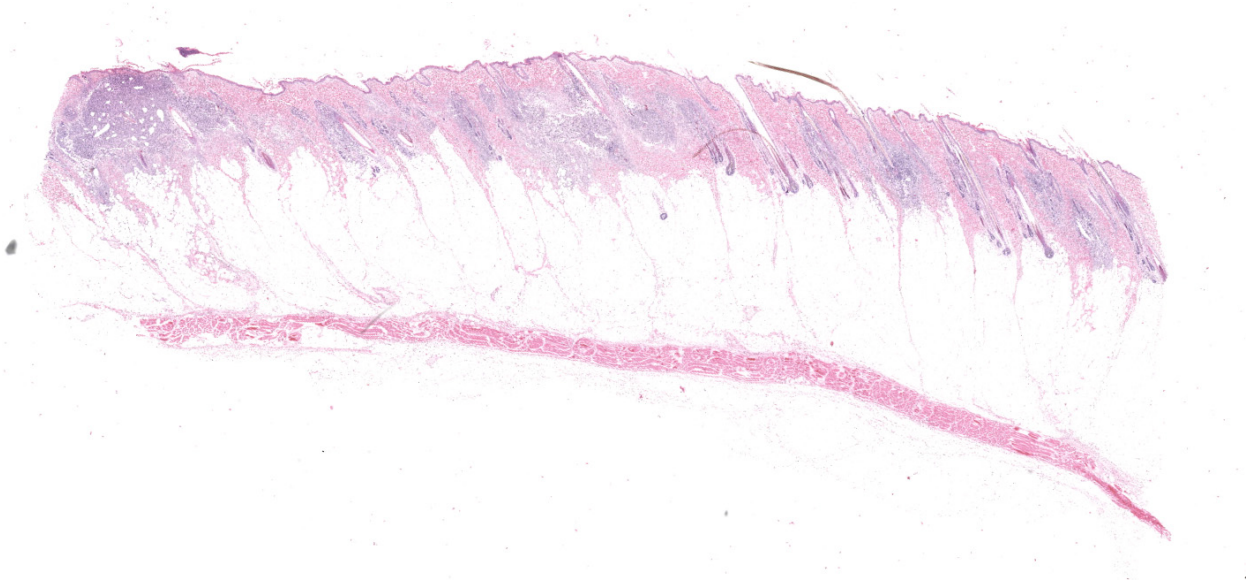
1. Bain BJ, Horny HP, Hasserjian RP, Orazi A. Chronic eosinophilic leukaemia, NOS. In: *WHO classification of tumours of Haematopoietic and Lymphoid tissues*. Herndon, VA: Stylus Publishing, LLC. 2017:71-79.
2. Barbui T, Thiele J, Gisslinger H, et al. The 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms: document summary and in-depth discussion. *Blood Cancer Journal*. 2018;8:15.
3. Boes KM, Durham AC. Chronic myeloid leukemia. In: Zachary JF, ed. *Pathologic Basis of Veterinary Disease*. 6th ed. St. Louis, MO: Elsevier; 2017:755.
4. Escobar-Alarcón DK, Reyes-Matute A, Méndez-Bernal A, et al. Chronic eosinophilic leukemia in an African hedgehog (*Atelerix albiventris*). *Braz J Vet Pathol*. 2016;9:34-38.
5. Hashish E, Merwad A, Elgaml S, et al. *Mycobacterium marinum* infection in fish and man: epidemiology, pathophysiology and management; a review. *Vet Q*. 2018;38:35-46.
6. Higbie CT, Eshar D, Choudhary S, et al. Eosinophilic leukemia in a pet African hedgehog (*Atelerix albiventris*). *J Exot Pet Med*. 2016;25:65-71.
7. Jacobson ER. Mycobacterium. In: Jacobson ER, ed. *Infectious Diseases and Pathology of Reptiles*. Boca Raton, FL: CRC Press; 2007:468-469.
8. Martínez-Jiménez D, Garner B, Coutermarsh-Ott S, et al. Eosinophilic leukemia in three African pygmy hedgehogs (*Atelerix albiventris*) and validation of Luna stain. *J Vet Diagn Invest*. 2017;29:217-223.
9. Nakamura S, Yasuda M, Ozaki K, Tsukahara T. Eosinophilic leukaemia and systemic Mycobacterium marinum infection in an African pygmy hedgehog (*Atelerix albiventris*). *J Comp Path*. 2020;181:33-37.
10. Patel AB, Franzini A, Leroy E, et al. *JAK2*^{ex13InDel} drives oncogenic transformation and is associated with chronic eosinophilic leukemia and polycythemia vera. *Blood*. 2019;134(26):2388-2398.
11. Sia TY, Taimur S, Blau DM, et al. Clinical and pathological evaluation of *Mycobacterium marinum* group skin infections associated with fish markets in New York City. *Clin Infect Dis*. 2016;62:590-595.
12. Tappe JP, Weitzman I, Liu S, et al. Systemic *Mycobacterium marinum* infection in a European hedgehog. *J Am Vet Med Assoc*. 1983;183:1280-1281.
13. Valli VE. Chronic eosinophilic leukemia and hypereosinophilic syndrome. In: Valli VE, ed. *Veterinary Comparative Hematopathology*. Ames, IA: Blackwell publishing; 2007:436-440.
14. Whitaker BR, Wright KM. Bacterial diseases. In: Divers SJ and Stahl SJ, eds. *Mader's Reptile and Amphibian Medicine and Surgery*. 3rd ed. St. Louis, MO: Elsevier; 2019:1001-1003.

CASE 3: X-27229-14 (4066657-00)

Signalment:
Approximately 1 year-old, male, pastel, farmed mink, *Neovison vison* (formerly *Mustela vison*)

History:
A new line of pastel mink was purchased in the spring of 2014. One of the purchased juvenile mink subsequently developed irregular, coalescing, areas of alopecia and scaling over the dorsal trunk. That animal was culled. Later that fall, 4 more animals (out of 900) developed similar skin lesions. One of these mink was euthanized (using carbon monoxide inhalation) and sent for necropsy. This condition does not appear highly contagious as other mink in contact with affected ones did not develop lesions and the incidence of lesions was low. The farm of origin has rarely seen similar cases. The current ranch is reportedly free of Aleutian disease.

Gross Pathology:
A young, adult, male, pastel (light tan colored) mink was examined. The carcass had been frozen and thawed prior to examination. Along the dorsal trunk, extending from the shoulder to the lumbar area were irregular, coalescing, serpiginous or track-like, partially alopecic areas where the exposed skin was scaly and scabby. The remaining carcass was grossly unremarkable.



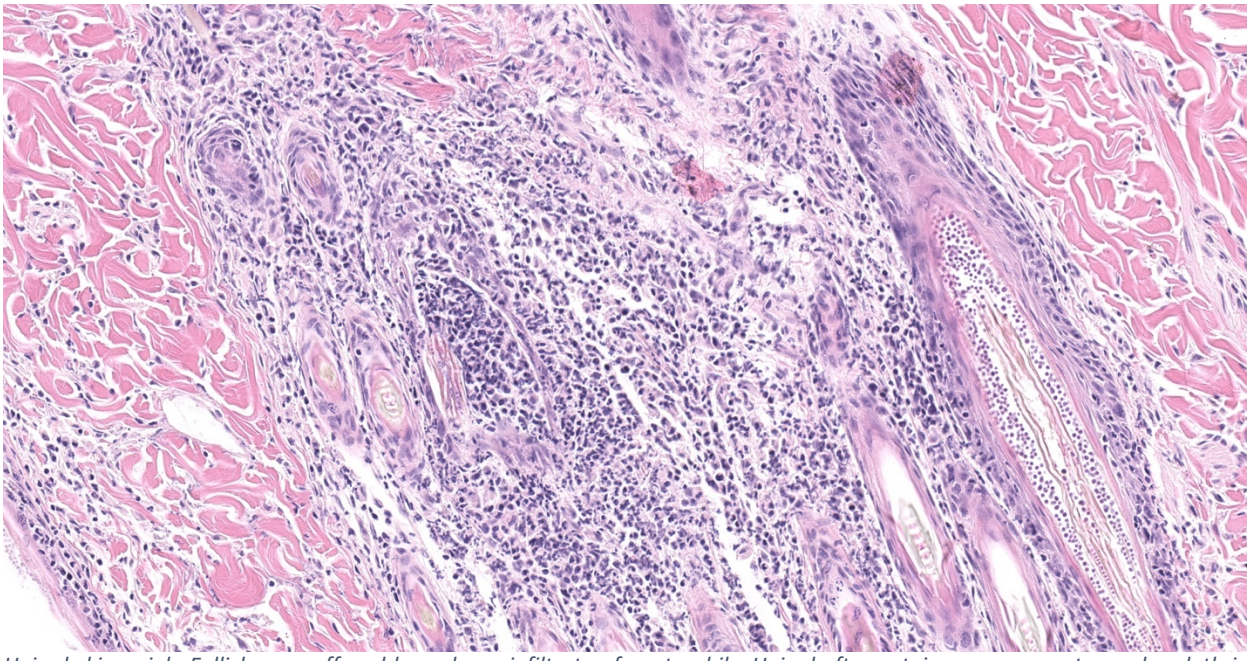
Haired skin, mink. There is a dense inflammatory infiltrate highlighting hair follicles (HE, 6X).

Laboratory results:
Bacterial culture of the affected skin yielded a moderate growth of normal flora and a heavy growth of fungi. The fungal isolates were subsequently cultured on both Sabouraud dextrose and potato-dextrose agar which produced powdery, white to cream-colored spores and hyaline hyphae with a distinct yellow pigmentation. Microscopic examination of these fungi identified macroconidia composed of 3-4 cells that were club-shaped with smooth walls. Spherical to pyriform or oblong, microconidia that formed dense grape-like clusters long the sides of mycelia were also noted. These findings were compatible with Trichophyton sp. Genomic DNA was extracted from the cultured isolates for gene amplification by PCR and sequencing that identified this organism as *Trichophyton equinum* (publication in process).

Microscopic description:
The epidermis was mildly hyperplastic and covered by increased amounts of dense, ortho- and parakeratotic keratin which contained occasional small intracorneal aggregates of degenerate neutrophils. The upper portions of the walls of hair follicles were similarly, mildly thickened. In scattered follicles, myriads of densely packed, round, basophilic, 1-2 micron in diameter, arthrospores encircled hair shafts (ectrothrix arthrospores). Fewer, similar endothrix arthrospores and pale staining endothrix hyphae were present within affected hair shafts. These follicles were surrounded by mild infiltrates of lymphocytes, plasma cells, few macrophages and rare neutrophils. Several adjacent hair follicles had ruptured and were replaced by dense, nodular aggregates of macrophages admixed with neutrophils and cell debris often centered on hair shaft fragments. The latter also often contained endothrix arthrospores and pale hyphae. Arthrospores and hyphae were highlighted with PAS and Grocott's methenamine silver stains. With PAS staining, hyphae appeared septate, non-branching, and measured approximately 1.0 um in width.

Contributor's morphologic diagnosis:
Haired skin, dorsal trunk: Moderate, multifocal, subacute, folliculitis/furunculosis with foci of nodular, pyogranulomatous dermatitis, mild, hyperkeratosis and numerous intralesional fungal arthrospores and hyphae (findings consistent with dermatophytosis or ringworm)

Contributor's comment:
Dermatophytosis (or ringworm) infections are relatively common and affect species of farmed and domestic animals. In farmed mink, outbreaks many are relatively rare and have been attributed to *Microsporum canis* and *Trichophyton mentagrophytes* infections. In most reports, kits are most frequently and severely affected. Lesions in adult mink are much less common.



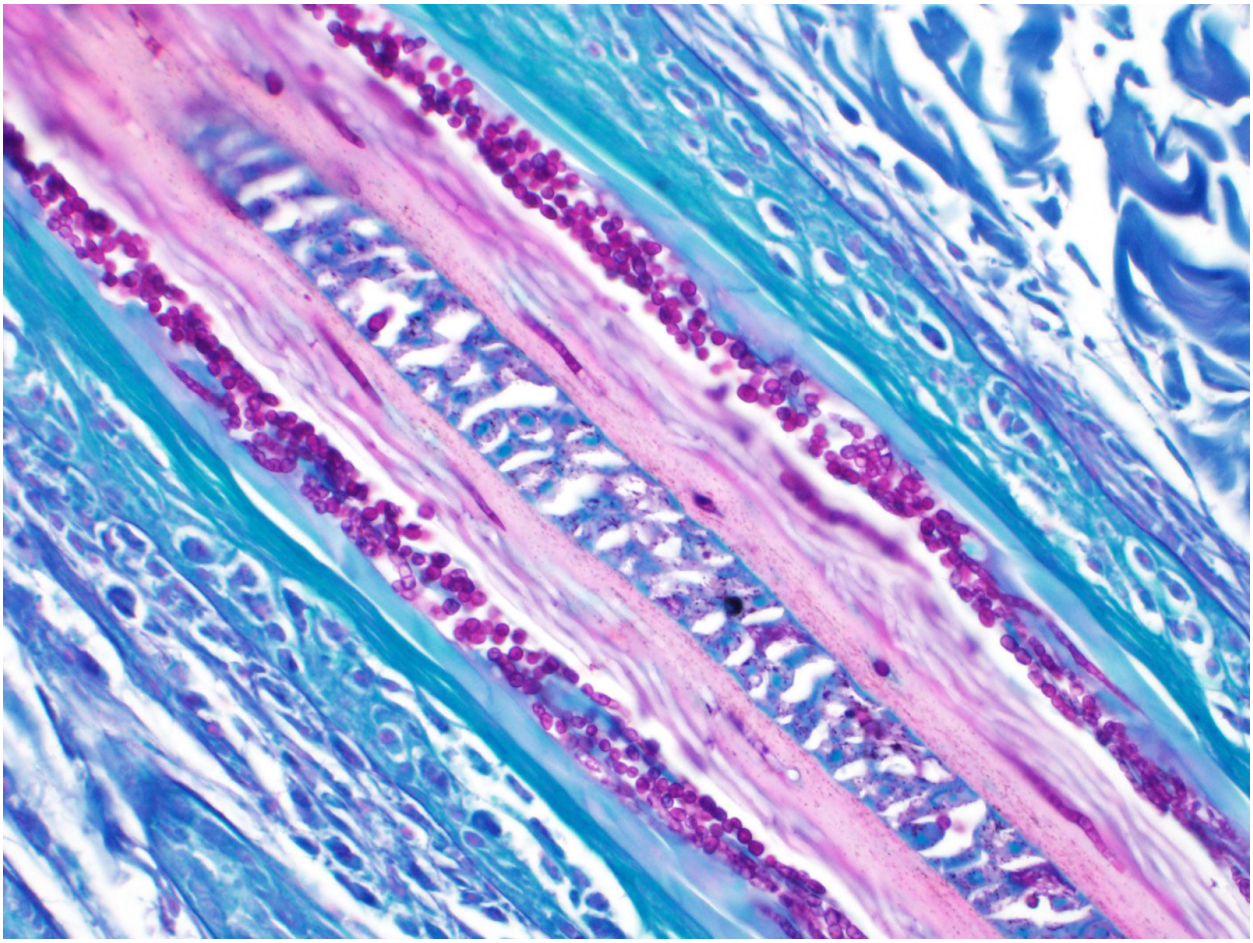
Haired skin, mink. Follicles are effaced by a dense infiltrate of neutrophils. Hair shafts contain numerous ecto- and endothrix arthrospores and fungal hyphae (in the hair shaft at lower right.) (HE, 180X)

Morbidity and mortality are generally low but, fungal infection can cause significant damage to pelts, and thus large outbreaks can have a significant financial impact on producers.[1,4]

Transmission of infection generally occurs via direct contact with infected animals or by fomites contaminated with hair or dandruff from an infected animal. Dermatophytes are keratinophilic and invade actively growing hair and follicular keratin. In most instances, patchy areas of alopecia and scaling are noted. Peripheral papules or erythematous rims generally surround more centrally located areas of clearing and healing.[3]

In most instances, dermatophytes are well-adapted to their hosts and have a preferential host range likely due to their specific nutritional requirements. In their preferred host, dermatophyte infection often induces minimal host inflammatory response. Many dogs and cats are asymptomatic carriers. Infection with dermatophytes that are not well adapted to a particular animal species may elicit a greater inflammatory reaction. In most cases, infections are self-limiting and healthy, immune-competent animals will eventually eliminate infection. Immunocompromised animals are at a greater risk of chronic and generalized disease. Most dermatophytes can cause zoonotic disease.

The microscopic lesions in this case are quite classic of dermatophytosis and this case is not much of a diagnostic challenge. What makes it interesting, is the identification of the causative agent via gene sequencing. *Trichophyton equinum*, as the name implies, is typically associated with dermatophytosis in horses with a worldwide distribution. Reports of *T. equinum* infection in species other than horses is rare and limited to dogs and cats and humans with direct contact with infected horses. We could find no report of *T. equinum* infection in mink in the literature. Interestingly, near the time this case was diagnosed, another outbreak of dermatophytosis was diagnosed in 3 week old, kits on a different ranch. These were all dark color phase mink and involved seven litters in 2 different sheds. No adult mink developed lesions. All the mink in each affected litter were euthanized. Microscopic lesions were identical to that described above and *T. equinum* was isolated from lesions. Testing for Aleutian disease in animals from both ranches was negative and there was no evidence of concurrent or underlying disease in any of the mink examined.



Haired skin, mink. Numerous arthrospores and fungal hyphae are present within the hair shaft. (PAS, 400X)

In both instances, culling of infected animals resulted in control of infection and no new cases were reported. The origin of the *T. equinum* infection in both these ranches remains a mystery. The first case involving the adult pastel mink may have been the result of infection arising from the source farm. However, the source of infection in the second outbreak involving the mink kits is unknown. There were 2 different ranches with no close contact. Contaminated straw or wood-shaving bedding and transmission from handlers were suggested possibilities. However, the bedding used had never been stored or been in direct contact with horses and no animal handlers on the ranch had lesions consistent with dermatophytosis. Contact with potentially infected cats and dogs and the ranch mink not considered likely.

Contributing Institution:
Department of Pathology/Microbiology
Atlantic Veterinary College, University of Prince Edward Island
www.avc.upei.ca

JPC diagnosis:
Haired skin: Dermatitis, folliculitis, and furunculosis, pyogranulomatous, multifocal, severe, with numerous endothrix and ectothrix arthrospores and hyphae.

JPC comment:
The contributor provides a concise summary on this case of dermatophytosis in mink, and subsequently published this case in 2015.[7] There are a number of different species specific dermatophytes that affect domestic species, with occasional exceptions. There are more than 30 species of dermatophytes that affect dogs and cats, but relatively few consistently cause

infection and disease. *Microsporum canis*, *Microsporum persicolor*, *Trichophyton* spp., *Trichophyton erinacei*, and *Microsporum gypseum* are the most often implicated agents in these species, with dogs occasionally being infected by multiple species of dermatophyte.[6]

Contrary to naming convention, *M. canis* is most commonly associated with cats. *Trychophyton mentagrophytes* is typically acquired from small rodents, and *M. gypseum* is assumed to be acquired from outdoor activities involving digging or rooting, since *M. gypseum* is saprophytic. In the event of disruption of the stratum corneum, fungal arthrospores adhere to keratinocytes and migrate to the follicular orifice. Through production of keratinolytic enzymes, endoproteases, and exoproteases, dermatophytes are able to hydrolyze keratin, penetrate the hair shaft, and avoid some of the body's host defenses and sometimes UV light.[5]

During the conference, there was discussion about culturing fungi. While Sabouraud's dextrose agar and potato-dextrose agar are commonly used for some species, dermatophyte test medium (DTM) is most commonly used for culturing dermatophytes. While most fungi metabolize carbohydrates, dermatophytes use nitrogen sources (keratin, proteins, amino acids) and produce alkaline byproducts. DTM contains Sabouraud's agar for a nutrient source, cycloheximide as a fungicide to prevent saprophytic fungal growth, an antibiotic (often chloramphenicol) to prevent bacterial growth, and phenol red to signal alkaline product production.[2]

During discussion about which species of dermatophytes typically affect which animals, the moderator introduced most of the group to the term fossorial, characterizing animals that dig and burrow. The analogous adjective to describe tree dwelling animals is arboreal. The discussion was enjoyed by all.

References:

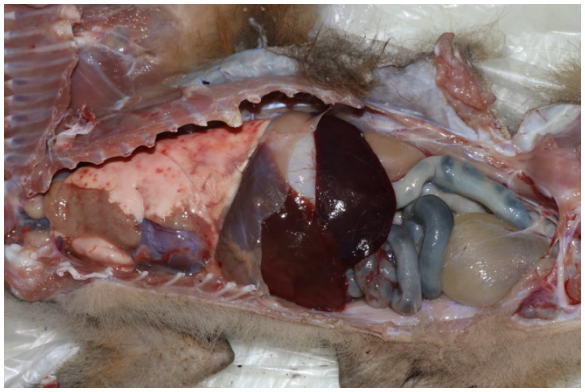
1. Bildfell RJ, Hedstrom OR, and Dearing PL. Outbreak of dermatophytosis in farmed mink in the USA. Vet Rec. 2004; 155:746-748.
2. Chengappa MM, Pohlman LM. Dermatophytes. In: McVey DS, Kennedy M, Chengappa MM, eds. *Veterinary Microbiology*, 3rd Ed. Ames, IA:John Wiley and Sons. 2013:324.
3. Gross TL, Ihrke PJ, Walder EJ, Affolter VK, eds. Pustular and nodular disease without adnexal destruction. In: *Skin Diseases of the dog and cat: Clinical and histopathologic diagnosis.* 2nd ed. Blackwell Publishing Company. 2005:406-419.
4. Hagen KW, Gorham JR. Dermatomycosis in fur animals: Chinchilla, ferret, mink and rabbit. US Department of Agriculture. Jan 1972.
5. Maudlin EA, Peters-Kennedy J. Integumentary System. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, Vol 1, 6th Ed. St. Louis, MO:Elsevier. 2016:649.
6. Moriello KA, DeBoer DJ. Dermatophytosis. In: Greene CE, ed. *Infectious Diseases of the Dog and Cat*, 4th Ed. St. Louis, MO:Elsevier. 2012:388-390.
7. Overy DP, Marron-Lopez F, Muckle A, et al. Dermatophytosis in farmed mink (*Mustela vison*) caused by *Trichophyton equinum*. *J Vet Diag Invest*. 2015; 27(5):621-626.

CASE 4: W18-0154-4 (4132734-00)

Signalment:
Juvenile male red fox (*Vulpes vulpes*)

History:
This wild, juvenile, male red fox (*Vulpes vulpes*) was found moribund, lying on a storm drain cover in Washington, DC. The animal was thin, minimally responsive and had generalized alopecia with severe thickening, crusting and scaling of the skin, especially along the face, ears and dorsum. The fox was humanely euthanized.

Gross Pathology:
This fox was is in good postmortem condition and thin body condition (2.0 kg) with scant adipose stores and exaggerated bone prominences. The fur was multifocally thinned and the skin over



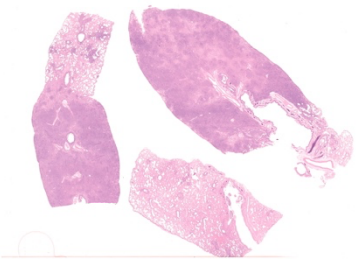
Lung, fox. The cranioventral portions of the left cranial and left caudal lungs lobes were consolidated, firm and pale brown. (Photo courtesy of: Smithsonian Institution's National Zoological Park www.nationalzoo.si.edu)

most of the body was malodorous and markedly thickened by pale tan fissured crusts. The cranioventral portions of the left cranial and left caudal lungs lobes were consolidated, firm and pale brown. The right lung was mottled pink to dark red. In the free wall of the right ventricle of the heart, there was a 1 x 0.5 cm, well-demarcated focus of pale brown discoloration (euthanasia artifact). Small bronchi contained mucus and abundant, 0.5-1.5 cm long by < 1 mm diameter white worms. The stomach and small intestine contained mucoid dark brown to black material. The colon contained formed dark green-brown feces. No other gross lesions were appreciated.

Laboratory results:
Worms collected from the bronchi were examined by Dr. JM Kinsella, HelmWest Laboratory and were identified as *Crenosoma vulpis* based on the cuticular rings around the anterior end of males and the spicules, gubernaculum and bursal rays of the female.

The brain was negative for Rabies virus via direct immunofluorescence.

Microscopic description:
Multifocally to regionally, the normal architecture of the lung is obscured by a florid, basophilic, densely cellular infiltrate that often completely fills the air spaces of bronchioles and surrounding alveoli. Within alveoli, inflammatory cells consist predominantly of neutrophils and foamy macrophages with fewer multinucleated giant cells and eosinophils, that are admixed with proteinaceous fluid (edema) and two types of nematode eggs and free larval nematodes. The more numerous eggs are approximately 70 by 35 um and embryonated, with a thin to inapparent wall and a 10 to 12 um diameter, coiled larva with small lateral alae. These larvae are also free within alveolar lumina. The second, less numerous, egg type is embryonated (but not larvated), approximately 70 by 35 um, with bipolar plugs, a prominent, eosinophilic, 5 um thick wall containing ridges, and granular eosinophilic egg contents. Within the lumina of smaller bronchi, there are adult male (~200 um diameter) and female (~400 um diameter) nematodes that have a cuticle with thin longitudinal ridges, coelomyarian musculature, accessory hypodermal chords, a large intestine lined by few multinucleate cells and a genital tract containing either sperm or developing eggs with both embryos and first stage larvae present. In most (but not all) slides, within the epithelium of a larger bronchi, there is a 100 to 150 um diameter adult nematode with a thin cuticle, a pseudocoelom and hologonic gonads. Small airway (bronchioles and small bronchi) lumina are often filled and sometimes occluded by a mix of neutrophils, fewer eosinophils, free larvae and mucus. Few neutrophils and eosinophils transmigrate the respiratory epithelium, which often has increased goblet cells (mucus metaplasia) and plump and piled cells with large nuclei and vesiculate chromatin (bronchiolar/bronchial epithelial hyperplasia). Serous submucosal glands in a large airway are tortuous, dilated and filled with clear to slightly proteinaceous fluid. Moderate numbers of plasma



Lung, fox. Three sections of lung are submitted for examination. Two of the three sections have are over 50% consolidated with severe inflammation, the third (center) demonstrates extensive alveolar edema. (HE, 5X).

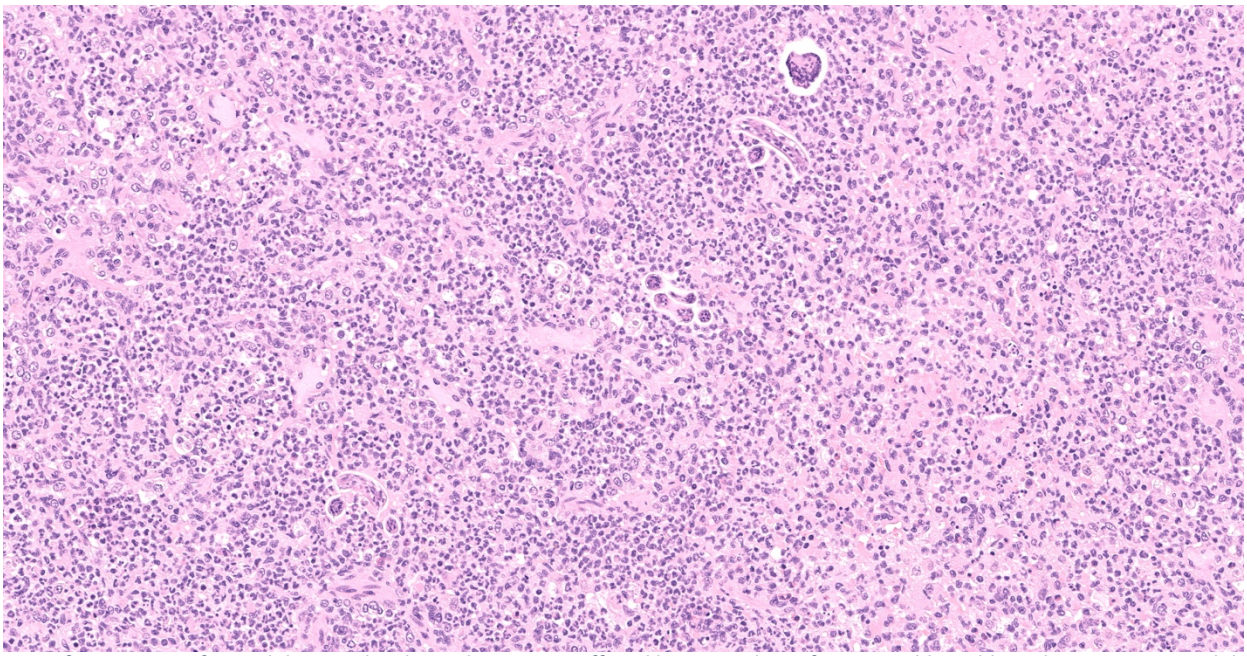
cells and fewer lymphocytes infiltrate the adventitia of airways and pulmonary vessels.

Contributor's morphologic diagnosis:
Lung: Severe, regionally extensive, chronic, pyogranulomatous and eosinophilic bronchopneumonia with intrabronchial adult metastrongylid and aphasmid nematodes, intra-alveolar and intra-bronchiolar metastrongylid and aphasmid larvae and eggs, bronchiolar epithelial hyperplasia and mucus metaplasia.

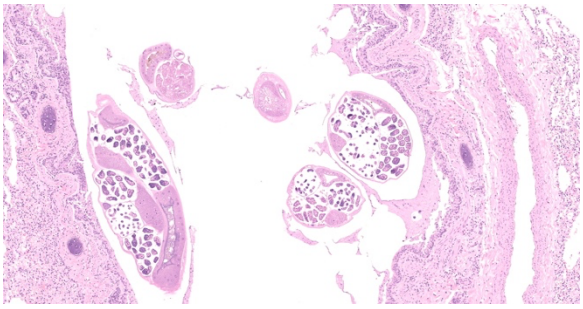
Contributor's comment:
A dual lungworm infestation causing bronchopneumonia was diagnosed. Based on the morphology and location of eggs and adults described, the larger intraluminal adult worms, larva and thin-walled larvated eggs are consistent *Crenosoma vulpis*, while the smaller intra-epithelial adult worm and thick-walled eggs with bipolar plugs and ridges are consistent with *Eucoleus aerophilus.*[9] *Crenosoma vulpis* identification was further confirmed based on examination of whole adult worms, which revealed cuticular ridges on the anterior end of males that is characteristic of this worm.

Crenosoma vulpis is a metastrongyle nematode known to infect domestic dogs and many wild carnivores in eastern North America and Europe, including red foxes, badgers (*Meles meles*), wolves (*Canis lupus*), raccoons (*Pryocyon lotor*)[3] and Virginia opossum (*Didelphis virginiana*).[7] The indirect life cycle involves the definitive mammalian host becoming infected when it eats a snail or slug (intermediate host) that harbors the third-stage larvae (L3).[1] L3 larvae then penetrate the gastrointestinal tract wall and migrate to the lungs, where they mature into adults that live in the lumen of the trachea, bronchi or bronchioles.[1] Thin-shelled eggs containing larva or free larvae deposited by adults become lodged in alveoli, and migrate up the airways until they are coughed up, swallowed and passed in the feces where they can infect the intermediate host.[1,3]

Eucoleus aerophilus is an aphasmid nematode that was first described in 1839 and named *Trichiosoma aerophilus*. Subsequent name and taxonomic designation changes (formerly *Thominx aerophilus* and *Capillaria aerophila*) have landed the organism in the *Eucoleus* genus.[2] Documented infestations have occurred in wildlife, domestic cats and humans in a wide geographic range.[8] Much of the biology and life



Lung, fox. In areas of consolidation, alveolar architecture is effaced by an exudate of innumerable viable and necrotic neutrophils and large numbers of foamy and debris-laden macrophages. Scattered throughout the section of low to moderate numbers of metastrongyle larvae. (HE, 200X).



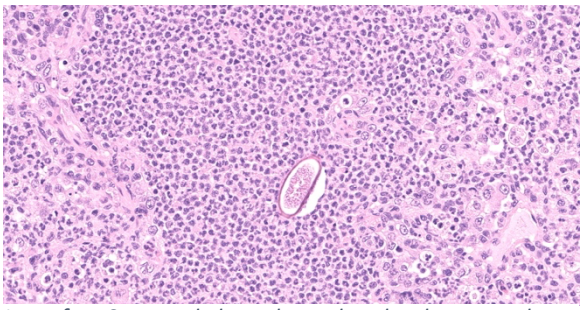
Lung, fox. Within the lumen of a bronchiole, there are cross sections of male and female metastrongyle adults. (HE, 5X).

history of this parasite is still poorly understood. *E. aerophilus* is thought to have a direct life cycle, where adults living in the epithelium of the respiratory tree deposit eggs that are coughed up, swallowed and passed in the feces.[3,13] Eggs may develop directly in the environment or be ingested by earthworms, which act as a facultative intermediate host.[3,13] Characteristic histologic features of aphasmids are bacillary bands and a stichosome, which were not visible in the few sections of adult worms in this case. But based on the size and location of adult worms (intra-epithelial in a bronchus), and presence of thick-walled eggs with bipolar plugs, *Eucoleus aerophilus* is considered most likely.

C. vulpis and *E. aerophilus* infestations are important causes of chronic pulmonary disease in both wild and domestic canids, especially in the northeastern United States and Canada where dual infestations by both worms are common in wild red foxes.[10] Clinically, infected animals often have a chronic cough and resemble animals afflicted with allergic bronchitis and chronic obstructive pulmonary disease (COPD).[10] Pathologic changes within the lungs of wild foxes are typically limited to airway-centered exudate (eosinophilic and catarrhal bronchitis/ bronchiolitis), BALT hyperplasia, submucosal bronchial gland hyperplasia, smooth muscle hypertrophy of airways and mucus metaplasia.[5,10] *C. vulpis* tends to inhabit smaller airways (small bronchi and bronchioles), whereas *E. aerophilus* prefers larger bronchi, as was the case in this fox.[10] Granulomatous pneumonia can occur in canids when *C. vulpis* larvae are aspirated into alveoli and bacterial pneumonia can complicate both types of infestations.[5] In dogs experimentally infected with *C. vulpis*, a marked granulocytic exudate filled alveoli (in addition to terminal bronchioles) in the early stages of infection as L3 larvae migrated into the lungs.[12] The heavy involvement of alveoli and lack of significant smooth muscle hypertrophy and submucosal gland hyperplasia in this case may reflect a more acute and fulminant course of disease in a juvenile animal with an immature immune system and severe concurrent ectoparasitism (this animal was also heavily infested with sarcoptic mange and in poor body condition at the time of euthanasia).

Metazoan parasites of importance in the respiratory tract of carnivores that must be distinguished from *C. vulpis* and *E. aerophilus* include: *Aelurostrongylus abstrusus*, which is similar to *C. vulpis* but parasitizes felids[5]; *Angiostrongylus vasorum* that reside the pulmonary arteries[5]; *Eucoleus* (=*Capillaria*) *boehimi* that reside in the nasal cavity and have smaller (50-60 x 30-35 um) eggs with a pitted rather than ridged wall[6]; *Paragonimus kellicotti*, which is a trematode[5]; and *Oslerus* (=*Filaroides*) *osleri,* which typically cause grossly evident submucosal nodules in the trachea and bronchi[5]; *Andersonstrongylus* (=*Filaroides*) *milks*i and *Filaroides hirthi*. *Andersonstrongylus milksi* and *Filaroides hirth* are metastrongyles that are similar in size to *C. vulpis* and also inhabit the bronchioles, thus differentiation of these three worms based on histology is problematic and examination of intact adults is preferred (as was done in this case).

This case highlights the importance of a thorough histologic evaluation with special attention paid to secondary or tertiary diseases that may be



Lung fox: Scattered throughout the alveolar parenchyma are few oblong, eggs of Eucoleus (Capillaria) aerophila, with bipolar plugs. (HE, 400X)

present in examined organs. Although parasite morphology and identification can at times be challenging for a pathologist, when multiple parasite lifestages are present care should be taken to ensure they do not represent separate species.

Contributing Institution:
Smithsonian Institution's National Zoological Park
www.nationalzoo.si.edu

JPC diagnosis:
Lung: Bronchopneumonia, pyogranulomatous, chronic, multifocal to coalescing, severe, with intraluminal metastrongyle and intraepithelial aphasmid adults, and intra-alveolar larvae, and metastrongyle and aphasmid eggs.

JPC comment:
The contributor provides a very thorough analysis of this case, and well illustrates the capacity of animals to have coinfections pathogenic organisms, nematodes in this case. As the contributor stated, *C. vulpis* and *E. aerophilus* have a wide geographic distribution. In particular, *C. vulpis*, *E. aerophilus*, and *Angiostrongylus vasorum* are particularly problematic for domestic dogs and wild canids, particularly red foxes, in central Germany. Across three Federal states (Thuringia, Rhineland-Palatinate, Hesse), 569 red foxes examined had prevalence of *C. vulpis* infections of up to 32.3%, *A. vasorum* of up to 14.1%, and *E. aerophilus* of up to 69.4%. In the examined red foxes, approximately 30.7% had dual infections with *E. aerophilus* and *C. vulpis*. A small subset of foxes (5%) had triple infections, with *A. vasorum* in addition to *C. vulpis* and *E. aerophilus*.[11]

Recent investigations of lungworm infections in red foxes have used geographic information systems (GIS) to visualize the cases, allow for geospatial analysis such as cluster analysis, and to attempt correlation of cases with other environmental factors.[4,11] Investigation in Slovakian red foxes found moderately good predictive value in a multivariate model using backward stepwise regression. The model indicated that infection of *A. vasorum* is positively correlated with areas of lower warm period precipitation and a higher share of arable land and permanent cultures.[4]

References:

1. Anderson R. Order Strongylida. In: *Nematode Parasites of Vertebrates: Their Development and Transmission*. Oxon, UK: CAB International; 1992:35–208.
2. Bowman A. Eucoleus aerophilus. *American Association of Veterinary Parasitologists*. 2014: retrieved from http://www.aavp.org/wiki/nematodes/aphasmidida/eucoleus-aerophilus/
3. Bowman DD. *Georgis' Parasitology for Veterinarians, 10th Edition*. St. Louis, MO: Elsevier; 2014.
4. Cabanova V, Miterpakova M, Druga M, Hunikova Z, Valentova D. GIS-based environmental analysis of fox and canine lungworm distributions: an epidemiological study of *Angiostrongylus vasorum* and *Crenosoma vulpis* in red foxes from Slovakia. *Parasitology Research*. 117:521-530.
5. Caswell J, Williams K. Respiratory System. In: *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. St. Louis, MO: Elsevier; 2016:465–591.
6. Conboy G. Helminth Parasites of the Canine and Feline Respiratory Tract. *Vet Clin North Am Small Anim Pract*. 2009 Nov;39:1109–1126.
7. K Rice J. Crenosoma vulpis larva (fox lungworm) in a juvenile opossum from Northern Virginia, USA. *Int Int J Avian Wildl Biol*. 2018 Apr 2;3:4–2.
8. Lalošević D, Lalošević V, Klem I, Stanojev-Jovanovic D, Pozio E. Pulmonary Capillariasis Miming Bronchial Carcinoma. *Am J Trop Med Hygeine*. 2008;78:14–16.
9. Lalošević V, Lalošević D, Čapo I, Simin V, Galfi A, Traversa D. High infection rate of zoonotic Eucoleus aerophilus infection in foxes from Serbia. *Parasite*. 2013;20.
10. Nevárez A, López A, Conboy G, Ireland W, Sims D. Distribution of *Crenosoma Vulpis* and *Eucoleus Aerophilus* in the Lung of Free-Ranging Red Foxes (*Vulpes Vulpes*). *J Vet Diagn Invest*. 2005 Sep;17:486–489.
11. Schug K, Kramer F, Schaper R, et al. Prevalence survey on lungworm (*Angiostrongylus vasorum*, *Crenosoma vulpis, Eucoleus aerophilus*) infections of wild red foxes (*Vuples vulpes*) in central

Germany. *Parasites and Vectors*. 2018;11:85.
12. Stockdale PHG, Hulland TJ. The Pathogenesis, Route of Migration, and Development of Crenosoma vulpis in the Dog. *Pathol Vet*. 1970 Jan 1;7:28–42.
13. Traversa D, Di Cesare A, Conboy G. Canine and feline cardiopulmonary parasitic nematodes in Europe: emerging and underestimated. *Parasit Vectors*. 2010 Jul 23;3:62.