Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

C o n f e r e n c e 6

29 September 2021

CASE I:R46-18-5 (JPC 4134356)

Signalment:
Adult domestic shorthair cat (*Felis catus*)

History:
A feral cat, trapped for spay, neuter and release was euthanized due to FIV positive status. The cat was submitted to the parasitology department of Ross University School of Veterinary medicine for nematode collection. Colonic nodules, seen during nematode collection, were excised and submitted to the pathology department for histological evaluation.

Gross Pathology:
There were multiple large (4-8mm in diameter), slightly raised, pale tan nodules in the colonic wall. The nodules appeared to be located in the submucosa, and were visible from both the serosal and mucosal surface. Small pits or depressions were seen on the mucosal surface of the nodules.

Laboratory results:
No laboratory findings reported.

Microscopic Description:
Colon. Expanding the submucosa, and elevating the mucosa, are several 4-8mm in diameter, discrete nodules composed of tubules of herniated hyperplastic mucosal epithelial cells. The latter form branching tubules, surrounded and separated by lymphocytes, plasma cells and occasional macrophages admixed with adult nematodes, eggs, and larvae. Occasionally tubules contain protein or necrotic cell debris. Adult nematodes and eggs are also within tubules. Occasionally larvae are within the tubules and interstitium of the overlying mucosa. Adult females are approximately 110µm in diameter and have platymyarian musculature, paired genital tracts, and intestine composed of uninucleate cells. Larvae are approximately 15-20µm in diameter. A rhabditiform esophagus is rarely identified within larvae. Eggs are ovoid, 30x50µm in

Figure 1-1. Colon, cat. Multiple discrete pale 4-8mm nodules are scattered within the colonic wall. (Photo courtesy of: Ross University School of Veterinary Medicine, PO Box 334, Basseterre, St. Kitts, https://veterinary.rossu.edu/)

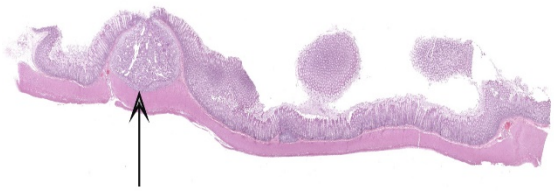

Figure 1-2. Colon, cat. A section of colon with an enlarged Peyer's patch (arrow) is submitted for examination. (HE, 5X)

diameter and contain morulae or larvae. The overlying mucosa is attenuated and mildly eroded.

Contributor's Morphologic Diagnoses:
Multifocal colonic epithelial nodular hyperplasia and lymphoplasmacytic colitis with intralesional *Strongyloides* sp. adults, eggs and larvae.

Contributor's Comment:
Strongyloides is a genus of nematode with a complex life cycle involving free living adult stages in the environment and intestinal parasitism of a wide variety of host species. The most important veterinary species are *S. papillosus* in ruminants, *S. westerii* in horses, *S. ransoomi* in swine and *S. stercoralis* in dogs, cats, humans and non-human primates, and *S. felis*, *S. planiceps* and *S. tumefaciens* in cats.[5]

Hosts become infected by percutaneous penetration, or ingestion, by third stage filariform larvae (L3), which migrate to the intestine, typically through the lungs.[5] L3 develop into adult, parthenogenetic females, typically localized within small intestinal mucosa, and reproduce asexually releasing eggs or first stage rhabditiform larvae (L1) in the feces. Environmental L1 can develop directly into infectious L3 larvae, or into free-living adult males or females which produce eggs and larvae by sexual reproduction. Likewise, their progeny may either develop into new generations of infectious L3 larvae or noninfectious free-living adults. Further-more, at least in *S. stercoralis*, L1 can develop to L3 within the intestine, and migrate through the intestinal wall, back to the small intestine to complete a new parasitic cycle within the same host (autoinfection).[5] Most infections are probably asymptomatic, but diarrhea and rarely death, can be associated with *Strongyloides* infection in young animals.[5]

Strongyloides sp. within colonic nodular epithelial hyperplasia was first described in two US cats, by Price and Dikmans 1927,[4] and later in a few case reports from the United States[1,2] and Brazil.[3] The pathology in all cases, has been similar, with female adult *Strongyloides* only observed within nodules in the colonic submucosa, and nodules histologically consisting of epithelial cells forming tubules, extending into the submucosa, supported by stroma infiltrated by lymphoid cells, and with sections of

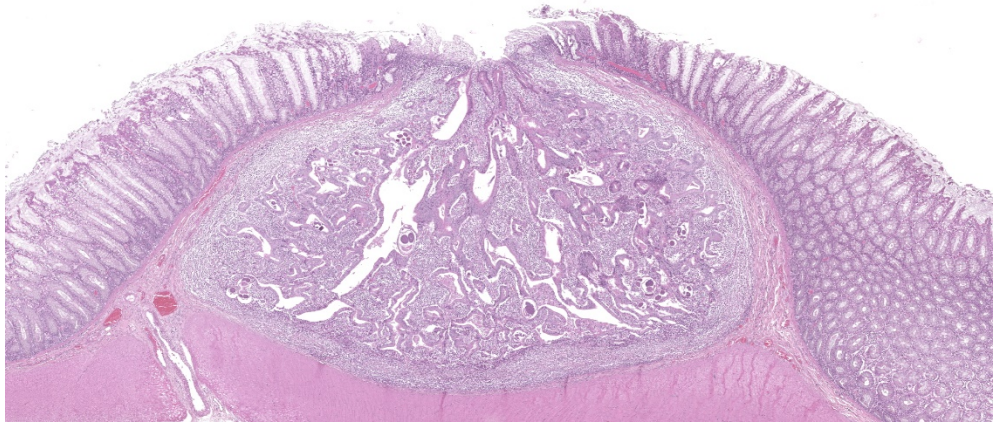

Figure 1-3. Colon, cat. Higher magnification of the Peyer's patch with deep herniation of colonic glands. Just left of center, "ultra-fly" nematode Joe Cool surveys his domain. (HE, 133X)

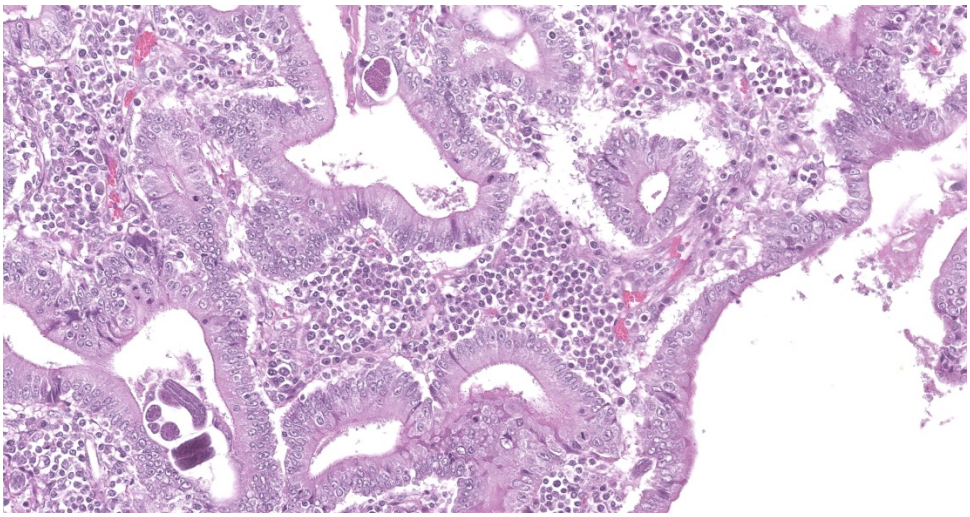

Figure 1-4. Colon, cat. High magnification of herniated colonic glands, with nematode cross sections within their lumina. (HE, 252X)

parasitic female nematodes and larvae throughout the tubules and stroma.

The location within colonic nodules, and associated pathology (nodular epithelial hyperplasia) is atypical for *Strongyloides* spp. in general. For all other species described, nematode stages are confined to the proximal small intestinal mucosa, and associated pathology includes villous atrophy, crypt hyperplasia, and eosinophilic to lymphoplasmacytic infiltrate in the lamina propria.[6]

The *Strongyloides* sp. observed within feline colonic nodules was designated as a separate species, *Strongyloides tumefaciens*, by the authors who first described it in 1927.[4] The species designation was based primarily on the unusual location and lesion, rather than on nematode morphology. Thus, the validity of this species is questionable, and a molecular genetic approach to this parasite's identification would seem warranted.

Contributing Institution:
https://veterinary.rossu.edu/

JPC Diagnosis: Colon: Crypt hyperplasia, focal, severe with crypt herniation and numerous rhabditoid adults, larvae and eggs.

JPC Comment:
The contributor provides a good review of the complex and unique lifecycle of the genus *Strongyloides* as well as the various species infected. This genus is of significant historical significance, particularly in livestock. However, it has been significantly suppressed in recent decades, likely due to extensive use of macrocyclic lactone anthelmintics and/or improved hygiene.[5]

Also known as threadworms, *Strongyloides* spp. are grouped within the Rhabditoidea superfamily and are small (3-8mm), slender nematodes discovered over 140 years ago in French troops returning from present day Vietnam.[5,7] Its name is derived from the Greek words 'strongylos' meaning 'round', and 'eidos' meaning 'similar', with the intention to show *Strongyloides* was close to the genus *Strongylus.*

The *Strongyloides* genus of nematodes represent one of the soil-transmitted helminthiases (STH), a WHO-recognized neglected tropical disease in humans. Of the estimated 30-100 million human infections, most are asymptomatic. However, people with an altered immune status, particularly because of administration of corticosteroids or HTLV-1 infection, can develop more intense infections resulting in hyperinfection, which can be fatal if not recognized.[7]

The validity of *S. tumefaciens* as a species has recently been called into question by a 2019 report describing six cats with colonic nodules containing nematodes that based location and histologic characteristics were consistent with *S. tumefaciens*, such as in this case. However, the report's authors determined the nematodes to be *S. stercoralis* based on sequencing analysis of a portion of the cytochrome c oxidase subunit 1 (cox1) gene. This report generated dis-cussion amongst participants, with the moderator advising that genes are largely conserved within genera and unique histologic characteristics of *S. tumefaciens*, such as a larger egg size, supports *S. tumefaciens*'s validity as a species.

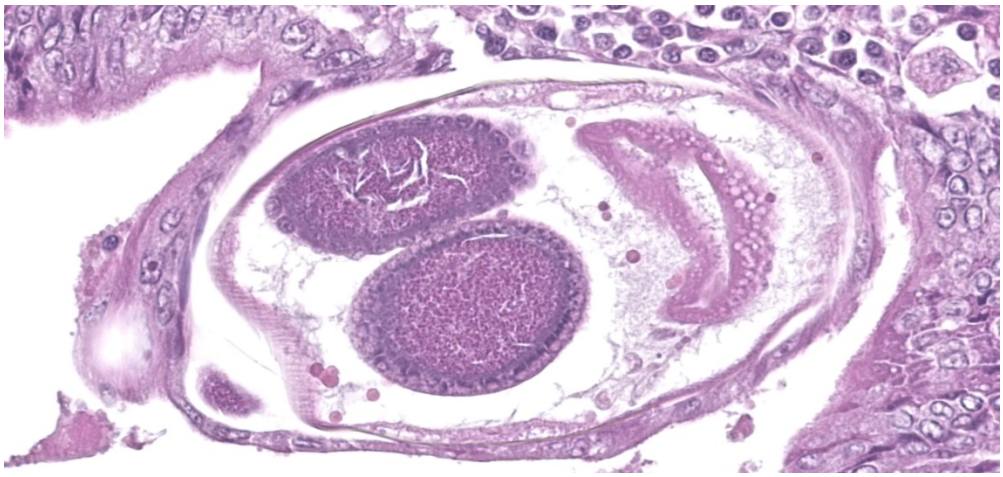

Figure 1-5. Colon, cat. High magnification of a nematode adult female contained within the epithelium. The female has a thin cuticle, low platymyarian-meromyarian musculature, a body

S. stercoralis classically results in the formation of characteristic nodules in the proximal small intestine and is known to infect dogs, cats, non-human primates, and humans.[8] *S. felis,* and *S. planiceps* have also been described in the proximal small intestine while *S. tumefaciens* has been described in the colon.

Each species within genus *Strongyloides* share similar morphologies, however, there variations as previously noted. For example, *S. stercoralis, S. felis,* and *S. tumefaciens* have straight ovaries while *S. planiceps* has spiraled ovaries. Both *S. stercoralis* and *S. felis* rhabditiform (L1) larvae are found in the feces while larvated eggs are found in the feces of animals infected with *S. planiceps.* Parasitic females of each species have a bluntly pointed tail.[8]

The topic of crypt herniation was also discussed amongst participants, particularly in regard to extension of hyperplastic crypts extending into underlying gut associated lymphoid tissue (i.e. Peyer's patches or GALT). The aforementioned report evaluated similar findings associated with lymphocytic populations within the lamina propria and submucosa. In this report, the authors determined via immunohisto-chemical staining that the majority of cells in all *Strongyloides*-associated nodules were immunopositive for CD3 (T-cells) and likely indicates these lymphoid nodules are part of an efferent T-cell dominated immune response against the nematodes rather than herniation into preexisting GALT. However, it is also possible that the presence of nematodes within GALT downregulates signals for B-cells, resulting in altered composition of the nodules. Although this phenomenon hasn't been described in *Strongyloides* spp., other parasites have been shown to produce proteins that interact with human B-lymphocytes and may play a rule in suppression of molecules resulting in activation of B-lymphocytes.[8]

References:

1. Lindsay DS, Blagburn BL, Stuart BB, Gosser HS. Strongyloides tumefaciens infection in a cat. 1987;1:12–13.
2. Malone JB, Butterfield AB, Williams JC, Stuart BS, Travasos H. Strongyloides tumefaciens in cats. *J Am Vet Med Assoc*. 1977 Aug 1;171:278–280.
3. Moura MAO, Jorge EM, Nascimento KKG do, et al. Colonic epithelial nodular hyperplasia associated with strongyloidiasis in cats in the Amazon region, Pará State, Brazil. *Ciência Rural*. 2017;47:1–4.
4. Price EW, Dikmans G, Others. Adenomatous tumors in the large intestine of cats caused by Strongyloides tumefaciens n. sp. *Proc Helminthol Soc Wash*. 1941;8.
5. Thamsborg SM, Ketzis J, Horii Y, Matthews JB. Strongyloides spp. infections of veterinary importance. *Parasitology*. 2017;144(3):274-284.
6. Uzal FA, Plattner BA, Hostetter JM. Chapter 1 Alimentary system. In: Maxie GM, ed. *Jubb, Kennedy and Palmer's Pathology of domestic animals volume 2, sixth edition*. 2015:1–257. (updated superscript in the contributor's comment)
7. Viney M. Strongyloides. *Parasitology*. 2017;144(3):259-262.
8. Wulcan JM, Dennis MM, Ketzis JK, Bevelock TJ, Verocai GG. Strongyloides spp. in cats: a review of the literature and the first report of zoonotic Strongyloides stercoralis in colonic epithelial nodular hyperplasia in cats. *Parasit Vectors*. 2019;12(1):349.

CASE II: 15-185 (JPC 4090739)

Signalment:
Adult female koala (*Phascolarctos cinereus*)

History:
Hyperkeratotic dermatitis.

Gross Pathology:
The haired skin over the ventro-medial aspects of the forelimbs, the dorsomedial aspects of the hindlimbs, and between the digits of all four feet is markedly thickened with alopecia and crusting (marked hyperplastic and hyperkeratotic dermatitis). Similar thickening and crusting of the skin with fissuring is noted in the perioral and perinasal haired skin.

Laboratory results:
Skin scrapings revealed numerous mites with morphologic features consistent with *Sarcoptes scabiei.* The adult mites measured up to 450µm in length with round or globoid bodies, very short jointed appendages, a posteriorly positioned anus, and numerous dorsal triangular spines.

Microscopic Description:
Haired skin: The epidermis is diffusely moderately to markedly thickened by irregular epidermal hyperplasia with mild intercellular edema (spongiosis), intracellular edema, and marked diffuse parakeratotic to orthokeratotic hyperkeratosis. Scattered within the thickened stratum corneum are numerous intracorneal arthropod mites, small to moderate numbers of coccoid bacteria, and multifocal to coalescing areas of neutrophilic and eosinophilic exudation and crusting. The arthropod mites (up to ~350 µm in greatest dimension) have a chitinous exoskeleton with dorsal spines, short jointed appendages, striated skeletal muscles, and internal gastrointestinal and reproductive tracts. The underlying superficial perivascular dermis is

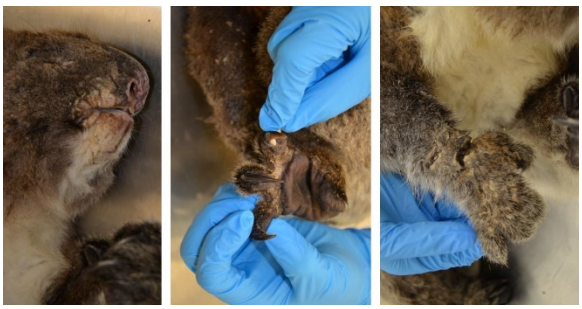

Figure 2-1. Haired skin, koala: There are multiple patches of alopecia, acanthosis, and hyperkeratosis on the skin of this the feet and legs, as well as the face. (Photo courtesy of: University of Adelaide, Roseworthy Campus, Mudla Wirra Road, Roseworthy, SA, Australia 5371, http://www.adelaide.edu.au/vetsci)

infiltrated by small numbers of neutrophils admixed with very small numbers of lymphocytes, plasma cells, macrophages, mast cells, and rare eosinophils. The superficial dermal and mid-dermal blood vessels are diffusely congested. In some sections (facial skin), the epidermis contains areas of erosion and ulceration (possible excoriations) with marked local fibrinosuppurative exudation and crusting and underlying superficial dermal edema.

Contributor's Morphologic Diagnoses:
Haired skin: Severe, diffuse, subacute to chronic, neutrophilic and hyperplastic dermatitis, with marked parakeratotic to orthokeratotic hyperkeratosis, neutrophilic exudation and crusting, moderate to large numbers of superficial coccoid bacteria, and numerous intracorneal mites (*Sarcoptes scabiei*, presumed).

Contributor's Comment:
Sarcoptic mange (referred to as scabies in humans) is a highly contagious skin disease caused by the parasitic mite, *Sarcoptes scabiei.* Sarcoptic mange has a broad host range and has been reported in over 100 species of animals including domestic dogs, livestock (e.g., cattle, pigs, goats, camelids), and wildlife (e.g., red foxes, wild dogs, coyotes, wolves, dingoes, deer, ibex, cheetahs, lions, bobcats, gorillas, wombats, koalas and wallabies).[2,6] Sarcoptic mange is a major disease of Australian wildlife, particularly in wombats (bare-nosed/ common and southern hairy-nosed), which also impacts humans, domestic animals, and other Australian wildlife.[2] Reports of the disease were previously rare in koalas but more recently several outbreaks of sarcoptic mange have been observed in Australia (Dr. Lucy Woolford, personal obs.).[7]

Sarcoptic mites parasitizing the various host species are largely morphologically indistinguishable, thus it is unclear if the mites parasitizing different mammalian host species are different species or if they are strains or variants (pathovars) of one species.[2,6] Some strains appear to parasitize a single host species while others parasitize multiple host species (e.g., dogs and rabbits), and infestations from prey-to-predator appear to occur in some ecosystems.[1,6] Cross-infestations between different host species have been shown to occur, but these documented spillover events have typically been self-limiting.[2]

Concurrent pyoderma is often observed in cases of sarcoptic mange, as seen in this case. *Sarcoptes scabiei* infestation results in injury to the epidermal layer caused by a combination of host scratching and the mechanical damage by the burrowing mites and provides an entry point for the invading bacteria. Localized complement inhibition by excreted mite products in the mite burrow may provide a protected microenvironment in which bacteria can initiate colonization without immediate attack by the host immune system and thus play a role in the development of pyoderma in scabies mite infested skin.[1,4,8]

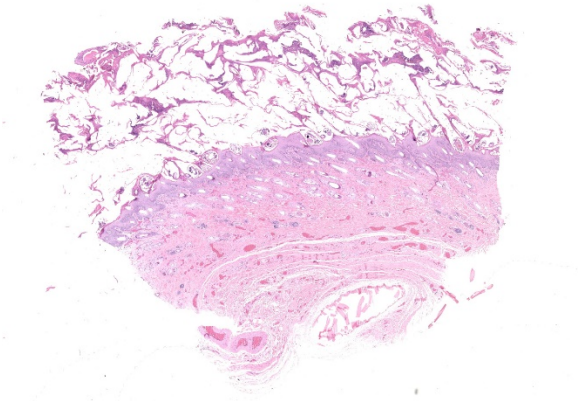

Figure 2-2. Haired skin, koala. The submitted section of haired skin is covered by a thick hyperkeratotic crust covering a moderately hyperplastic epidermis. (HE, 4X)

Contributing Institution:
University of Adelaide, School of Animal and Veterinary Sciences
http://www.adelaide.edu.au/vetsci/

JPC Diagnosis:
Haired skin: Epidermal hyperplasia and hyperkeratosis, diffuse, marked, with mild eosinophilic dermatitis and numerous intracorneal mites and eggs, etiology consistent with *Sarcoptes scabiei*.

JPC Comment:
Sarcoptes scabiei is a submacroscopic burrowing mite in which male and female adults, larvae, protonymphs, tritonymphs, and eggs are found within the epidermis, extending to the level of the stratum granulosum, and consume living cells and tissue fluid.[5] As noted by the contributor, there are no definitive means to taxonomically distinguish the various strains that tend to demonstrate a high degree of host specificity. Thus *S. scabiei* as a species is divided into morphologically indistinguishable variants with a high degree of host specificity and low degree of cross infectivity. As a result, varieties are named for their predominant host species, such as *S. scabiei* var. *canis, S. scabiei* var *suis*, etc.[5]

Infection occurs either via direct or indirect transmission. Direct transmission occurs via contact between an infected host and an uninfected animal since larvae and nymphs leave their burrows and wander the skin's surface. Alternatively, indirect transmission may occur since *S. scabiei* are capable of surviving off the host for potentially up to three weeks in low temperature and high relative humidity environments, such as former dens of infected animals.[2,5] Host species population density also tends to influence the parasite's prevalence since transmission is dependent on direct and indirect contact.[5]

Following exposure, *S. scabiei* burrows into the skin, usually to the level of the stratum granulosum, using a combination of its mouthparts (chelicerae and gnathosoma) and cutting hooks on its legs. Digestive enzymes may also play a role in burrowing and feeding but have not yet been described.[5]

A significant component of *S. scabiei*'s pathogenesis is due to host hypersensitization as a result of mechanical disruption of the skin, excretions and secretions of the mite, and the massive amount of antigenic material produced (including dead mites, molted skins of adults and immature life stages, and egg shells). Both type I and type IV hypersensitivity reactions have been demonstrated in domestic animals such as the dog and pig;

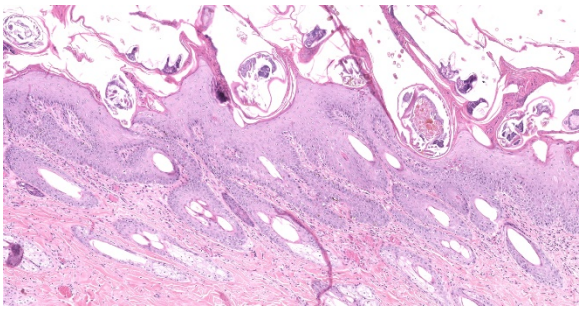

Figure 2-3. Haired skin, koala. Above the hyperplastic epidermis are numerous cross-sections of adult mites contained within the hyperkeratotic scale. (HE, 90X)

however, only type I hypersensitivity reactions were demonstrated in experimentally infected red foxes, which was also seems to occur in coyotes, even in advanced cases. Initial lesions in many hosts are localized and nonpruritic. After a period of several weeks an allergic response develops, resulting in generalized urticarial eruptions and severe pruritus. Depending on the host, this may or may not be accompanied by a delayed hypersensitivity response, which is associated with low numbers of mites within the lesion. In contrast, recently exposed, previously naïve new hosts as well as anergic and malnourished animals may develop nonpruritic, severe crusting dermatitis with severe hyperkeratosis, serosanginous exudate, and large numbers of mites.[5]

Numerous epizootics of sarcoptic mange in wildlife have been documented, frequently resulting in devastatingly high losses, such as a 70% of the southern Texas coyote population in one epizootic. During the 1960s and 1970s an epizootic in Fenno-scandia red foxes resulted in a 50% population decline overall and up to 90% mortality in some areas. Similar epizootics have been reported in multiple species across the globe and seem to cycle every 30-45 years in some areas. Despite very high losses, many populations recover over time. However, remnant or isolated populations, even species not listed by the Convention on

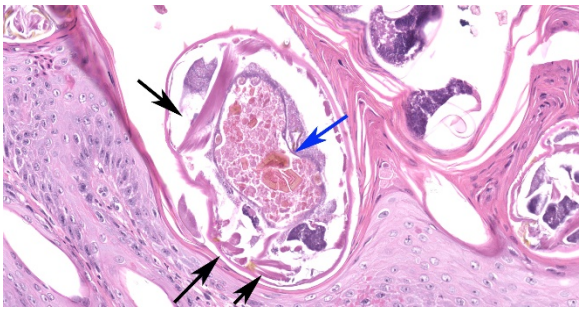

Figure 2-4. Haired skin, koala. Cross sections of the adult mites demonstrate a spiked chitinous exoskeleton, striated muscle (black arrows) and a gastrointestinal tract containing ingested keratin (blue arrow). (HE, 300X)

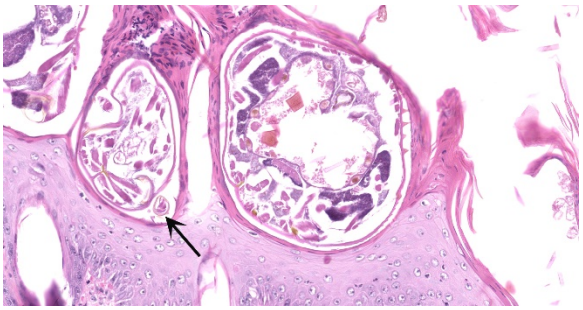

Figure 2-5. Haired skin, koala. Another characteristic features of arthropods in tissue section are joint appendages. There is also a lot of striated muscle to move those legs in this section. (HE, 300X)

International Trade in Endangered Species of Wild Fauna and Flora (CITES), can be severely affected by sarcoptic mange. As an example, naïve red foxes on the island of Borrnholm in Denmark completely disappeared following introduction of sarcoptic mange. Remnant populations of endangered species identified to be at particular risk include the Bonobo chimpanzee (*Pan panisci),* Ugandan mountain gorillas, cheetahs, wild dogs, chamois, Iberian Ibex, and the highly endangered European mouflon (*Ovis orientialis musimoon)*.[5]

References:

1. Bergström FC, Reynolds S, Johnstone M, Pike RN, Buckle AM, Kemp DJ, Fischer K, Blom AM. Scabies mite inactivated serine protease paralogs inhibit the human complement system. J Immunol. 2009;182(12):7809-17.
2. Fraser TA, Charleston M, Martin A, Polkinghorne A, Carver S. The emergence of sarcoptic mange in Australian wildlife: an unresolved debate. Parasit Vectors. 2016;9(1):1.
3. Gakuya F, Rossi L, Ombui J, Maingi N, Muchemi G, Ogara W, Soriguer RC, Alasaad S. The curse of the prey: Sarcoptes mite molecular analysis reveals potential prey-to-predator parasitic infestation in wild animals from Masai

Mara, Kenya. Parasit Vectors. 2011;4(1):1.
4. Holt DC, Fischer K. Novel insights into an old disease: recent developments in scabies mite biology. Curr Opin Infect Dis. 2013;26(2):110-5.
5. Pence DB, Ueckermann E. Sarcoptic manage in wildlife. *Rev Sci Tech*. 2002;21(2):385-398. (Rearranged contributor's, which were initially in order of use…not alphabetized)
6. Rider SD, Morgan MS, Arlian LG. Draft genome of the scabies mite. Parasit Vectors. 2015;8(1):1.
7. Speight K, Whiteley PL, Woolfod L, Duigan PJ, Bacci B, Lathe S, Boardman W, Scheelings TF, Funnell O, Underwood G, Stevenson MA. Outbreaks of sarcoptic mange in free-ranging koala populations in Victoria ans South Australia: A case series. Aus Vet J (in press).
8. Swe PM, Reynolds SL, Fischer K. Parasitic scabies mites and associated bacteria joining forces against host complement defence. Parasit Immunol. 2014;36(11):585-93.

CASE III:18-0933b Case 1 (JPC 4125007)

Signalment:
Red kangaroo (*Osphranter rufus)*

History:
Culled female free-ranging red kangaroo examined as part of investigations into mass mortalities.

Gross Pathology:
No significant external findings

Laboratory results:
No laboratory findings reported.

Microscopic Description:
Kidney: Renal tubular epithelial cells, concentrated in the corticomedullary region and medullary rays are frequently hypertrophied, characterised by karyomegaly and cytomegaly and large cytoplasmic vacuoles. Vacuoles often contain protozoal macrogametes, sporonts, and sporozoites (parasitopherous vacuoles). Macrogametes, 6- 8 um in diameter, are most frequently observed within hypertrophied cells that bulge into the tubular lumina. Intraepithelial sporoblasts and basophilic crenated sporocysts containing closely packed sporozoites are also common. Sporonts (with circumferential nuclei) are rarely observed, intraepithelial shizonts are not observed. Epithelial cells of parasitized tubules are sometimes degenerate or attenuated, but minimal appreciable inflammation is observed.

Contributor's Morphologic Diagnoses:
Kidney: renal coccidiosis (*Klossiella* sp)

Contributor's Comment:
Protozoal parasites within the renal tubules of this kangaroo are morphologically consistent with *Klossiella* sp. This parasite is widely spread in both wild and captive Australian marsupials and is thought to be largely non-pathogenic in otherwise healthy individuals.[1-5,8]

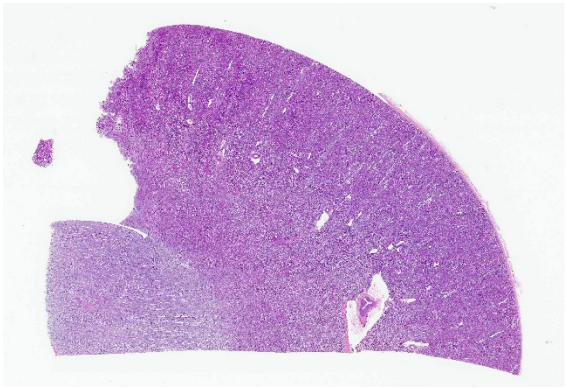

Figure 3-1. Kidney, kangaroo. A section of kidney is submitted for examination. No lesions are visible at this magnification. (HE, 5X).

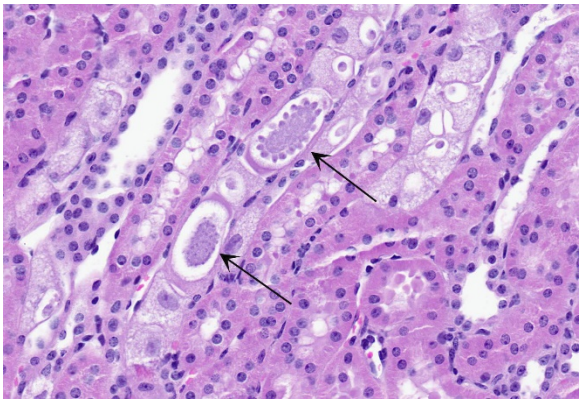

Figure 3-2. Kidney, kangaroo. Epithelial cells within collecting ducts are markedly hypertrophic. Within their vacuolated apical cytoplasm, many have parasitophorous vacuoles containing variably mature sporoblasts budding from a residual body (endopolygeny) (arrows). (HE, 400X)

Klossiella spp. are apicomplexan coccidians with a global distribution, known to infect the kidneys of mammals. Usually infecting the renal tubular epithelium, it may also be found within glomerular endothelial cells in some cases. There are 19 recognized species in this genus and additional reports without species characterization.[9] The life cycle is assumed to be direct with schizogony and sporogony occurring in the host's renal tissue, and infection likely acquired by the oral route after contamination of food and drinking water with urine containing sporocysts.[9] Species identification is based on the morphology of the different life-cycle forms and the parasitized tissues; although not definitively determined in this case, the host and parasite morphology suggest this species is most likely *K. rufi*.[3]

Contributing Institution:
The University of Adelaide, Veterinary Diagnostic Laboratory
www.adelaide.edu.au/vetsci

JPC Diagnosis:
Kidney, tubular epithelium: Hypertrophy, multifocal, severe, with numerous intra-epithelial apicomplexan sporonts and gametes, etiology consistent with *Klossiella* sp.

JPC Comment:
Apicomplexans of the genus *Klossiella* have been reported in multiple species, including mice (*K. muris*), guinea pig (*K. cobayae*), horse, zebra, jackass, burro, donkey (*K. equi*), South American opossum and mouse opossum (*K. tejarai*), Australian water rat (*K. hydromyos*), African bats (*K. killicki*), African muridae (*K. mabokensis*), snakes (*K. boyae*), birds, and six different species in Australian marsupials.[5]

Klossiella sp. are intracellular apicomplexan protists that infect renal epithelium. Following ingestion of an infective sporocyst, sporozoites are released and move to the kidney, where they enter tubular epithelial cells and become trophozoites. Trophozoites form schizonts and merozoites, and from these schizonts, gametes are thought to form. Fertilized gametes are believed to develop into sporonts, which bud to form sporoblasts. Each sporoblast undergoes successive divisions to form sporocysts that contain sporozoites. Mature infective sporocysts are surrounded by a

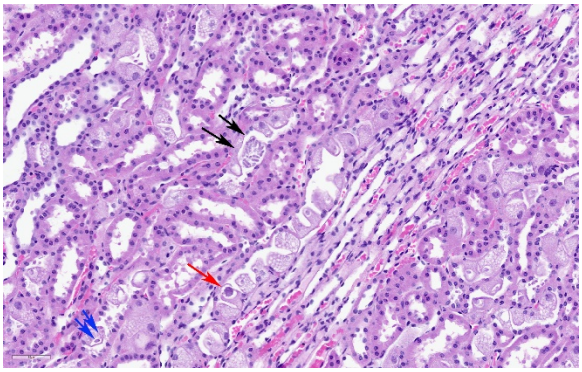

Figure 3-3. Kidney, kangaroo. Other life stages include a schizont with fully mature sporoblasts (black arrows), macrogametocytes (red arrow), and microgametocytes (blue arrows). (HE, 400X)

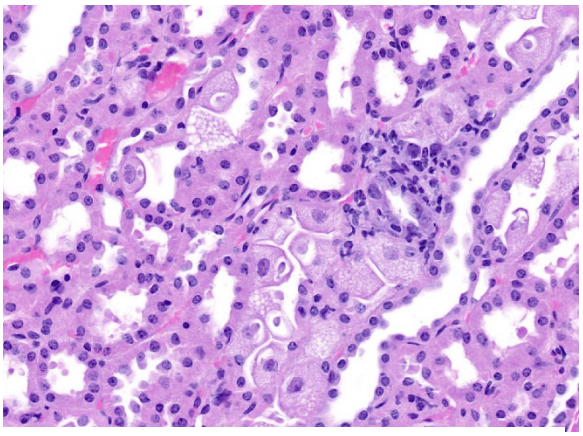

Figure 3-4. Kidney, kangaroo. There are rare regenerating tubules surrounded by few neutrophils and lymphocytes (HE, 400X)

thick wall and pass from the body into the urine to be later ingested by another host, completing the cycle.[7]

Most coccidians infect the gastrointestinal tract in their natural hosts; however, genus *Klossiella*'s renal tropism is not unique. In contrast to *Klossiella,* renal coccidiosis due to *Eimeria truncata* is known to be a highly fatal disease of geese, sometimes wiping out entire flocks within a few days. Symptomatic geese are typically extremely weak and emaciated. Grossly, kidneys of severely affected birds are greatly enlarged and discolored, corresponding with the microscopic features of enormous numbers of coccidia within tubules, which are sometimes extensively destroyed.[6] Multiple species of the genus *Eimeria* infect the avian renal tubular epithelium, including *E. somateriae* in long tailed ducks, *E. boschadis* in mallard ducks, and a novel species in great-horned owls.[7,9]

References:

1. Ardiaca M, Bennett MD, Montesinos A, Juan-Salles C, Soriano-Navarro M: Klossiella Dulcis N. Sp. (Apicomplexa: Klossiellidae) in the Kidneys of Petaurus Breviceps (Marsupialia: Petauridae). *J Zoo Wildl Med* 2016:47(2)**:**622-627.
2. Barker IK, Munday BL, Harrigan KE: Klossiella spp. in the kidneys of peramelid, petaurid, and macropodid marsupials. *Z Parasitenkd* 1975:46(1)**:**35-41.
3. Barker IK, Munday BL, Hartley WJ: Klossiella (Apicomplexa, Klossiellidae) in petaurid and macropodid marsupials in Australia. *J Protozool* 1985:32(3)**:**520-522.
4. Bennett MD, Woolford L, O'Hara AJ, Nicholls PK, Warren KS, Friend JA, et al.: Klossiella quimrensis (Apicomplexa: Klossiellidae) causes renal coccidiosis in western barred bandicoots Perameles bougainville (Marsupialia: Peramelidae) in Western Australia. *J Parasitol* 2007:93(1)**:**89-92.
5. Duncan, M. Perissodactyls. In: Terio KA, McAloose D, St. Leger J, eds. *Pathology of Wildlife and Zoo Animals*. San Diego, CA; Elsevier Inc., 2018: 450-451.
6. Farr MM, Wehr EE. Eimeria truncata associated with morbidity and death of domestic goslings. *Cornell Vet*. 1952;42(2):185-187.
7. Gardiner CH, Fayer R, Dubey JP, eds. *An Atlas of Protozoan Parasites in Animal Tissues*, 2nd ed. Armed Forces Institute of Pathology, Washington, DC, 1998:61-62.
8. Jankovsky JM, Brand M, Gerhold RW. Identification of a Novel Renal Coccidian (Apicomplexa: Eimeriidae) from the Great-Horned Owl (Bubo virginianus), USA. *J Wildl Dis*. 2017;53(2):368-371.
9. Taylor JL, Wagner JE, Kusewitt DF, Mann PC: Klossiella Parasites of Animals - Literature-Review. *Veterinary Parasitology* 1979:5(2-3)**:**137-144.
10. Wobeser G. Renal coccidiosis in mallard and pintail ducks. *J Wildl Dis*. 1974;10(3):249-255.

CASE IV:

Signalment:
Four-year-old Aberdeen Angus cow, bovine (*Bos taurus taurus*)

History:
A four-year-old Angus cow presented a clinical history of neurologic signs, which included aggressiveness and isolation from the herd, anorexia, depression and ataxia. Antibiotic treatment (doxycycline) was administered for three days; nonetheless, the clinical condition progressed, and after five days paddling movements, opisthotonus and lateral decubitus were noted. Euthanasia was elected, and the entire head was shipped to the laboratory by the field veterinarian. The referred cow was one of 300 beef cattle that were managed in a cow-calf operation in the state of Rio Grande do Sul, Brazil. The animals were kept in flooded pastures, in a paddock of irrigated rice stubble.

Gross Pathology:
Grossly, the leptomeninges covering the brain, especially the ventral surfaces of the mesencephalon and rhombencephalon presented multifocal, severely thickened dark red areas. Furthermore, the right olfactory bulb showed a soft well-demarcated grayish area of 2.5 cm in diameter. Similar areas, ranging from 0.5 to 2 cm in diameter were noted bilaterally in the piriform lobes, right hippocampus, frontal lobe cortex and fornix. The remaining organs were not available for evaluation.

Laboratory results:
Brain sections were processed for immunohistochemistry (IHC), to search for *Acanthamoeba spp., Balamuthia spp.* and *Naegleria spp.*, as previously described.[1] The amoebae stained positively for *Naegleria fowleri* through IHC, while immunostaining for *Acanthamoeba* spp. and *Balamuthia* spp. was negative.

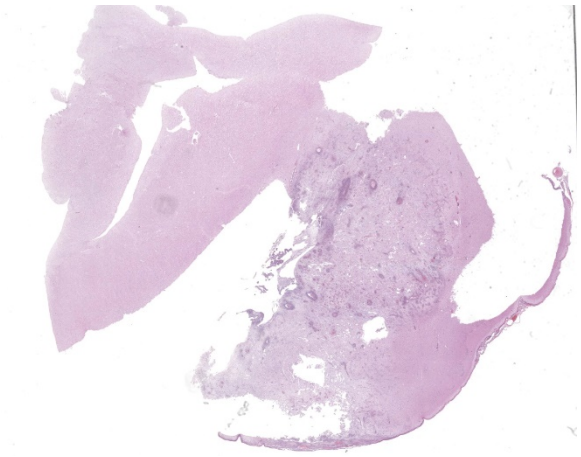

Figure 4-1. Cerebrum, ox. Multiple sections of cerebrum are submitted for examination. At subgross magnification, the section at right demonstrates extensive necrosis within the neuropil, characterized by rarefaction, loss of tissue, and marked perivascular hypercellularity (HE, 5X)

Microscopic Description:
The submitted slides present one section of brain (piriform lobe or mesencephalon), and some slides also display choroid plexus sections. In the brain, there is a focally extensive, well-demarcated area of marked necrosis, surrounded by a halo of severe inflammatory infiltrate of viable and degenerate neutrophils, fewer foamy macrophages (gitter cells), lymphocytes, plasma cells, eosinophils and rare multinucleated giant cells. The referred inflammatory cells are also noted scattered within the necrotic area, mainly in perivascular regions, markedly expanding Virchow-Robin spaces, as well as extending and moderately expanding the adjacent leptomeninges.

A large number of round to oval amoebic trophozoites measuring around 12 to 15 µm in diameter were seen within the necrotic areas, around and within blood vessels, both in the wall and in the lumen, frequently

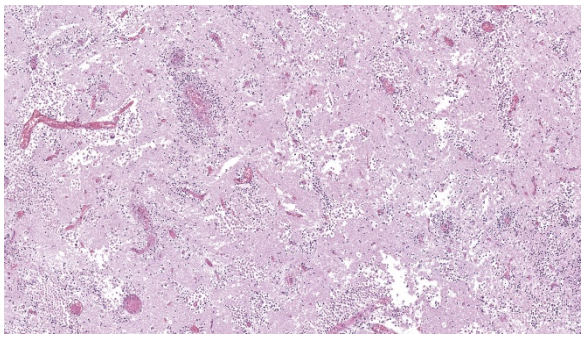

Figure 4-2. Cerebrum, ox. Higher magnification of the necrotic area demonstrating marked inflammation in areas of rarefaction as well as in perivascular areas. (HE, 88X)

forming clusters with numerous organisms. These amoebae had abundant granular and pale eosinophilic cytoplasm, eccentric nuclei measuring approximately 3 µm in diameter and had a single 1 to 2 µm hyperchromatic karyosome. The nuclei were surrounded by a clear thin halo.

Furthermore, marked multifocal fibrinoid necrosis and hyalinization of blood vessel walls, characterized by accumulation of amorphous proteinaceous material and inflammatory infiltrate of neutrophils, was noted within the necrotic area. Thrombosis was frequently observed in vessels showing fibrinoid necrosis hyalinization. Moreover, multifocal moderate inflammatory infiltrate comprised predominantly by lymphocytes, plasma cells and fewer neutrophil was noted in the neuropil adjacent to the necrotic area. Similar inflammatory infiltrate was also observed markedly and diffusely expanding the choroid plexus.

In addition to the piriform lobes, similar demarcated lesions were noted affecting both the white and grey matter of the right olfactory bulb, frontal brain cortex, thalamus, mesencephalon, and hippocampus. In the cerebellum, the leptomeninges and Virchow-Robins spaces were diffusely and markedly expanded by a similar inflammatory infiltrate described in the piriform lobe. Furthermore, multifocal areas of severe hemorrhage were seen in the neuropil, as well as multifocal areas of gliosis, which were also noted in the brainstem.

Contributor's Morphologic Diagnoses:
Necrotizing and pyogranulomatous meningoencephalitis, marked, focally extensive, acute, associated with severe multifocal thrombosis, vasculitis and numerous amoebic trophozoites. Lymphohistiocytic and neutronphilic choroid plexitis, acute, marked, diffuse.

Contributor's Comment:
Free-living ameboflagellates of the genera *Acanthamoeba*, *Balamuthia* and *Naegleria* have been associated with disease in people and animals.[1,2,4,5,6,7] *N. fowleri* infection is known to cause a rare entity called primary amoebic meningoencephalitis.[3] Most cases of this condition have been reported in people, and the majority of the cases have been described in the USA.[8] This protozoon reaches the brain through the olfactory nerve[10] causing a highly fatal disease that is restricted to the central nervous system, frequently in immunocompetent individuals.[10] *N. fowleri* is a widely distributed thermophilic free-living amoeba that is commonly found in fresh and hot water.[12] The drinking water has been suggested as a potential source of infection in previous reports of *N. fowleri* infection in cattle.[2] In the present case, amoebae were not searched in the environment and sources of water; however, it is plausible to think that the epidemiological characteristics present on the farm, including high temperatures and the use of pastures in seasonally flooded areas, may have contributed to the occurrence of the disease. After invading the nasal mucosa, *N. fowleri* is believed to reach the encephalon through the olfactory nerve, consequently causing initial damage to the rhinencephalon.[12] The initial lesion is

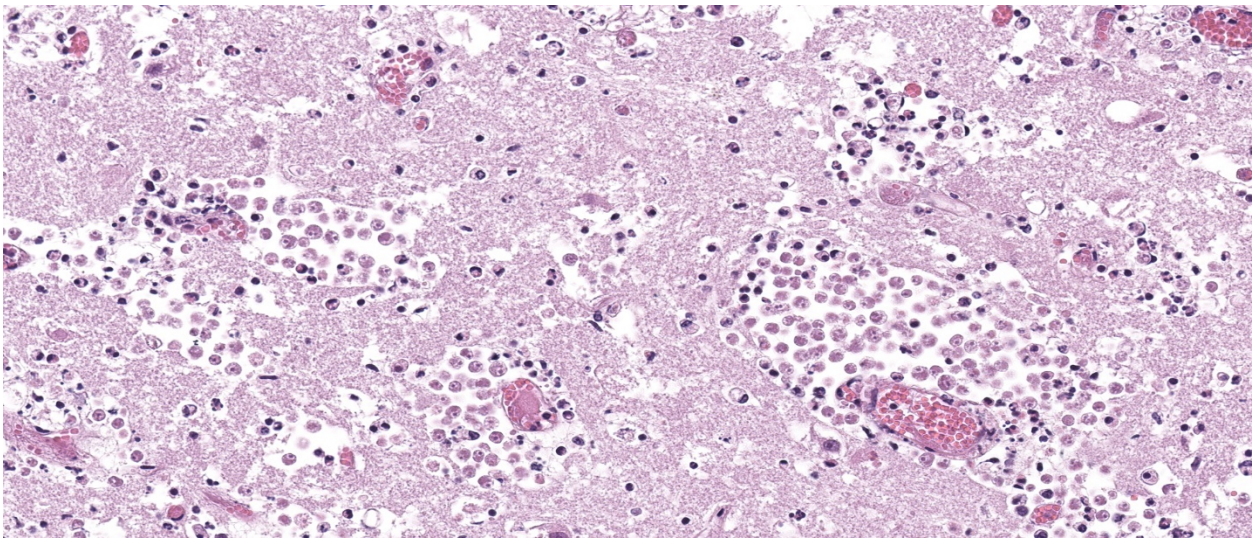

Figure 4-3. Cerebrum, ox: Large numbers of round 10-15um amebae occupy perivascular and rarefied areas within the neuropil. (HE, 250X)

frequently observed as an area of encephalic softening located in the olfactory bulb, and is most commonly unilateral.[2] After this initial lesion, *N. fowleri* tends to spread and cause disseminated lesions in the brain.[2]

Histologic findings were characterized by severe vascular lesions, including vasculitis and thrombosis. In the present case, these seemed to be caused by direct damage induced by *N. fowleri*, since great numbers of parasites were observed within the wall of blood vessel, associated with necrosis and neutrophilic inflammation. The referred vascular lesions may have preceded the marked necrotic changes observed, since the latter were interpreted to be the result of infarction, associated with direct damage induced by tissue spread of these agents. Histological visualization of amoebic trophozoites may be challenging.[2] Frequently these amoebas may resemble foamy macrophages in areas of suppurative inflammation and necrosis.[2] Thus, immunohistochemistry is crucial for establishing the diagnosis, as well as to distinguish these organisms from other amoebas, which are important differential diagnosis in such cases, including *Acanthamoeba* sp. and *Balamuthia mandrillaris.*[1]

Contributing Institution:
Faculdade de Veterinária
Universidade Federal do Rio Grande do Sul
Setor de Patologia Veterinária
http://www.ufrgs.br/patologia

JPC Diagnosis:
Cerebrum: Vasculitis and meningoencephalitis, necrotizing, multifocal, severe, with thrombosis and numerous amebic trophozoites.

JPC Comment:
The contributor provides an excellent review of *Naeglaria fowleri*, one of several mitochondria-bearing, free-living eukaryotic amebae, in addition to *Acanthamoeba* spp. and *Balamuthia mandrillaris,* each known to cause infection of the central nervous system (CNS) of humans and other animals.[14] Knowledge that free-living amoebae are capable of causing human disease dates back more than 50 years, prior to which time they were regarded as harmless soil organisms or, at most, commensals of mammals.[12] In humans, several species of the genus

Acanthamoeba cause an insidious and chronic disease, granulomatous amebic encephalitis (GAE), principally in immunocompromised hosts, including those infected with HIV/AIDS. *Balamuthia mandrillaris* causes GAE in both immunocompromised and immune-competent hosts, mostly in very young or very old individuals. In addition, both etiologies may cause disseminated disease in the lungs, skin, kidneys, and/or uterus. In contrast, *N. fowleri* causes an acute and fulminating, necrotizing infection of the CNS known as primary amebic meningo-encephalitis (PAM). Children and young adults with history of exposure to warm fresh water are most often affected by PAM.[14]

As described by the contributor, *N. fowleri*'s route of infection is via direct penetration of the olfactory neuroepithelium via the rhinocerebral route. Upon reaching the olfactory bulb, *N. fowleri* elicits a significant immune response through activation of the innate immune system, including macrophages and neutrophils. The parasite enters the body in the trophozoite form, which has special structures known as food cups. These structures allow the normally free-living organism to ingest bacteria and fungi, but also tissue of parasitized hosts. In addition, the pathogenicity of *N. fowleri* is dependent upon the release of cytolytic molecules, including acid hydrolases, phospholipases, neuraminidases, and phospholipolytic enzymes which contribute toward host cell and nerve destruction. The combination of the pathogenicity of *N. fowleri* and the intense immune response resulting from its presence results in significant nerve damage and subsequent CNS tissue damage, which often results in death.[3]

Other free-living amoeba may also gain entry to the brain via the rhinocerebral route, however, hematogenous spread following primary lung or skin infection is thought to be more common. An example of this was suggested following a retrospective review of five cases of amoebic meningoencephalitis in gorillas and other old world primates at the San Diego Zoo and San Diego Wild Animal Park between 1965 and 1995 caused by *B. mandrillaris*. Although the route of infection was unknown, the lack of olfactory lobe

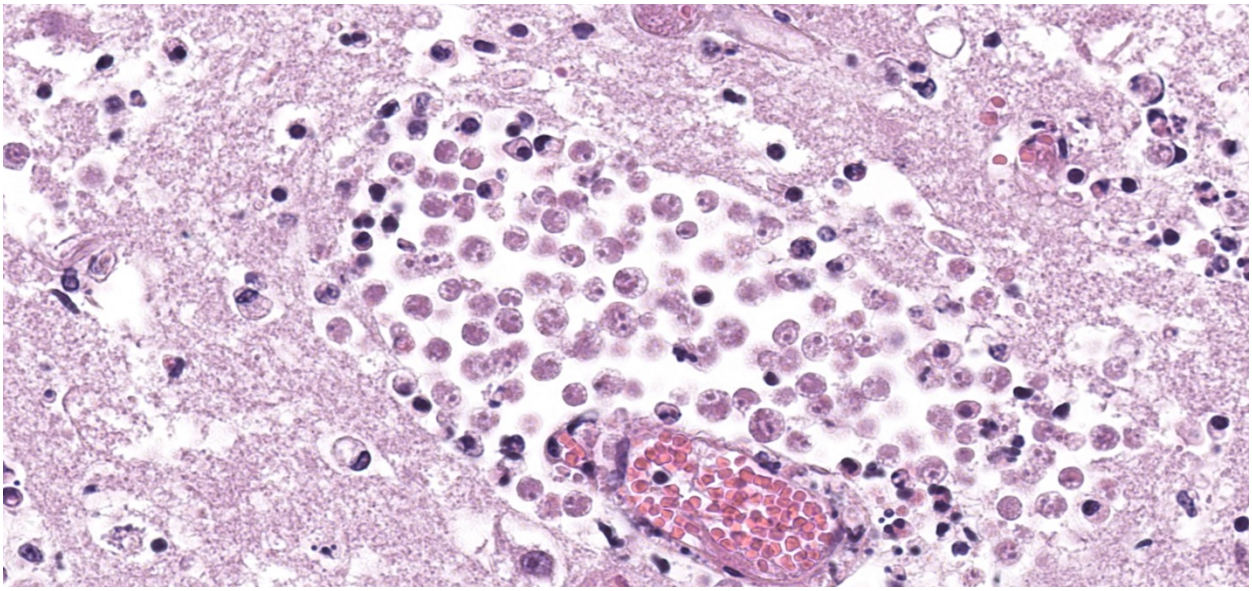

Figure 4-4. Cerebrum, ox: Higher magnification of the amebae admixed with few viable and necrotic neutrophils. The karyosome is prominent in these amebae. (HE, 400X).

involvement was inconsistent with the rhinocerebral route of infection.[9]

Another significant genus warranting discussion is *Entamoeba*. Species of this genus inhabit a range of invertebrates and vertebrates, often as commensals in the intestinal tract and less commonly as pathogens. Pathogenic species have an arsenal of virulence factors to enable tissue invasion, including lectins for attachment to intestinal epithelium, mechanisms to cause cytotoxicity of epithelial cells (including induction of apoptosis), secretion of "amoebapore" proteins to lyse cells, a vast array of cysteine proteinases to degrade extracellular matrix, oxygen detoxification mechanisms to allow deep invasion, and finally, various tools to evade the host immune response. *E. histolytica*, one of the most well-known species of this genus, causes serious disease in primates and is the etiologic agent of human amoebic dysentery, a significant cause of morbidity and mortality in the third world. Another well-known species is *E. invadens*, which variably affects captive reptiles. In snakes it may be either innocuous or cause severe necrotizing enterohepatitis whereas in turtles it is generally a commensal. Finally, *E. ranarum,* is rarely associated with intestinal or hepatic disease in captive amphibians.[10]

Although amebae were typically readily visible with standard HE staining, periodic acid–Schiff reaction (PAS), readily stains the glycogen-laden cytoplasm and can be a useful tool for their identification.[10]

References:

1. Benterki MS, Ayachi A, Bennoune O, Régoudis E, Pélandakis M. Meningoencephalitis due to the amoeboflagellate *Naegleria fowleri* in ruminants in Algeria. *Parasite* 2016; 23(11): 1-4.
2. Daft BM, Visvesvara GS, Read DH, Kinde H, Uzal FA, Manzer MD. Seasonal meningoencephalitis in Holstein cattle caused by *Naegleria fowleri*. *J Vet Diagn Invest* 2005; 17:605-609.
3. Grace E, Asbill S, Virga K. Naegleria fowleri: pathogenesis, diagnosis, and treatment options. *Antimicrob Agents Chemother*. 2015;59(11):6677-6681.
4. Jonckheere JFD. Origin and evolution of the worldwide distributed pathogenic amoeboflagellate *Naegleria fowleri*. *Infect Genet Evol* 2011; 11: 1520-28.
5. Lozano-Alarcón F, Bradley GA, Houser BS, Visvesvara GS. Primary amebic meningoencephalitis due to *Naegleria fowleri* in a South American tapir. *Vet Pathol* 1997; 34(3):239-43.
6. Martinez AJ, Visvesvara GS. Free-living, amphizoic and opportunistic amebas. *Brain Pathol* 1997; 7:583-598.
7. Morales JA, Chaves AJ, Visvesvara GS, Dubey JP. *Naegleria fowleri*-associated encephalitis in a cow from Costa Rica. *Vet Parasitol* 2006; 139:221-223.
8. Pimentel LA, Dantas AFM, Uzal F, Riet-Correa F. Meningoencephalitis caused by *Naegleria fowleri* in cattle of northeast Brazil. *Res Vet Sci* 2012; 93:811-812.
9. Rideout BA, Gardiner CH, Stalis IH, Zuba JR, Hadfield T, Visvesvara GS. Fatal infections with Balamuthia mandrillaris (a free-living amoeba) in gorillas and other Old World primates. *Vet Pathol*. 1997;34(1):15-22.
10. Schuster FL, Visvesvara GS. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. *Intern J Parasitol* 2004; 34:1001-1027.
11. Shilton CM, Šlapeta J, Shine R, Brown GP. Pathology Associated With an Outbreak of Entamoebiasis in Wild Cane Toads (*Rhinella marina*) in Tropical Australia. *Vet Pathol*. 2019;56(6):921-931.

12. Schuster FL, Visvesvara GS. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. *Int J Parasitol*. 2004;34(9):1001-1027.
13. Siddiqui R, Ali IKM, Cope JR, Khan NA. Biology and pathogenesis of *Naegleria fowleri*. *Acta Trop* 2016; 164: 375-394.
14. Visvesvara GS. Infections with free-living amebae. *Handb Clin Neurol*. 2013;114:153-168.