Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

C o n f e r e n c e 9

17 November 2021

CASE I: 576/20 (JPC 4161127)

Signalment:
3 year old, female, dog, German shepherd (*Canis lupus familiaris*)

History:
The animal began to move with difficulty two months before death. Neurological and CT examination raised the suspicion of the cauda equine syndrome. The animal underwent surgery that, like postoperative recovery, went smoothly. Two weeks after the surgery, the animal again began to move with difficulties and uncoordinated. Clinical examination revealed pharyngitis, rhinitis, chorioretinitis, and partial retinal ablation. Several differential diagnoses have been proposed that included neoplasia, bacteremia, fungal infection, autoimmune diseases and vascular anomalies (hypertension, vasculitis). Steroid therapy has been started but the animal developed convulsive seizures after two days and due to the deterioration of the condition, the animal was euthanized at the owner request.

Gross Pathology:
The necropsy revealed severe bilateral granulomatous nephritis. Multiple splenic nodules interpreted as granulomas or abscesses were found. In the lungs, diffuse congestion, edema, marginal emphysema and multifocal to coalescing hemorrhages. In heart, eccentric hypertrophy of the left ventricle and multifocal epicardial hemorrhages were noted. In the liver and brain, severe congestion were present.

Laboratory results:
E. coli and *Cryptococcus sp*. were isolated by postmortem microbiological examination of kidney samples. Mucicarmine special staining reveal positive staining of yeasts with narrow based budding.

Microscopic Description:
Kidney: Multifocal to coalescing randomly distributed, renal tissue (cortex, medulla, and papilla) and perirenal adipose tissue is infiltrated by a myriad of yeasts admixed

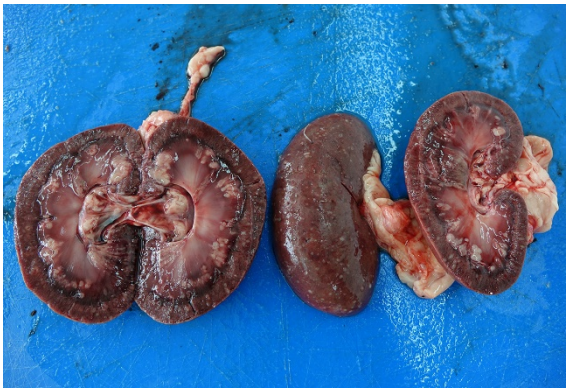

Figure 1-1. Kidney, dog. Numerous granulomas are scattered throughout the cortex and medulla but are concentrated at the corticomedullary junction. (Photo courtesy of Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55 , 10000 Zagreb Croatia. http://www.vef.unizg.hr/)

with numerous neutrophils, macrophages and a variable number of lymphocytes and plasma cells. The inflammatory cells and yeast along with cellular debris surround the remaining vital tubular epithelial and interstitial cells. The described yeasts are oval to round, 5-10 µm in diameter, with a thin wall (1-2 µm) and are surrounded by clear tick capsule (10-20 µm). Rarely, narrow-based budding of the yeast is visible. Multifocal, predominantly in the areas of pronounced lymphoplasmacytic infiltration, proliferation of fibroblasts is present (granulation tissue). Tubular epithelial and glomerular cells multifocally (predominantly in distal tubules) exhibit signs of degeneration and necrosis. Renal tubules and periglomerular spaces are filled with highly proteinaceous fluid. The renal cortex and to a lesser extent, the medulla are diffusely congested and edematous with multifocal hemorrhage.

Contributor's Morphologic Diagnoses:
Kidney: Nephritis, pyogranulomatous and lymphoplasmacytic, multifocal to coalescing, severe, with numerous yeasts (morphology of the yeasts is consistent with *Cryptococcus spp*.).

Contributor's Comment:
The presented slide is a sample from a case of disseminated systemic cryptococcosis with macroscopic and histological pyogranulomatous inflamation in the kidneys, lungs, liver, pancreas, spleen, heart, intestine, eye, brain, spinal cord and lymph nodes.

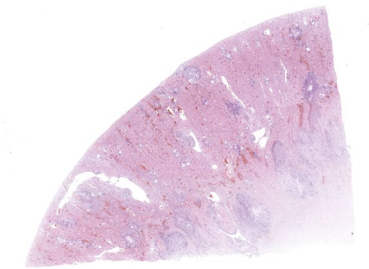

Figure 1-2. Kidney, dog. A wedge-shaped section of demonstrates multiple areas of hypercellularity in the cortex and medulla – inflammation is most prominent at the corticomedullary junctions. (HE, 6x)

Cryptococcus has a worldwide distribution and can affect many animal species and humans. Systemic mycosis is rare and more commonly affects cats than dogs.[1,2] Cryptococcosis in dogs and cats is caused by *Cryptococcus neoformans* and *Cryptococcus gattii,* which are dimorphic, encapsulated, basidiomycetous fungi.[11,12] *Cryptococcus neoformans* is usual cause of disease in temperate climates and *C. gatti* was found mainly in tropical climates, but is now considered to have a global distribution. *C. neoformans* and *C. gatti* exist in soil, pigeon or other avian guano, and decaying organic matter in a filamentous form known as a teleomorph, with the name *Filobasidiella neoformans* and *F. bacillosporus*, respectively; this form of the organism undergoes both sexual as well as asexual reproduction in the environment. Dogs are primarily infected with *C. neoformans* (except in regions with high endemicity for *C. gatti*, such as the Pacific Northwest of North America), whereas *C. gatti* is more frequent in cats.[2]

Immune suppression, such as from corticosteroid therapy or pre-existing infections, often underlies *C. neoformans* infections, but *C. gatti* is a primary pathogen and may infect hosts without known immune deficiencies. Consequently, in cats, the infection frequently occurs in animals that are not immunosuppressed, and feline immunodeficiency virus infection does not worsen the prognosis.[1, 2]

The main virulence factors of *Cryptococcus* are the capsule and the production of melanin. The thick capsule, composed of glucuronoxylomannan and other mannose-rich polysaccharides, impairs phagocytosis, activates complement, and may suppress T-

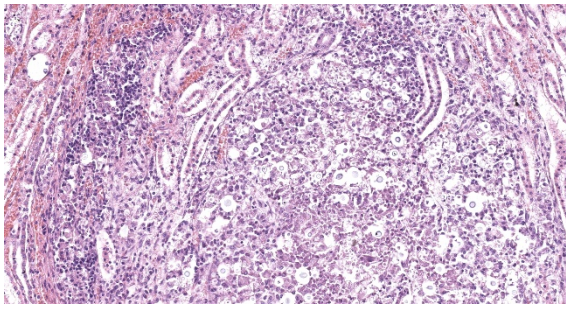

Figure 1-3. Kidney, dog. Inflammatory foci efface tubules and are composed pf large numbers of macrophages surrounding and engulfing encapsulated yeasts. (HE, 200X)

cell responses. The role of the capsule in pathogenicity is visible in rare *Cryptococcus* strains without capsule that provoke severe granulomatous reaction and are minimally pathogenic. The ability to synthesize melanin and enzyme phenoloxidase (laccase) is important for scavenge oxygen radicals produced by macrophages. Cryptococcal yeasts also secrete eicosanoids and mannose protein that modulate immune and inflammatory responses, and production of superoxide dismutase.[2] In addition to the fact that in most animals cryptococcosis is more common in immunosuppression, *Cryptococcus neoformans* and *gatti* themselves can cause severe immune-suppression.[9] The cryptococcal capsular polysaccharide, glucuronoxylomannan effectively inhibits phagocytosis and interferes with migration of leukocytes from the bloodstream into tissues by causing them to shed selectin. It can also deplete complement and directly inhibits T-cell responses.[8, 15] There is a shift from a Th1 to a Th2 immune response, the Th1 response being normally required for organism clearance. The cryptococcal urease enzyme was shown to promote accumulation of immature dendritic cells within the lung, and an associated shift in the immune response to a non-protective Th2-cytokine dominated response.[8] Immunity to *Cryptococcus* is dependent on delayed-type hypersensitivity reactions. IFN-γ and other cytokines elicit and activate macrophages and perhaps neutrophils to kill the yeast by using reactive nitrogen and oxygen intermediates. Cytotoxic T-cells directly limit viability or proliferation of this pathogen.[2] Recent data indicate that presence of testosterone in men, but not β-estradiol in women, may influence capsule growth and reduce phagocytosis of yeast by macrophages.[1] The infection usually occurs by inhalation of basidiospores or desiccated yeast from contaminated dust. Yeasts are replicated into alveoli and are subsequently spread, hematogenously (via macrophages) or locally to other organs (brain, eyes, lymph nodes, skin etc.). Cutaneous cryptococcosis is probably the result of local innoculation and cryptococcal mastitis is the consequence of ascending infection.[2, 10]

Most infected animals do not develop clinical disease. The disease differs between species; in cats it affects mostly the respiratory tract (rhinitis, pneumonia) wheras in dogs the disease is more disseminated with central nervous system or ocular involvement.[2,6] Due to inhalatory route of infection, the lungs and nasal cavity are the most commonly affected organs. Many cases are characterized by chronic nasal disease, with sneezing and serous or mucopurulent discharge. Facial swelling is a common feature of cryptococcal rhinitis of cats. Infection may spread locally from the nasal cavity to involve the skin, oral mucosa, paranasal sinuses, or brain, meninges (through the ethmoid bone, via the cranial nerves or hematogenous spreeding) and eyes.[2,11] Less commonly disease has a systemic form with more variable clinical manifestations and this form is more common in dogs. Wider systemic dissemination usually affects the lymph nodes, lung, and distant visceral organs such as liver, spleen, urinary and gastrointestinal tract.

Macroscopic lesions are most commonly in the form of gelatinous masses, granulomas, ulcerating nodules, or depositions of viscous gelatinous exudate on the surfaces of serosal or meningeal membranes. Skin lesions are often nodular and ulcerative. Visceral lesions consist of multifocal discrete white gelatinous lesions. Gross lesions in the brain are often subtle, but may include gelatinous material in meninges and ventricles.[2, 3, 5, 7, 11, 13, 14, 16]

Histologically, the lesion is remarkable for the large numbers of yeast with abundant nonstaining capsular material that gives a "soap bubble" appearance to the lesion. The inflammatory, mostly granulomatous, reaction is often mild probably due to masking effect of capsule that protect yeasts from phagocytosis. *C. neoformans* yeast bodies are 4-8 µm diameter, plus a capsule that varies from 1-30 µm. Occasional yeast have single buds that are attached by a thin stalk; this narrow-based budding differentiates *Cryptococcus* from *Blastomyces*. Special staining methods, such as periodic acid-Schiff (PAS) and Gomori's methamine silver, demonstrate the organisms, and the capsule can be stained with mucicarmine and alcian blue stain. The inflammatory response is consistent of neutrophils, macrophages, multinucleate giant cells, lymphocytes, plasma cells and eosinophils, which is dependent upon host immune status.[2,16]

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JPC Diagnosis:
Kidney: Nephritis, granulomatous, multifocal, marked, with numerous extracellular and intrahistiocytic yeasts, etiology consistent with *Cryptococcus* sp.

JPC Comment:
The contributor provides an excellent review of *Cryptococcus neoformans* and *C. gatti*, two serious fungal pathogens within phylum Basidiomycota. The range of species susceptible to cryptococcosis is extraordinarily diverse and includes single-celled *Acanthamoeba*, terrestrial and marine mammal wildlife, small companion animals, horses, production animals, and humans.[4]

This entity was first described as a human pathogen in 1894 by pathologist Otto Busse and surgeon Abraham Buschke when they isolated a '*Saccharomyces-like*' organism from a bone infection in a young woman. In 1901, the organism was renamed *Cryptococcus neoformans* because it did not produce ascospores, a defining characteristic of the genus *Saccharomyces.*[9]

The epidemiology of cryptococcosis in humans has shifted dramatically over the last century. Fewer than 300 cases were reported worldwide during the 1950s. This number dramatically increased in the following years due to the increase in the numbers of patients with AIDS or other states of immune compromise. A 2006 study estimated there were over one million annual cases of

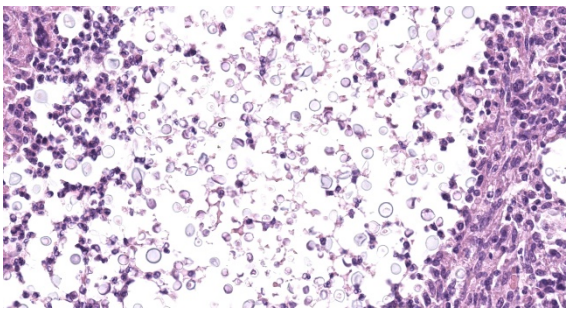

Figure 1-4. Kidney, dog. High magnification of Cryptococcus sp. yeasts. (HE, 400X)

cryptococcal meningitis worldwide, predominantly within sub-Saharan Africa and other developing regions where treatment is inhibited by lack of infrastructure and cost. Over half these cases result in patient death, yielding fatalities comparable to both tuberculosis and diarrheal disease in these regions.[9]

Historically, pathogenic *Cryptococcus* isolates were treated as a single species, *C. neoformans*. Heterogeneity between isolates within this species was suspected based on the recognition for four serotypes (A, B, C, and D). This was subsequently confirmed by the discovery of two different teleomorphs, *Filobasidiella neoformans* (*Cryptococcus neoformans*) and *Filobasidiella bacillospora* (*Cryptococcus gattii*). This division was further verified by whole-genome sequencing. In 2002, serotypes B and C were formally reclassified as *C. gatti*, while *C. neoformans* included all serotype A, AD, and D strains. It is possible additional species will be identified given molecular typing methods have demonstrated multiple genetically diverse monophyletic clades within both species.[4]

As noted by the contributor, *Cryptococcus neoformans* occurs worldwide and is associated with avian excreta protected by light and is the predominant cause of human and animal infections. This pathogen is often associated with pigeon guano, however, birds are unlikely to be a reservoir given 0 to 2.2% of samples taken from the feet, crop, and cloacal swabs of Colombiforms and water birds in multiple studies were positive for the pathogen. Instead, it is most likely *C. neoformans* propagates in the nitrogen-rich guano where they grow, rather than being transferred via an avian alimentary tract. In addition, the core body temperature of birds may be as high as 42-44ºC, which inhibits crytptococcal growth and multiplication.

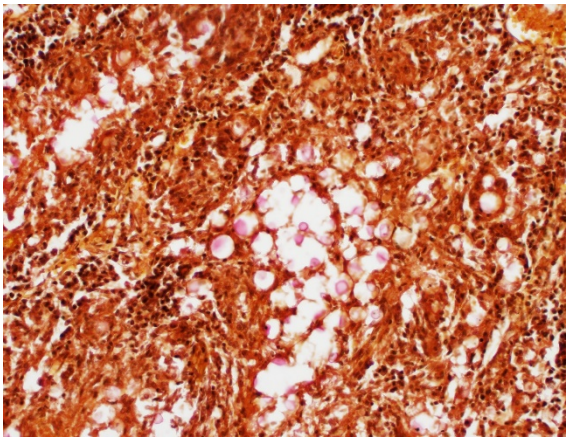

Figure 1-5. Kidney, dog. Cryptococcal yeasts stain positively with mucicarmine. The yeasts stain due to retraction of the capsule during processing. (Mucicarmine, 400X)

Interestingly, *C. neoformans* can complete its lifecycle, including mating, on pigeon guano whereas *C. gatti* does not. *Cryptococcus gatti* has historically been found in tropical and subtropical regions and is associated with various species of trees, notably eucalyptus, in addition to carob and olive trees within the Mediterranean basin.[4,9]

Given both koalas and *C. gatti* share an intimate association with a common environmental niche (i.e. eucalyptus trees), it is not surprising the former has a comparatively high, if not the highest prevalence of cryptococcosis in comparison to other species, with approximately 3% of koala necropsies being attributed to the pathogen. Cryptococcosis occurs in both captive and free-ranging koalas, most commonly affecting the upper and/or lower respiratory tract and spreading to regional lymph nodes in 20% of these cases without further tissue involvement. However, approximately a third of all cases in this species are disseminated, often following a longstanding respiratory illness. Neurotropism is common, as in other species, with 32% of cases in koalas having CNS involvement.[4]

Marine mammals, including delphinids, porpoises, pinnipeds such as harbor seals and sea lions, and baleen whales such as the

southern right whale are also susceptible hosts for various mycoses including cryptococcosis. Porpoises and dolphins are the most commonly infected marine mammals. These animals are likely at risk for infection when close to land and exposed to infective propagules (likely spores) in effluent and runoff that has washed into the ocean. Those with blow holes are particularly vulnerable, given they inhale an enormous tidal volume with marked inspiratory effort following an explosive expiration, without the benefit of filtration of inspired air via sinonasal protective mechanisms. As a result, large inoculums of inspective spores can be carried deep into the lower respiratory tract.[4]

Since around 2000, cryptococcosis has emerged as a cause of disease in both marine mammals and humans, particularly in the vicinity of Vancouver Island and the Pacific Northwest of the United States and Canada. Wordwide, *C. gatti* has been identified in 45/54 cases of symtomatic cryptococcosis, followed by *C. neoformans* in five animals, and one case of the less pathogenic *C. albidus* in a stranded California sea lion. The *Cryptococcus* species was not identified in the remaining three cases. A common finding in all marine mammal cases was tropism for the lungs, lymph nodes, and gastrointestinal tract, with the lung as the logical portal of entry for infection. Similar to other species, extension of the fungus to the brain has also been reported.[4]

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CASE II: 19-0830-64 (JPC 4161168)

Signalment:
Adult (3-year old) limousine female sheep (*Ovis aries*)

History:
A ewe from a flock of 300 meat sheep and 70 dairy sheep was presented for postmortem examination. Several animals (ewes and rams) were introduced into the flock during the previous years and months. The sheep were had been raised exclusively outdoors, in harvested fields and woods.

Over the past few months, 6 ewes developed similar symptoms, with chronic and progressive dyspnea, nasal discharge, characteristic stertorous breathing and increased appetite for salt. Progressive cachexia led to euthanasia. Three of these animals were necropsied and similar lesions were found.

Gross Pathology:
The animal was cachectic. The main gross lesion was a bilateral mass in the ethmoid region and slight protrusion into the nasopharynx. The mass was exophytic, about 6 cm wide, rather firm, with a lumpy, wet surface focally covered with fibrin. The diaphragm was thickened (hypertrophy), secondary to obstruction of the respiratory tract.

Laboratory results: No laboratory findings reported.

Microscopic Description:
The submitted sample is neoplastic tissue with no normal structures. The tissue is grossly lobulated, composed of a tubulopapillar proliferation of rather monomorphous epithelial tumor cells. Tumor cells are medium-sized (25 µm wide), cuboidal when forming tubules and more prismatic or pseudostratified in papillary structures. Intercellular junctions are inapparent. Cytoplasm is abundant and eosinophilic, with small PAS-positive and Giemsa stain-metachromatic apical secretory granules (mucins). Nuclei are round, large,

Figure 2-1. Presentation, sheep. The sheep is dyspneic with nasal discharge and drooling. (Photo courtesy of: Département des Sciences Biologiques et Pharmaceutiques, Ecole Nationale Vétérinaire d'Alfort, 7 avenue du Général De Gaulle, 94704 Maisons Alfort Cedex, www.vet-alfort.fr)

generally in a basal position, with a small nucleolus. Anisokaryosis is moderate. Mitotic figures are rare. Some mucin admixed with cell debris is present within glandular lumens. The stroma is scarce, focally infiltrated by lymphocytes and plasma cells. Small necrotic foci are present (more obvious in one of the submitted sections). No emboli could be observed.

Contributor's Morphologic Diagnoses:
Enzootic nasal tumor.

Contributor's Comment:
Although normal nasal mucosa is not present on slides, the presence of a tubulopapillary adenocarcinoma with mucin secretion in a sheep is suggestive of enzootic nasal tumors (ENTs). ENTs are transmissible epithelial tumors of sheep and goats, geographically widespread, except for Australia, New Zealand and United Kingdom[8], with an incidence in affected flocks of 0.1 to 15%.[1, 8] Adults and lambs older than 6 months can be affected.

The tumors arise from the ethmoid turbinates, fill the caudal nasal cavity and can be unilateral to bilateral.[1] Their growth is compressive, leading to deviation of the nasal septum and facial deformity.[8] The duration of disease varies from 3 weeks to more than 1 year.[7] In goats, edematous inflammatory polyps can grow proximally to the tumor and protrude from the nostrils.[8] Those polyps can be more obvious than the tumor itself at necropsy and should not be misinterpreted.

Histologically, the tumor is a well-differentiated glandular tissue, with tubular, papillary and acinar arrangements made of cuboidal to pseudostratified, non-ciliated, epithelial cells. The fibro-vascular stroma is scant and variably infiltrated by lymphocytes. The tumor is histologically similar in sheep and goats, and considered as adenoma or as low-grade adenocarcinoma.[8] Metastases are not reported.[8]

ENTs are caused by species specific retroviruses: ovine ENTV-1 and caprine ENTV-2. Both are closely related to the Jaagsiekte sheep retrovirus (agent of the ovine pulmonary adenocarcinoma) and to sheep endogenous retroviruses.[2, 6] These are examples of retrovirus-induced epithelial tumors. Recently, a case of nasal adenocarcinoma associated with Jaagsiekte sheep retrovirus has been described in a sheep.[4]

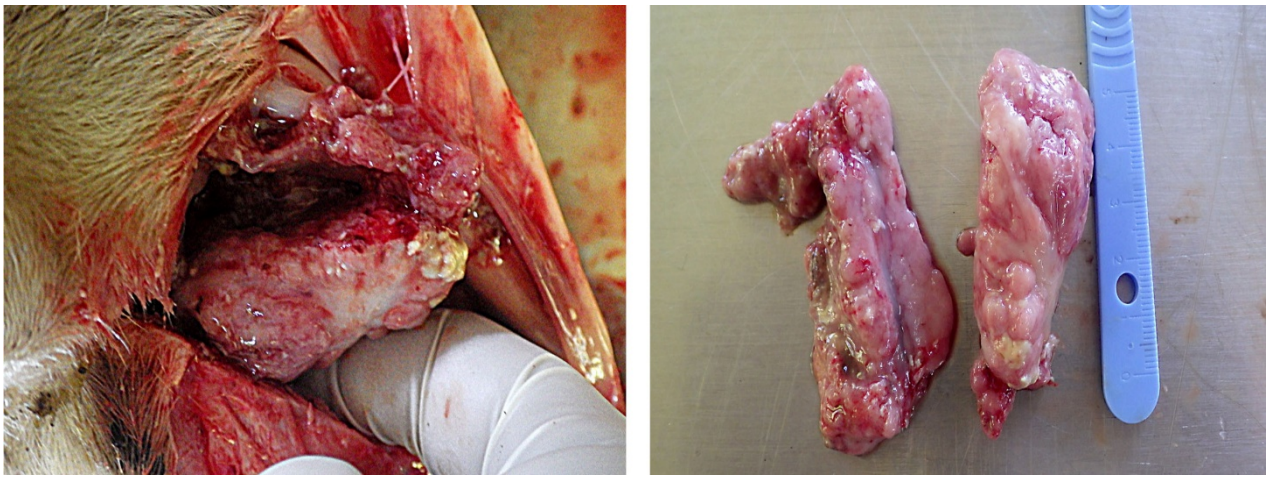

Figure 2-2. Respiratory mucosa, sheep. There is a 6cm bilateral exophytic mass within the in the ethmoid region that is covered with fibrin. (Photo courtesy of: Département des Sciences Biologiques et Pharmaceutiques, Ecole Nationale Vétérinaire d'Alfort, 7 avenue du Général De Gaulle, 94704 Maisons Alfort Cedex, www.vet-alfort.fr)

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JPC Diagnosis:
Nasal cavity: Respiratory adenocarcinoma.

JPC Comment:
Peyton Rous successfully transmitted a tumor from a hen to a chicken in 1909 via injection of cell-free extracts, one of the first indications of the association between viruses and neoplasia. This discovery served as a starting point for additional discoveries over the following decades in the field of virology.[5]

ENTV-1, ENTV-2, and jaagsiekte sheep retrovirus (JSRV), are oncogenic beta retroviruses. These enveloped RNA viruses depend on DNA-polymerase for their replication. In order to achieve this, the viral DNA or provirus integrates into the cellular genome during the early steps of the viral cycle and remain in the host DNA. Other oncogenic retroviruses include bovine leukemia virus, feline leukemia virus, avian leucosis virus (chickens), Rous sarcoma virus (chickens), walleye dermal sarcoma virus, mouse mammary tumor virus, murine leukemia virus, koala retrovirus B, and human T-lymphotropic virus.[5]

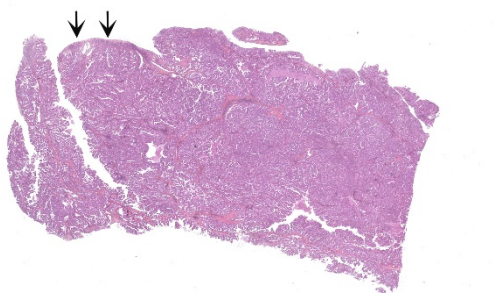

Figure 2-3. Nasal cavity, sheep. There is an exophytic epithelial neoplasm which is partially covered by respiratory epithelium (arrows). (HE, 60X)

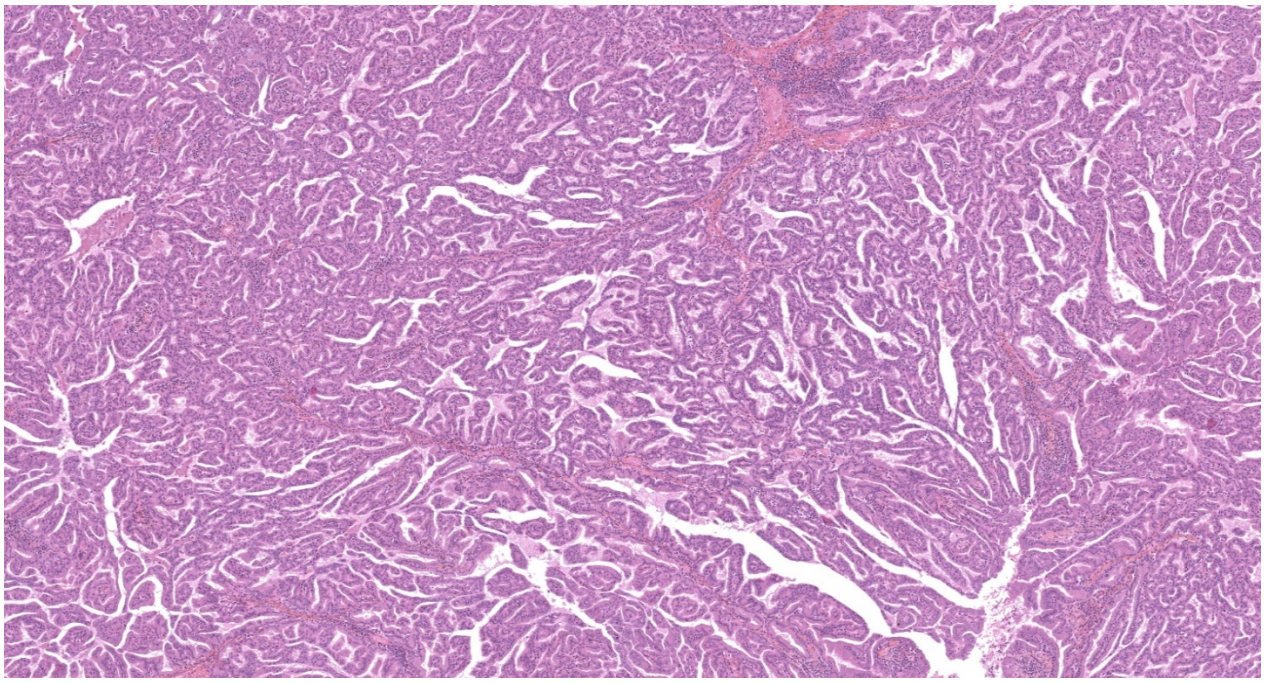

Figure 2-4. Nasal cavity, sheep. The neoplasm is composed of neoplastic epithelium that forms contiguous acini with occasionally papillary projections. (HE, 125X)

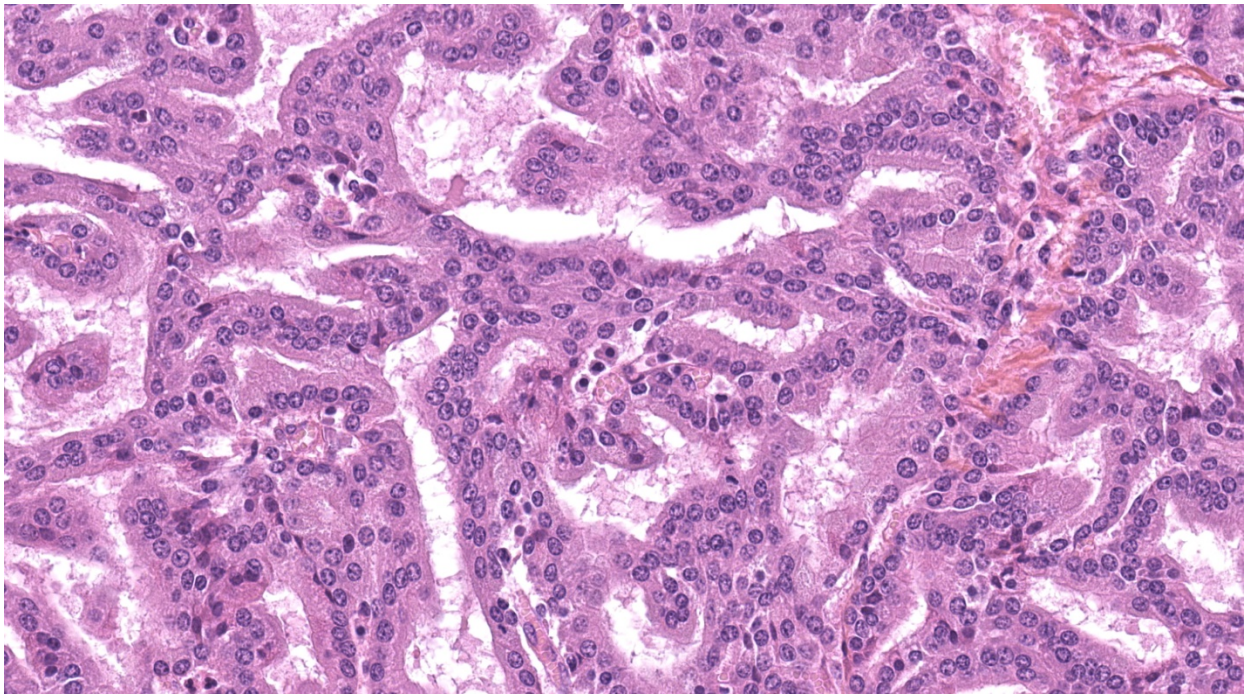

Figure 2-5. Nasal cavity, sheep. High magnification of ciliated neoplastic epithelium. (HE, 460X)

The respiratory route of transmission of oncogenic retroviruses in small ruminants was reported as early as 1934. In addition, JRSV can infect animals very early in life, with virus detectable at birth, suggesting in utero transmission. JRSV has also been detected in colostrum, which suggests the virus can be spread to newborns via colostrum and milk. The incubation period in naturally infected animals range from months to two to four years. This may vary depending on the type of infection, experimental inoculation having a shorter incubation period in comparison to spontaneous infection.[5]

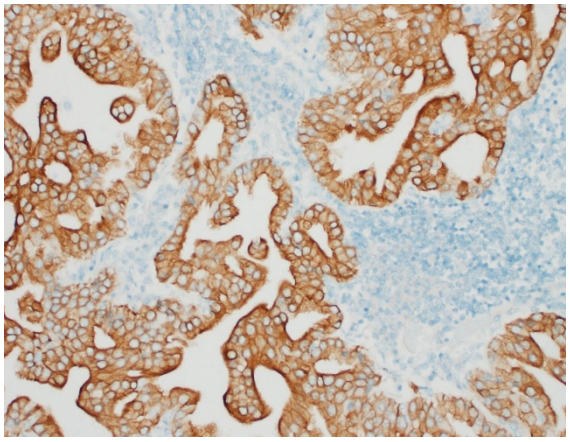

Figure 2-6. Nasal cavity, sheep. Neoplastic cells demonstrate strong cytoplasmic immunoreactivity for cytokeratin. (anti-AEI/AE3, 400X)

As noted by the contributor, a case of an ovine nasal adenocarcinoma associated with JSRV was recently identified in an 8 year old Belclare ewe in Ireland. The neoplasm had gross and microscopic features and immune-histochemistry results consistent with ENTV-1. However, differential PCR using primers specific to regions of divergent sequences between the viruses produced results negative for ENTV-1 and positive for JSRV. This was a significant finding, particularly given JSRV is endemic in sheep in the British Isles, whereas ENTV-1 has not been reported. Therefore, PCR in combination with immunohistochemistry is necessary to reach an accurate etiologic diagnosis, which is of significant importance in regions currently free of ENTV-1, such as the British Isles, Australia, and New Zealand.[4]

In addition to the aforementioned retroviral induced tumors, transmissible tumors affecting the paranasal sinuses of Rocky Mountain bighorn sheep have also been described. These tumors were previously hypothesized to be caused by ENTV-1, JSRV, or a closely related oncogenic retrovirus. However, screening via PCR and IHC of naturally occurring bighorn sheep sinus tumors were negative for these viruses and virus particles were not detected by electron microscopy in nasal secretions or cell cultures of sinus tumor tissues. Similar to ENTV-1, metastasis has not been reported with this entity. Transmissibility of the tumors was confirmed in 2016 after researchers inoculated tumor material and associated exudates from a naturally occurring sinus tumor into four Rocky Mountain Bighorn sheep lambs and four domestic lambs. Within 18 months of inoculation, all four inoculated domestic sheep (100%) and one of the four inoculated bighorn sheep (25%) developed tumors within the ethmoid sinuses or nasal conchae. The low transmission of sinus tumors to bighorns as compared to domestic sheep may suggest species differences in susceptibility to this disease. Therefore, measures to prevent transmission to domestic sheep may be warranted, including exclusion of domestic sheep from the natural range of bighorn sheep.[3]

There was spirited discussion amongst conference attendees in regard the classification of this neoplasm as a carcinoma rather than an adenocarcinoma due to the presence of neoplastic ciliated respiratory epithelial cells. Glandular epithelium is not typically ciliated and therefore "carcinoma" may be considered by some to be the more appropriate diagnosis in this case.

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CASE III: 18-7537 (JPC 4135935)

Signalment:
3 year old spayed female, Boxer cross dog (Canis lupus familiaris)

History:
The dog lost the ability to bark twelve months prior to being referred to the Western College of Veterinary Medicine Veterinary Medical Centre for further investigation of a previously identified laryngeal or tracheal mass. On physical examination the dog was BAR but a marked abdominal component was observed with inspiration. The dog was eating and drinking normally without any episodes of vomiting or regurgitation. The dog had experienced respiratory dyspnea with excitement and episodes of tonic-clonic seizures during these episodes. A survey of cervical radiographs revealed a poorly circumscribed, large, homogenous soft tissue opacity that deviated the larynx and trachea ventrolaterally and to the right. During anesthetic induction for advanced imaging studies (CT and MRI) two large masses could be observed, one tonsillar and one laryngeal. The caliber of the trachea was narrowed and required the placement of a small endotracheal tube. Due to respiratory complications the advanced imaging studies could not be completed. The owner elected euthanasia when informed of the advanced nature of the mass and the need for a temporary or permanent tracheostomy tube. The owner consented to incisional biopsy of the laryngeal mass following euthanasia. Imprint cytology was performed and the biopsy was submitted for histopathologic examination. A complete post mortem examination was declined.

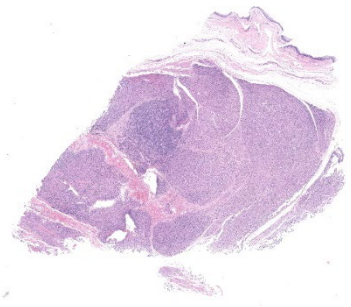

Figure 3-1. Larynx, dog. There is an infiltrative neoplasm within the laryngeal submucosa. (HE, 5X)

Gross Pathology:
Received a 0.7 x 0.5 cm brown, soft to firm mass. Four sections were submitted for routine processing.

Laboratory results:
No laboratory findings reported.

Microscopic Description:
The sections are partially covered by oral mucosa and in the submucosa there are lobules that are separated by thick bands of fibrovascular connective tissue stroma. Lobules comprise sheets of round to polyhedral to elongate cells with distinct to indistinct cell borders and a low to moderate nuclear to cytoplasmic ratio. The nucleus is centric, round with a coarsely stippled chromatin pattern and single, small round nucleolus. The cytoplasm is moderate to high, bright pink and appears smooth to finely granular. Pink intranuclear inclusion bodies are frequent. Anisokaryosis and anisocytosis are moderate. Multinucleate forms (up to 6 nuclei) are seen. Mitoses are rare (2 in 10 random 40x objective fields). Some of the elongate forms appear to have fine cytoplasmic cross striations and in some the nuclei are almost linearly arrayed (strap cells). There are also focal aggregates of smaller round cells with a high nuclear to cytoplasmic ratio and hyperchromatic nuclei. The tumor cells are present in the stroma supporting bundles of skeletal muscle myocytes. There are foci of hemorrhage in

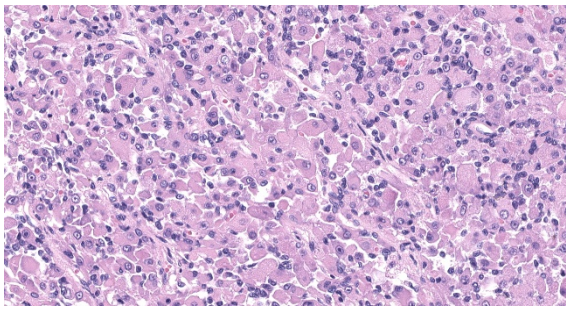

Figure 3-2. Larynx dog: The neoplasm is composed of numerous polygonal to spindle cells with abundant granular eosinophilic cytoplasm and variably sized nuclei. (HE, 380X)

the mass. Hemosiderophages are scattered throughout the arrangements of tumor cells.

Contributor's Morphologic Diagnoses:
Tumor of skeletal muscle origin – laryngeal rhabdomyosarcoma most likely.

Contributor's Comment:
The histologic findings in the biopsy from the laryngeal mass coupled with the clinical findings, and immunohistochemical stains are most consistent with a laryngeal rhabdomyosarcoma (RMS). RMSs are malignant neoplasms of striated muscle, which originate from muscle progenitor mesenchymal cells or from myocytes undergoing neoplastic transformation.[1,2] The majority of RMSs in dogs have occurred in tissues that do not normally contain striated muscle, such as the pharynx, gingiva, urethra, trachea, larynx and the jawbone.[1] In veterinary medicine RMSs can be classified into subtypes based on histologic characteristics: embryonal, alveolar, botryoid (aka: botryoid embryonal) and pleomorphic RMS. The clinical relevance of this tumor subtyping has not been established in veterinary medicine.[1] The variation in phenotype, age of onset and cellular morphology makes the diagnosis and classification of RMS difficult. The diagnostic features of skeletal muscle differentiation are not always evident on light microscopic examination and immunohistochemistry and ultrastructural examination are often needed to confirm the diagnosis.[1]

Canine laryngeal rhabdomyoma-sarcoma is considered a rare, distinct clinical entity in dogs, being locally invasive but rarely metastatic.[1,6] Complete excision is often difficult due to local invasion and recurrence of these tumors often lead to euthanasia. Although most are histologically benign, they may cause death or result in euthanasia due to laryngeal obstruction, as in this case. Affected dogs typically present with dysphonia, aphonia, stridor and dyspnea. Due to the limited number of cases age, sex and site predilection have not been recognized.

Canine laryngeal rhabdomyoma and rhabdomyosarcoma have been diagnosed as laryngeal oncocytoma in the past. Laryngeal rhabdomyomas-sarcomas can be distinguished from laryngeal oncocytomas by positive staining by myoglobin and desmin or by the ultrastructural presence of myofibers in addition to intracytoplasmic glycogen (PAS+ and diastase-sensitive) and numerous mitochondria.[1,2]

The use of immunohistochemical stains for desmin, α-actins, myogenin and MyoD1 and ultrastructural identification of sarcomeric structures can be used to determine if relatively undifferentiated tumors could be RMS.

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JPC Diagnosis:
Larynx: Rhabdomyoma.

JPC Comment:
In humans, rhabdomyosarcoma (RMS) is the most common soft tissue tumor of adults and children. Unfortunately, histologic features that clearly distinguish between rhabdomyoma and rhabdomyosarcoma have not been well documented in veterinary literature.[3] As previously noted by the contributor, the World Health Organization Classification categorizes human RMS as embryonal (includes the botryoid subtype), alveolar, pleomorphic, or spindle/cell sclerosing. The prognosis varies according to category and subtype, with botryoid subtypes being associated with a more favorable outcome in comparison to the poor prognosis associated with alveolar RMS. Although the same classification scheme is applied to canine RMS, the prognostic significance of category and subtype are undetermined.[5]

Diagnosis of RMS based solely on histologic evaluation can present a challenge, as RMS cells can vary widely in morphology, presenting as round, or well-differentiated or undifferentiated mesenchymal cells. As noted by the contributor, immunohistochemical stains for MyoD1 and myogenin are commonly used in human medicine to distinguish RMS from other mesenchymal or embryonic tumors, which is in contrast to less specific markers typically used in veterinary pathology, including muscle-specific actin, desmin, and vimentin to diagnose RMS, which can potentially result in inaccurate diagnosis.[5]

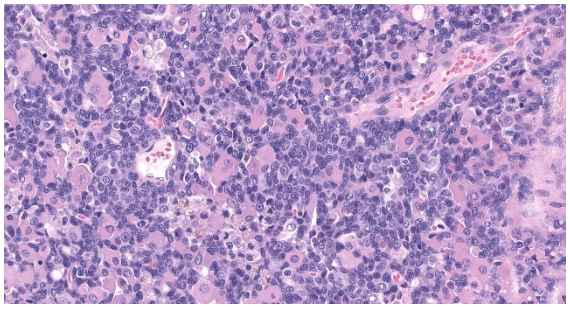

Figure 3-3. Larynx, dog: Regionally, neoplastic cells are surrounding by smaller less differentiated cells resembling satellite nuclei. (HE, 380X)

Myogenin and MyoD1 are both transcriptional regulatory proteins that stimulate early stages of myogenesis.[4, 5] Both proteins are present in fetal skeletal muscle cells and regenerating muscle cells, demonstrating nuclear immunoreactivity; normal adult skeletal muscle is negative. Given these transcriptional regulatory proteins are expressed early in skeletal muscle differentiation, expression of MyoD1 and myogenin has been demonstrated to be the greatest in less differentiated rhabdomyosarcomas in humans.[5]

A recently published study evaluating the immunohistochemical reactivity of canine RMS found myogenin and MyoD1 to be useful for diagnosing RMS in conjunction with histopathologic characteristics and desmin immunoreactivity. Of the 13 cases diagnosed as RMS in the study, 100% were MyoD1 positive and five were positive for myogenin.[5]

Although cost prohibitive, transmission electron microscopy remains the gold standard for the diagnosis of RMS. Key features include poorly developed cytoplasmic myofilament tangles associated with numerous polyribosomes and short electron-dense bands or plaques.[5]

In this case, approximately 10% and 5% of neoplastic cells demonstrate strong nuclear immunoreactivity for MyoD1 and myogenin, respectively. There is diffuse, weak to moderate cytoplasmic immunoreactivity for muscle specific actin, whereas 30% of neoplastic cells have weak to strong cytoplasmic immunoreactivity for desmin. Neoplastic cells are diffusely negative for smooth muscle actin and AE1/AE3

(pancytokeratin), inconsistent for leiomyosarcoma and oncocytoma, respectively. These findings, combined with the histomorphologic features, are consistent with a skeletal muscle neoplasm.

There was vigorous discussion amongst conference participants in regard to the diagnosis of canine laryngeal rhabdomyoma versus rhabdomyosarcoma in this case. Unfortunately, histologic features that clearly distinguish between the two have not been well documented. Features that should raise the possibility of malignant biological behavior include a high percentage of undifferentiated cells, invasive growth, and a high mitotic count.[3] The majority of participants preferred the diagnosis of rhabdomyoma based on lack of invasiveness at the near margin (although the lateral and deep margins could not be assessed) and low mitotic count. However, a significant minority preferred the diagnosis of "skeletal muscle neoplasm" due to the overlap of histomorphologic features shared between the two entities.

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CASE IV: 20040779 (JPC 4166757)

Signalment:
1-year-old, castrated male, domestic longhair cat (*Felis catus*)

History:
This patient presented at a private clinic with a fever (105.3°F), icterus, and thrombocytopenia. The animal progressed to a comatose state within 48 hours. Humane euthanasia and necropsy were performed off-site, and selected tissues were received at Oklahoma Animal Disease Diagnostic Laboratory (OADDL).

Gross Pathology:
No gross findings reported.

Laboratory results:
No laboratory findings reported.

Microscopic Description:
Lymph node: Multifocally expanding blood vessels and extensively scattered within subendothelial spaces, cortical and medullary sinuses, are numerous, discrete, enlarged macrophages, distended up to 55 µm by multiple intracytoplasmic, irregularly round, developing schizonts, containing numerous,

1-3 µm, uninucleate merozoites. Affected blood vessels are congested, and lumina are frequently marginated and occasionally occluded by the schizont-laden macrophages. Small numbers of these vessels are lined by plump, hypertrophic endothelial cells. The connective tissue stroma within the medullary cords is loose and separated by increased amounts of clear space (edema).

Contributor's Morphologic Diagnoses:
Lymph node: Histiocytosis, intravascular and parenchymal, diffuse, severe with intrahistiocytic schizonts (consistent with *Cytauxzoon felis*).

Contributor's Comment:
The microscopic features of the lymph node are consistent with a diagnosis of cytauxzoonosis, which is an emerging, tick-borne, life-threatening disease that affects domestic cats and wildlife felids. Since the first cases reported in Missouri in 1976, this disease has been identified in domestic cats from 17 states from within the United States, mainly in the southern and southeastern regions, and also in a few other countries to include Europe, South America, and Asia.[13, 15, 16]

The main etiologic agent, *Cytauxzoon felis*, is an apicomplexan protozoa belonging to order *Piroplasmida*, family *Theileriidiae*.[7] *C. felis* infects a variety of wild felids including bobcat (*Lynx rufus*), Texas cougars (*Puma concolor stanleyana*) and Florida panthers (*Puma concolor coryi*), which act as natural reservoirs or incidental hosts in North America.[4,12] Infected bobcats rarely have clinical illness but develop a persistent, non-fatal parasitemia as a life-long carrier of this parasite.[14] In contrast, domestic cats (*Felis catus*) infected with *C. felis* usually show clinical signs at approximately 5-14 days after exposure to an infected tick.[16] Clinical presentation is typically non-specific, with an acute onset of anorexia, lethargy, and fever.[7] Other clinical findings exhibited by infected domestic cats, may include increased vocalization, respiratory distress, weakness, icterus, vomiting, abnormal mentation, or moribund.[7,13] The duration of illness is extremely rapid and more than 90% of infected domestic cats succumb within days despite medical intervention.[3] Domestic cats surviving acute illness, may become asymptomatic carriers with long-term parasitemia for years.[8]

Transmission of *Cytauxzoon felis* to a felid host requires a tick vector. Although *C. felis* has been recovered from both *Amblyomma americanum* (lone star tick) and *Dermancentor variabilis* (American dog tick), *Amblyomma americanum* has been viewed as the primary vector for natural transmission of *C. felis* in North America, because the geographic distribution of this tick appears to overlap with ranges of reported *C. felis* infections, in domestic cats.[1,5] In the life cycle of *C. felis*, zygotes develop in tick guts during the sexual phase of reproduction.[16] The zygotes mature into mobile ookinetes and migrate to the salivary glands, where sporogony occurs, and then infectious sporozoites are inoculated into the dermis of the felid hosts during tick feeding.[13] In the peripheral blood circulation, sporozoites directly enter into mononuclear

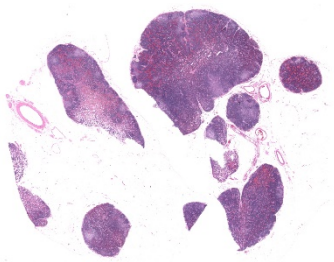

Figure 4-1. Lymph nodes, cat. Multiple lymph nodes are submitted for examination. At low magnification, there is mild reactive hyperplasia, areas of pallor within the cortex, and draining hemorrhage. (HE, 5X)

phagocytic cells and develop into nucleated schizonts. During schizogony, numerous merozoites are formed within mature schizonts that significantly expand the cytoplasm of phagocytes, with an increase in size up to 250 µm in diameter.[16] Upon rupture of infected phagocytes, merozoites are released and subsequently taken up by circulating erythrocytes by endocytosis. The intra-erythrocytic merozoites (piroplasms) are 1-2 µm, often "signet-ring" or occasionally "safety pin" shaped, and capable of undergoing asexual reproduction by binary fission and then infecting other erythrocytes.[16] Repetition of the asexual cycle results in parasitemia in the host animals. The parasite-laden erythrocytes are ingested by the tick vector, and the merozoites are released from the erythrocytes into the tick guts.[13] After gametogenesis and fusion of male and female gametocytes, the life cycle of *C. felis* is completed.[13,16]

The schizogenous phase of parasitic replication within the host's mononuclear phagocytic cells is responsible for the primary clinical illness.[7] In cats with *C. felis* infection, the large, schizont-laden macrophages are scattered throughout the body via blood circulation, which may occlude vascular lumina in various organs, particularly lung, liver, spleen, and lymphoid tissues.[13] Hepatomegaly, splenomegaly and enlarged lymph nodes are often observed at necropsy.[2] Additionally, disruption of vascular endothelia resulting in disseminated intravascular coagulation (DIC), is a common complication in naturally infected cats.[16] Vascular obstruction, anoxia, severe systemic immune response against the parasites, and cell death, are contributory to multiorgan failure resulting in death of infected cats.[6,13] Although the invasion of merozoites into erythrocytes sometimes leads to hemolysis, this parasitic phase (intra-erythrocytic piroplasmosis) is not considered a main contributor to illness.[8,13]

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JPC Diagnosis:
Lymph nodes and perinodal fibroadipose tissue: Apicomplexan schizonts, intra-histiocytic, intra- and extravascular, numerous, with diffuse mild reactive hyperplasia and draining hemorrhage, etiology consistent with *Cytauxzoon felis*.

JPC Comment:
The contributor provides a detailed and thorough overview of *Cytauxzoon felis*, an emerging tick-borne disease of domestic and wild felids first reported as a parasite of domestic cats in Missouri by Wagner in 1976.[10,13]

Cytauxzoon spp. are closely related to *Thelieria* spp. and are classically differentiated based on leukocyte predilection in their vertebrate hosts. *Thelieria* spp. undergo schizogony in lymphocytes with multiple

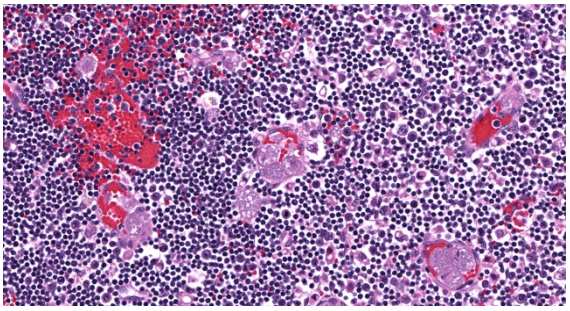

Figure4-2. Lymph node, cat: There are numerous schizonts of Cytauzoon felis within macrophages within vessel lumina, paracortex, and sinuses. (HE, 400X)

fission in erythrocytes whereas *Cytauxzoon* spp. undergo schizogony within histiocytes and binary fission within erythrocytes. However, differentiation between these genera based on host leukocyte predilection may be inadequate and in need of reconsideration.[10]

C. felis has historically been considered to be highly fatal in domestic cats. However, recent studies have found there is a significant subpopulation of subclinically infected domestic cats in the United States. Using PCR amplification with *C. felis* specific primers, 902 blood samples from healthy asymptomatic cats from Arkansas, Missouri, and Oklahoma were evaluated. 15.5%, 12.9%, and 3.4%, respectively, were infected with *C. felis* in one study.[11] A similar study of 1104 healthy cats in Kansas found 25.8% were subclinically infected.[17] Similar to the bobcat, transmission of *C. felis* from subclinically infected domestic cats to naïve cats via *A. americanum* has been observed in multiple studies. Therefore, it is likely these chronically infected cats serve as an important reservoir of infection to other domestic cats.[10]

An additional interesting case of likely *C. felis* transmission was reported in Switzerland, involving a subclinical blood donor. Following transfusion, the recipient demonstrated intra-erythrocytic inclusions. *C. felis* was confirmed in both donor and recipient using PCR and sequencing of 1637 bp of the 18S rRNA gene with 100% nucleotide sequence identity. Therefore, it may be prudent to screen healthy blood donor cats for this agent by PCR, particularly given the aforementioned rates of subclinically infected domestic cats in endemic areas.[9]

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