Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2022-2023

Conference #25 **10 May 2023**

CASE I:

Signalment:
A 2-year-old, male, mixed breed dog (*Canis familiaris*)

History:
This dog was presented to the clinic with one-week history of firm and multicentric swelling of the face and muzzle, acute vision loss, generalized lymphadenomegaly, fever, and asymmetric testicular swelling. The dog was neutered, and the testicles were submitted for histopathology with a presumptive diagnosis of neoplasia. After neutering, the dog was treated with steroids and doxycycline, facial swelling and lymphadenomegaly decreased after treatment. Once therapy was discontinued, facial swelling and lymphadenomegaly waxed and waned for approximately two months. Three months after neutering, the dog developed multiple nodules over the thigh region and stertorous respiration was noticed on physical exam. Skin nodules were submitted for histopathology (slides not included in this WSC submission).

Gross Pathology:
Both testicles (3.5 and 4.5 cm in greatest dimension) were received in 10% buffered formalin for histopathology. Diffusely and bilaterally, the epididymis was markedly enlarged by multiple, expansile, pale tan, firm masses. Similar masses expanded the testicular parenchyma, and elevated and effaced the visceral vaginal tunic. White bar = 1 cm.

Laboratory Results:

Clinical Pathology
Thrombocytopenia and leukopenia were documented. Cytologic examination of FNA material from the submandibular lymph nodes and swollen skin of the muzzle revealed histiocytic inflammation.

Serology
Serum samples were submitted to different laboratories to test for Rocky Mountain spotted fever, ehrlichiosis, brucellosis, Lyme disease, bartonellosis, and leishmaniasis. All serologic tests came back negative.

Microbiology
Fungal and aerobic bacterial cultures of fresh samples of skin (nodules from thigh region) and popliteal lymph node failed to detect microorganisms.

Microscopic Description:
Testicle: Broad, coalescing, areas of angiocentric and interstitial inflammation obliterate the testicular parenchyma and visceral vaginal tunica, and obscure and efface approximately 40% of the epididymal ducts. Inflammatory cells are composed of numerous epithelioid macrophages and lesser numbers of degenerate neutrophils, plasma cells, and lymphocytes. Macrophages are often arranged in concentric collections (granulomas) that, in some areas, encompass aggregates of degenerate neutrophils admixed with necrotic cellular debris (pyogranulomas). In addition, multiple macrophages contain a

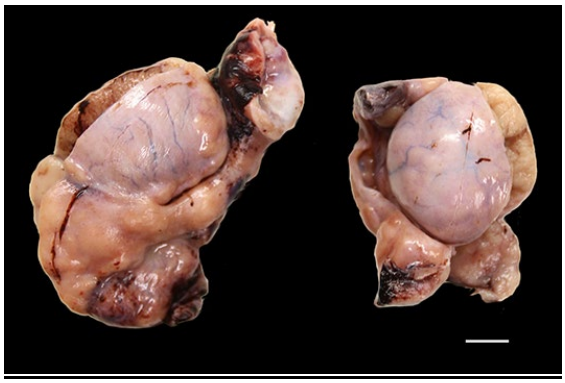

Figure 1-1. Testes, dog. The epididymis and to a lesser extent, the testis is expanded by numerous yellow-tan nodules. (Photo courtesy of: Wyoming State Diagnostic Laboratory www.uwyo.edu/wyovet/)

large, 4 to 7 µm, cytoplasmic, clear vacuole. Macrophages often display mitotic figures and cytological atypia, including pleomorphic nucleus and anisokaryosis. There are low numbers of binucleate and multinucleate cells among inflammatory leukocytes. A few areas of lytic necrosis are scattered among inflammatory foci. The lumen of seminiferous tubules and epididymal ducts lacks spermatids and spermatozoa.

The following special stains (not submitted) failed to highlight microorganisms in replicate and appropriately controlled sections: GMS, PAS, Brown & Hopps, Giemsa, Steiner's, acid fast, and Gimenez.

Haired skin (thigh nodules) and popliteal lymph node (not submitted): The dermis, subcutaneous tissue, and lymph node parenchyma were effaced by coalescing areas of inflammation with similar microscopic features as those described above in the testicles. Immunohistochemical staining for CD1a, CD4, and CD11c of frozen skin sections (thigh nodules) was performed. Dermal infiltrates of round cells were positive for CD11c and CD4. The immunoreactivity for CD1a was reported as ambiguous.

Contributor's Morphologic Diagnoses:
Testicle: Epididymitis and orchitis, angiocentric and interstitial, granulomatous, pyogranulomatous, and lymphoplasmacytic, severe, multifocal with histiocytic atypia and testicular atrophy

Haired skin (thigh nodule, not submitted): Dermatitis and panniculitis, angiocentric to interstitial, granulomatous, pyogranulomatous, and lymphoplasmacytic, moderate to severe, diffuse with histiocytic atypia

Popliteal lymph node (not submitted): Lymphadenitis, pyogranulomatous, severe, diffuse with histiocytic atypia

Contributor's Comment:
The dog in the present case had granulomatous and pyogranulomatous inflammation with histiocytic atypia in the skin, draining lymph nodes, and testes. Based on the immunohistochemical results from skin nodules and negative results from ancillary tests, the final diagnosis was systemic reactive histiocytosis. The initial presentation of this case was strongly suggestive of infectious disease and infection by either *Leishmania* sp or *Brucella* sp was suspected. Multiple infectious diseases were ruled out with a combination of

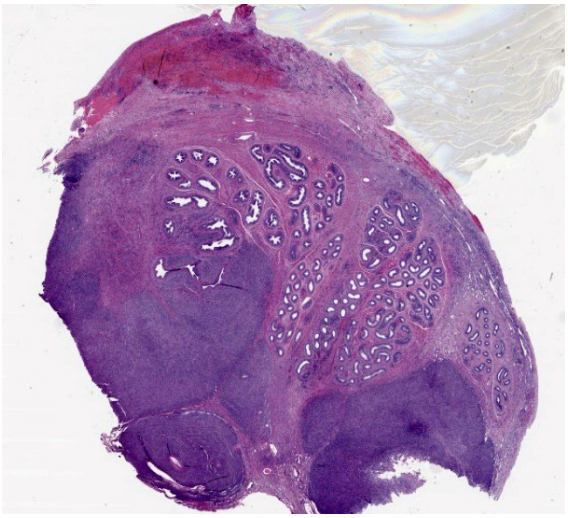

Figure 1-2. Epididymis, dog. There are nodular infiltrates effacing approximately 50% of epididymal tubules. (HE, 7X)

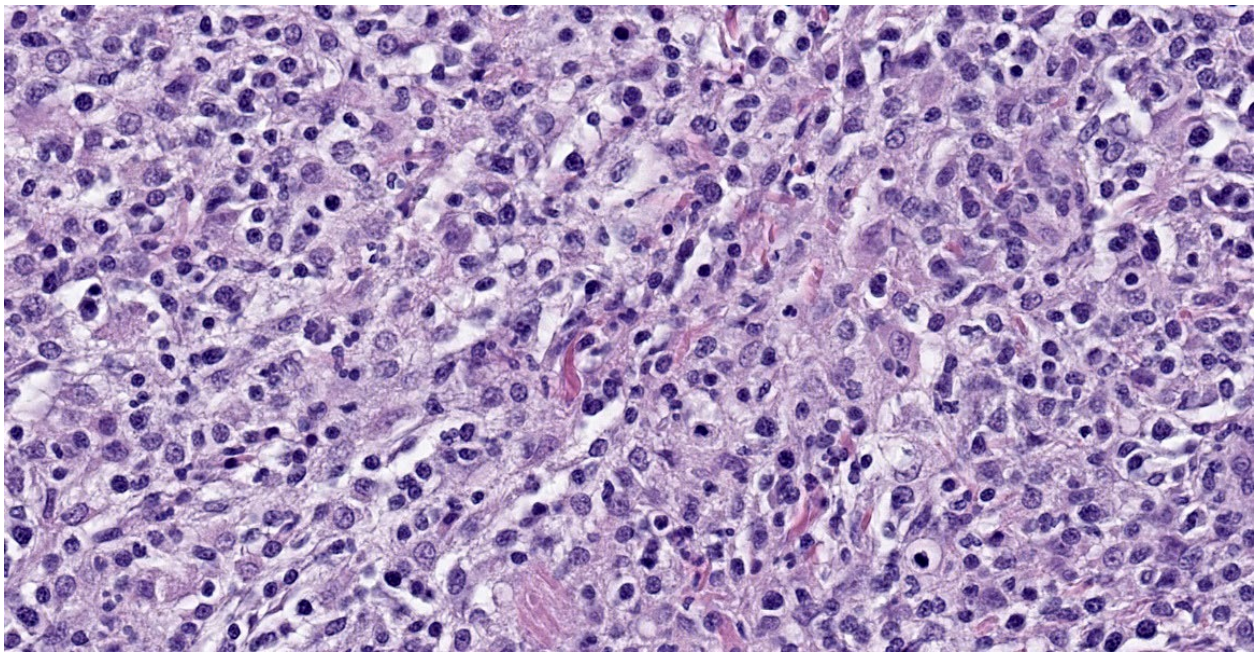

Figure 1-3. Epididymis, dog. The cellular infiltrate is composed of large numbers of macrophages with fewer lymphocytes and neutrophils. (HE, 450X)

special stains, bacteriology, and serology. No immunohistochemistry for dendritic cell markers was done on sections of testis (i.e., they were received in formalin), but immunohistochemistry of frozen haired skin samples was considered sufficient for the diagnosis of systemic reactive histiocytosis.

Reactive histiocytosis is a proliferative disorder of interstitial dendritic cells that has been well characterized in dogs,[3] and is uncommonly reported in other veterinary species.[1] In dogs, reactive histiocytosis has two clinical manifestations: cutaneous and systemic. While cutaneous reactive histiocytosis is confined to skin and draining lymph nodes, systemic reactive histiocytosis involves extracutaneous sites such as nasal and ocular mucosa, and internal organs. Involvement of the testicles, however, is less common than other extracutaneous sites. In the present case, intraocular and pulmonary involvement was suspected due to acute vision loss and stertorous respiration.[4] Systemic reactive histiocytosis was first described in the Bernese Mountain dog but has now been reported in multiple dog breeds. The diagnosis of reactive histiocytosis is based on immunohistochemistry for dendritic cell markers. Histiocytes in both cutaneous and systemic reactive histiocytoses express markers of dendritic cells (CD1a, CD11c/CD18, CD90, and MHC class II) and a marker of dendritic cell activation (CD4).[3] In addition, macrophages lack expression of E-cadherin. It is important to bear in mind that most of these markers are assessable in frozen tissue sections only (CD90, MHC class II, and E-cadherin are the exceptions).

After the diagnosis of systemic reactive histiocytosis was made, the dog of the present case has been on an anti-inflammatory dose of steroids and, according to the practitioner, the dog has shown great improvement.

Contributing Institution:
Wyoming State Diagnostic Laboratory
www.uwyo.edu/wyovet/

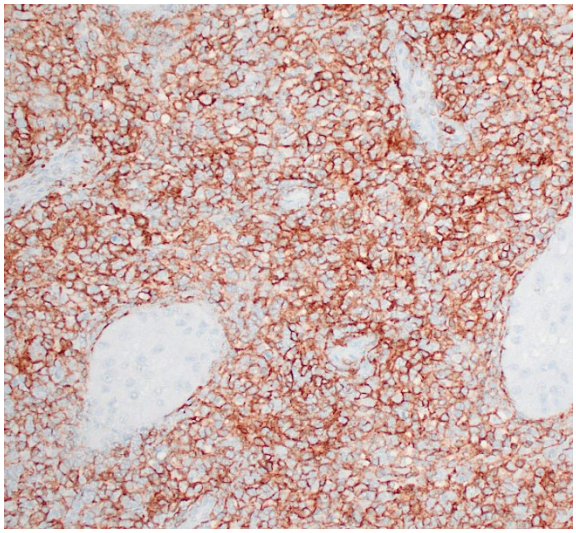

Figure 1-4. Epididymis, dog. The macrophages within the infiltrate stain strongly positive for IBA-1 (as you know, macrophages do.) (anti IBA-1, 400X)

JPC Diagnosis:
Epididymis: Epididymitis, lymphohistiocytic, chronic, multifocal to coalescing, moderate.

JPC Comment:
This case illustrates the challenge of diagnosing proliferative histiocytic diseases in dogs, which often require immunohistochemical staining for a definitive diagnosis.

Proliferative histiocytic diseases in dogs are typically of dendritic cell origin.[5] Dendritic cells arise from CD34+ precursors in the bone marrow and differentiate into *interstitial dendritic cells* or epidermal dendritic cells (*Langerhans cells*). Proliferative diseases of interstitial dendritic cells include cutaneous and systemic reactive histiocytoses and histiocytic sarcoma. The main proliferative diseases of canine Langerhans cells are cutaneous histiocytoma and cutaneous Langerhans cell histiocytosis.

Since these diseases all originate from dendritic cells (excepting the hemophagocytic form of histiocytic sarcoma), they express common dendritic cell markers: CD1a, CD11c, CD18, and MHCII.[3,5] Dendritic cells, which are professional antigen presenting cells, use CD1a and MHCII for antigen presentation for T cells. CD11c and CD18 are components of the beta-2 integrin heterodimer CD11c/18, one of the adhesion molecules expressed by leukocytes.[3]

A few additional immunohistochemical stains can assist in differentiating the diseases under the dendritic cell umbrella. Langerhans cells express E-cadherin, which they use to bind homotypically to the epithelium, so canine cutaneous histiocytoma and Langerhans cell histiocytosis are both expected to have E-cadherin immunoreactivity. Canine cutaneous and systemic reactive histiocytosis are diseases of activated interstitial dendritic cells and thus express CD4, a marker for activation. They also express CD90 (Thy-1), which is not expressed by Langerhans cells.[3]

In this section, the main histologic differentials are histiocytic sarcoma, histiocytic inflammation (i.e from dimorphic fungus), and systemic reactive histiocytosis. The relatively bland cellular population lacks atypia and bizarre nuclei, making histiocytic sarcoma less likely. Differentiating between primary inflammation and reactive histiocytosis is not possible based solely on this H&E section, so conference participants decided to morph

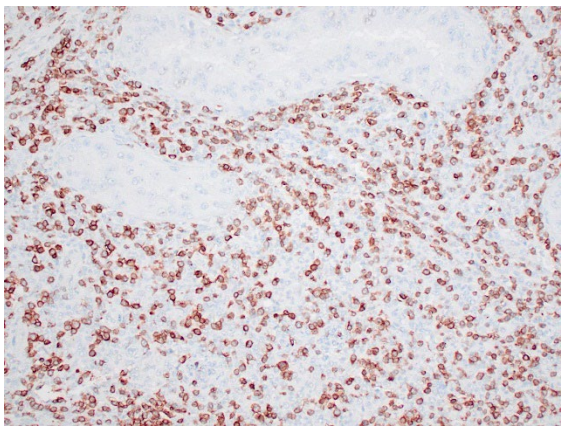

Figure 1-5. Epididymis, dog. Lymphocytes within the infiltrate stain strongly positive for CD3 within the (anti-CD3, 400X)

lymphohistiocytic epididymitis. The clinical history, CD4 immunoreactivity of cutaneous lesions noted by the contributor, and lack of infectious agents on special stains, microbiologic, and serologic testing, however, are consistent with the diagnosis of systemic reactive histiocytosis.

In the multifocal cutaneous lesions of reactive histiocytosis, another differential to consider is canine cutaneous Langerhans cell histiocytosis, which is histologically similar to a histiocytoma but multifocal in distribution. These can generally be differentiated on H&E sections by their growth patterns: they are "top heavy" lesions, with the base of the tumor smaller than the top and tend to track adnexal structures.[2] Reactive histiocytosis lesions are generally bottom-heavy, extend into the subcutis, and are oriented along vasculatures.[2]

This week's moderator, Dr. Rachel Neto from Auburn University, explained that even though Langerhans cells live within the epithelium, they are thought to arise from precursors within the dermis, and cellular proliferations in Langerhans cell histiocytosis and cutaneous histiocytoma occur in the superficial dermis. She also explained that Langerhans cells are dependent on epidermal growth factors, and the closer they are to the epidermis, the stronger their expression of E-cadherin.

References:

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CASE II:

Signalment:
12-year-old neutered male European Shorthair cat (*Felis catus*)

History:
The cat had a clinical history of respiratory dyspnea and cough.

Gross Pathology:
The entire pulmonary lobe was submitted for histopathology.

Laboratory Results:
No laboratory findings reported.

Microscopic Description:
Lung. About 70% of the parenchyma is effaced by a multifocal to coalescing, severe proliferative process. Alveolar spaces are filled with numerous round cells of 25 micrometer in diameter, with distinct cell borders, high nuclear-to-cytoplasmic ratio, and small to moderate amounts of finely granular eosinophilic cytoplasm. Nuclei are round, central, with finely stippled chromatin and one or more visible nucleoli. Anisocytosis and anisokaryosis are mild and occasional mitoses with atypical features are present. Admixed to these cells, there are multifocal

aggregates of large alveolar macrophages with foamy cytoplasm, or scant cell debris and hypersegmented neutrophils. In about the 20% of the section and mainly the periphery of the lesions, the alveolar spaces are multifocally dilated and merged, with disrupted and blunted septae (emphysema). Lining the alveoli there are plump and cuboidal pneumocytes (type II pneumocyte hyperplasia). Smooth muscle fibers of terminal bronchioles are diffusely increased in size (hypertrophy). The interstitium is multifocally and markedly expanded by haphazardly arranged eosinophilic collagen fibers embedding fibrocytes (fibrosis), small caliber capillaries and rare macrophages containing coarsely granular brown-black pigment (anthracosis), and numerous lymphocytes; bronchial glands are increased in number (hyperplasia) and bronchial walls are collapsed multifocally. Blood vessels are multifocally engorged with erythrocytes (hyperemia) and the tunica media of medium and small arteries is moderately thickened by several layers of smooth muscle cells (arteriolar hypertrophy).

Immunohistochemistry (slides not submitted, pictures submitted as supporting material on DVD): vimentin+, Iba+, e-cadherin+, CK-.

Contributor's Morphologic Diagnosis:
Lung. Multifocal to coalescing, alveolar histiocytosis with emphysema and smooth muscle hyperplasia, with fibrosis and arteriolar hypertrophy.

ND. Feline Pulmonary Langerhans cell Histiocytosis

Contributor's Comment:
Feline pulmonary Langerhans cell histiocytosis (FPLCH) is a rare disease of older cats (>10 years) that causes progressive respiratory insufficiency that leads to death secondary to pulmonary parenchymal infiltration with Langerhans cells (LCs).[1,7,9,13] Clinical signs vary from acute respiratory distress, including tachypnea, labored breathing, and open mouth-breathing, to prolonged chronic pulmonary disease.[7,9,13] FPLCH is thought to represent a neoplastic process because of cellular morphological characteristics and extrapulmonary invasion, reported in pancreas and kidney[2,6], and the pulmonary one is the only form of Langerhans cells proliferation described in this species, with no cutaneous manifestation reported.[1]

Similar to the pulmonary lesions in canine LCH, histiocytic proliferations affect first the peribronchial parenchyma, causing multinodular-to- diffuse proliferations that vary from 2-5 mm in diameter and affect all lung lobes.[1] As the disease progresses, nodules coalesce, and lesions extend to the pleural surface. In advanced cases, the whole lungs tend to be affected, and all lobes appear diffusely firm.[13] However, there is a higher degree of cellular and nuclear pleomorphism. Aggregates of histiocytes are usually cohesive, and proliferating cells extend from the peribronchial parenchyma into the surrounding alveolar septa and alveoli, eventually effacing the pulmonary parenchyma.

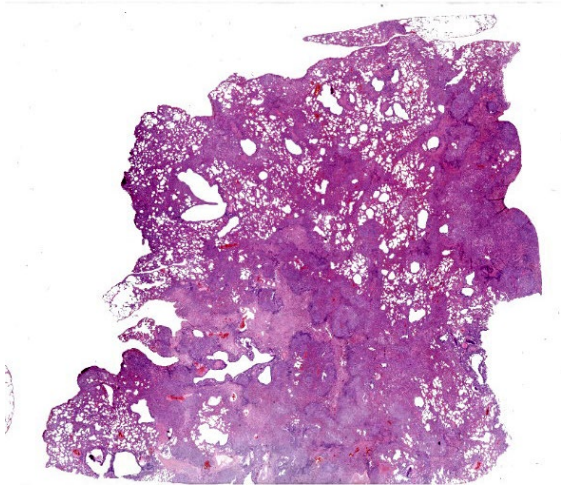

Figure 2-1. Lung, cat. A single section of consolidated lung is submitted. There are areas of emphysema and dilated airways. (HE, 7X)

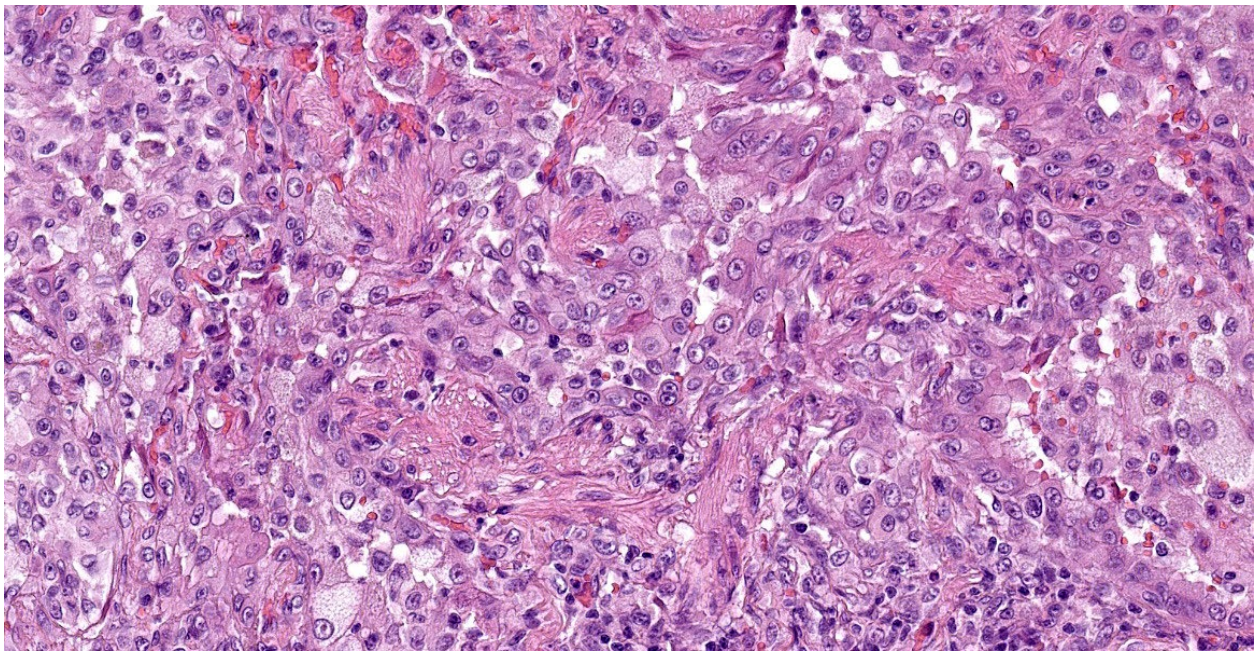

Figure 2-2. Lung, cat. Alveoli are filled with numerous histiocytes with prominent nuclei and nucleoli. There is marked smooth muscle hyperplasia. (HE, 381X)

Ultrastructurally, Birbeck granules, which represent the internalized cell surface receptor langerin (CD207) cross-linked by an antibody, have been reported in the cytoplasm of proliferating histiocytic cells, confirming a Langerhans cell origin. This is in contrast to canine Langerhans cells, which do not have Birbeck granules.[7,13]

Immunohistochemically, histiocytic cells of feline LCH are positive for CD18 and E-cadherin and negative for CD204 in formalin-fixed tissues.[9] In the current case, FPLCH was diagnosed on the basis of E-cadherin positive neoplastic cell, with variable pattern and intensity of immunolabeling among pulmonary lobes (see attached figures: 1, e-cadherin, 2 Iba1).

In a recent work by Hirabayashi et al., that accurately characterized histiocytic cell types of cats by immunohistochemistry and immunofluorescence, non-neoplastic histiocytes were mostly positive for Iba-1 and HLA-DR, while the immunoreactivity of other antibodies varied among histiocytes that resided in different organs. Dermal interstitial Dendritic Cells (iDCs) and macrophages were positive for CD204 and negative for E-cadherin. Epidermal LCs were negative for CD204 and positive for E-cadherin. However, antibodies for CD1a, langerin/CD207, and S100, which are regarded as specific markers of human LCs, were not available for detecting LCs on formalin-fixed, paraffin-
embedded tissue sections of the cat, as well as those of the dog.[9,12] Double-labeling immunohistochemistry of the lymph nodes revealed E-cadherin-positive CD204-negative histiocytes and E-cadherin and CD204-double positive histiocytes in the sinus, which may be veiled cells and LC-like cells, respectively.[6] Neoplastic histiocytic cells were immunohistochemically positive for Iba-1 and HLA-DR in all cases of Feline Progressive Histiocytosis (FPH) and Histiocytic Sarcoma (HS). Immunoreactivity for CD204, CD163, and E- cadherin varied among cases. Of the HS cases, the neoplastic cells of more than half of cases presented with the iDC/macrophage immunophenotype (CD204+/E-cadherin-), while a minor number presented with the LC immunophenotype (CD204-/E-cad-

herin+), and with the LC-like cell immunophenotype (CD204+/E-cadherin+). Feline HS with the LC immunophenotype may correspond to Langerhans cells histiocytosis (LCH) or LC sarcoma (LCS) in humans.[1] Among FPH cases of the study, the neoplastic cells of the majority of cases presented with the iDC/macrophage immunophenotype (CD204+/E-cadherin-) and a minor part with the LC immunophenotype (CD204-/E-cadherin+). Moreover, the epitheliotropism of neoplastic cells was reported in some E-cadherin-positive cases. In a previously reported case of FPLCH, E-cadherin expression by ne oplastic cells was decreased in extrapulmonary metastatic lesions, while in one of the HS cases in the Hirabayashi study, the E-cadherin immunoreactivity of neoplastic cells varied in different organs.[2,6] These results suggest that the expression of E-cadherin by feline neoplastic histiocytes could be altered by their microenvironments.

PLCH in humans has been associated with tobacco smoke, especially in young adults.[1,3] Tobacco smoke supposedly causes damage to the bronchial epithelium, which prompts the release of peptides that stimulate alveolar macrophages to secrete cytokines that activate resident antigen-presenting LCs.[3] Genetic and other factors, such as prior treatment with chemo-therapeutic agents, may also be involved in the pathogenesis of PLCH.[1]

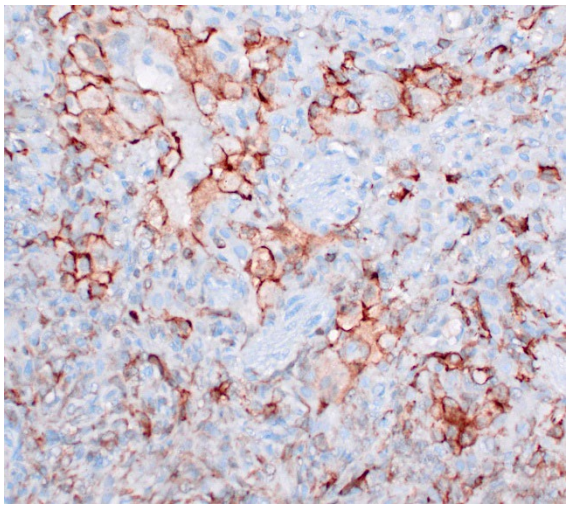

Figure 2-3. Lung, cat. Cells within alveoli demonstrate strong immunopositivity for IBA-1. (anti-IBA-1, 400X)

Contributing Institution:
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Veggiano (PD), Italy
https://www.clinicaveterinariasanmarco.it/

JPC Diagnosis:
Lung: Histiocytosis, alveolar, chronic, diffuse, severe, with fibrosis, type II pneumocyte hyperplasia, and smooth muscle hyperplasia.

JPC Comment:
This case of feline pulmonary Langerhans cell histiocytosis provided participants with an opportunity to review proliferative histiocytic disease in cats and complements the first case in this week's conference, a case of canine systemic reactive histiocytosis.

As in dogs, proliferative histiocytic disease of cats generally arises from dendritic cells – either interstitial dendritic cells or epidermal dendritic cells (Langerhans cells). Feline pulmonary Langerhans cell histiocytosis is the only proliferative disease of Langerhans cells in cats, as a feline equivalent canine cutaneous histiocytoma has not been documented. Proliferative diseases of interstitial dendritic cells include feline progressive histiocytosis and histiocytic sarcoma (except the hemophagocytic form, which likely arises from macrophages).

Feline progressive histiocytosis and histiocytic sarcoma can both affect the lung and are histologic differentials for this section. FPH occurs in older cats and starts as single to multiple raised, alopecic, nonpainful and

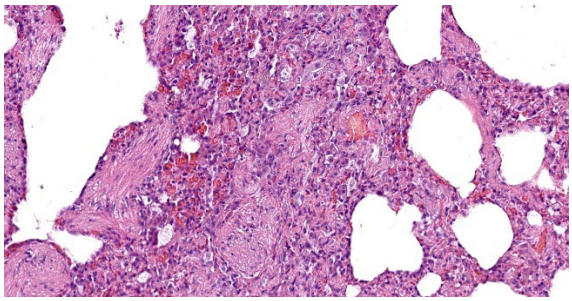

Figure 2-4. Lung, cat. There is marked airway smooth muscle hyperplasia. (HE, 460X)

nonpruritic nodules in the skin. The lesions do not regress, but wax and wane, and in advanced cases, can spread to internal organs, such as the lungs, liver, and spleen.[4,8,9] Histiocytic sarcoma can occur as a primary (localized) or metastatic (disseminated) neoplasm in the lung of cats, though it is less common in cats than in dogs.[9] Histiocytes of both FPH and histiocytic sarcoma are expected to be negative for E-cadherin, which is normally expressed by Langerhans cells, and allows these differentials to be ruled out in this case.[8,9]

Langerhans cells were first discovered by German physician Paul Langerhans (1847-1888) while working in Rudolf Virchow's laboratory at the Berlin Pathologic Institute. Langerhans was also the first to describe the pancreatic islets, now known as the islets of Langerhans, which he discovered nestled among pancreatic acini in the rabbit pancreas. Langerhans did not discover the function of these cells which bear his name; his academic career ended early after a pulmonary infection drove him to relocate to Portugal and return to medical practice. In this coastal location, he also developed a keen interest in marine biology, and several species of marine worms bear his name. Langerhans died when he was only 41 due to a severe kidney infection.[11]

References:

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CASE III:

Signalment:
11-month-old female spayed Golden Retriever (*Canis lupus familiaris*)

History:
Female, spayed, Golden Retriever presented with chronic anterior uveitis, possible vitritis, possible iris mass, and secondary glaucoma in the right eye. The right eye is blind; thus, removal with histopathology of the right eye was elected.

Gross Pathology:
Uveitis with secondary glaucoma

Laboratory Results:
No laboratory findings reported.

Microscopic Description:

Right eye: Within the cornea, there is a small number of small-caliber vessels originating from the limbus and coursing through the peripheral stroma (neovascularization). The anterior chamber contains a large amount of hemorrhage (hyphema) admixed with eosinophilic homogeneous to polymerized material (proteinaceous fluid) and small numbers of polymorphonuclear cells. The iris, iridiocorneal angle, and ciliary body are markedly expanded and almost completely effaced by an unencapsulated, poorly demarcated, infiltrative, densely cellular mass. The mass is composed of pleomorphic neoplastic spindle to oval cells arranged in extensive sheets supported by a moderate amount of fibrous to collagenous matrix. Multifocally, the neoplastic cells are separated and lined upon, small, thin trabecula composed of deeply eosinophilic granular to glassy extracellular material (osteoid) that is variably mineralized. Multifocally, neoplastic cells are present in lacuna and are embedded within a basophilic matrix (cartilage matrix). Neoplastic cells have a moderate amount of cytoplasm, variably discernible cell boundaries, a large oval nucleus with stippled chromatin, and 1-4 prominent nucleoli. Neoplastic cells

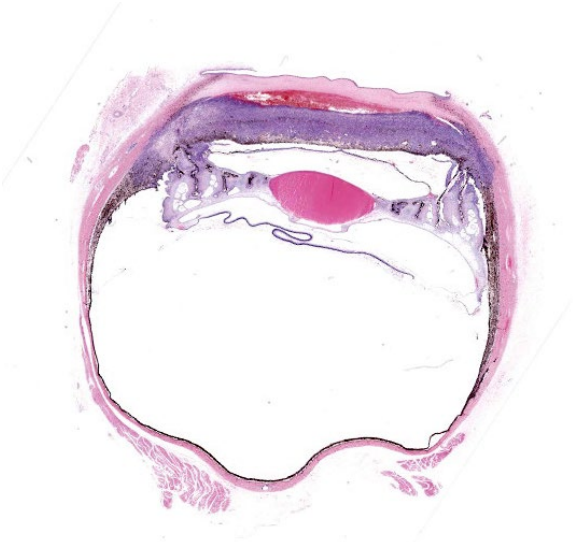

Figure 3-1. Globe, dog. A neoplasm infiltrates and expands the iris, ciliary body, anterior uvea, sclera, and bilaterally effaces the filtration angle. There is hemorrhage within the anterior segment. (HE, 6X)

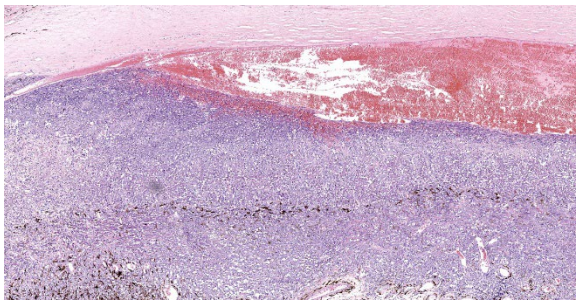

Figure 3-2. Globe, dog. The neoplasm is composed of short, haphazardly arranged bundles of plump spindle cells. The cornea is at top. There is hemorrhage between the cornea and the neoplasm. (HE, 37X)

demonstrate marked anisocytosis, anisokaryosis, and have a high mitotic index (20 per ten 0.237mm^2 fields). 50-60% of the mass is lack differential staining (coagulative necrosis) or a complete loss of cellular architecture with replacement by eosinophilic karyorrhectic and cytolytic cellular debris (lytic necrosis). The iris is multifocally adhered to the anterior lens capsule (posterior synechia). The iridocorneal angle is collapsed and effaced by neoplastic cells. The ciliary body is mostly effaced by neoplastic cells and neoplastic cells are observed extending up the zonules of Zinn to the lens capsule. There is a distinct layer of fibrous connective tissue extending from the surface of the ciliary body over the posterior lens capsule (cyclitic membrane). The non-tapetal retina is diffusely thin with marked hypocellularity of all layers, especially the ganglion and inner nuclear layers (retinal degeneration). The tapetal retina is somewhat spared, but there is hypocellularity of the ganglion and inner nuclear layer (ganglion cell layer degeneration). There are aggregates or emboli of neoplastic cells and necrotic cell debris within the scleral veins (vascular invasion).

Contributor's Morphologic Diagnosis:
Right eye: Osteoblastic osteosarcoma, anterior uvea, with neovascularization, hyphema, cyclitic membrane, secondary glaucoma, and retinal degeneration, Golden Retriever, *Canis lupus familiaris*, canine

Contributor's Comment:
Osteosarcoma is the most common tumor of the appendicular skeleton in dogs and cats.[10] They are locally invasive and readily metastasize to the regional nodes and elsewhere, especially lungs. Extraskeletal osteosarcomas are less common and occur in soft tissue and visceral organs, without any primary bone involvement.[9] Organs commonly affected are the adrenal gland, kidney, mesentery, ileum, liver, spleen, reproductive organs, and the eye.[8] Osteosarcoma in the eye, as a primary process, occurs with even more rarity in dogs and cats.[1,5] This ocular tumor has been reported most in large breed dogs between the age of 9 – 11.5 years.[5] Common clinical signs include exophthalmos[5], hyphema, and blepharospasm.[11]

Histologically, extraskeletal osteosarcomas appear similar to other skeletal osteosarcoma.[7] Within the eye, the tumors are observed in the iris and ciliary body. Tumors are composed of pleomorphic spindle cells or stellate cells, with occasional multinucleated cells resembling osteoclasts, in an abundant extracellular collagenous matrix (osteoid).[5,11] Anaplastic osteoblasts are characteristic of osteoblastic osteosarcoma[2], such as in this case. Other nuclear characteristics include hyperchromic, often eccentric nuclei with varying amounts of basophilic cytoplasm.[3]

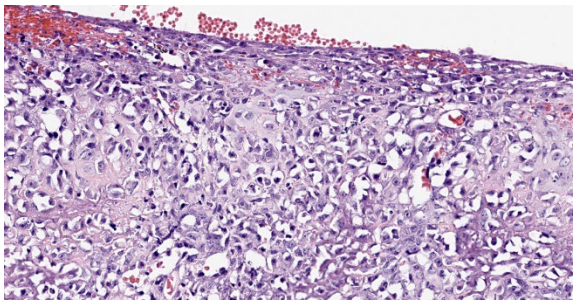

Figure 3-3. Globe, dog. Neoplastic cells are separated and surrounded by pink osteoid matrix. (HE, 318X)

Osteoblastic osteosarcomas are further characterized into 'productive' or 'non-productive' osteosarcoma to indicate the amount of bone production: minimal, lacy strands, irregular islands, and larger accumulation.[3] In dogs, the most common subcategory of skeletal osteosarcoma is moderately productive osteoblastic osteosarcoma.[3] Only one small scale study noted osteoblastic osteosarcoma as being the most frequent type of extraskeletal osteosarcoma in the dog, but was not further subcategorized.[8]

Similar to humans, extraskeletal osteosarcomas are highly malignant, and metastasis is common. However, this occurs less often to the lungs when compared to skeletal osteosarcomas.[9] Decreased survival times result from late detection of tumors and restricted surgical resection of tumors from some sites.[9]

In comparison, ocular osteosarcomas in cats can arise after trauma to the eye.[12] Feline post-traumatic osteosarcoma can occur several months to several years after penetrating injury to the eye. The tumor starts at the lens and progresses towards lining the interior of the eye. It then extends to the scleral venous plexus or optic nerve[10], to diffusely affect the globe.[4,11] The lining of the eye by the tumor is a key feature in differentiating metastatic osteosarcoma vs. primary ocular osteosarcomas. Metastatic rates in these feline cases have occasionally been documented to be 60% with metastasis reaching the brain even post enucleation.[12]

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JPC Diagnosis:
Eye, anterior uvea and sclera: Osteosarcoma.

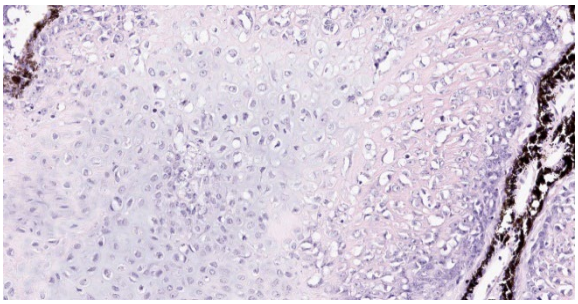

Figure 3-4. Globe, dog. In some areas of the neoplasm, neoplastic cells are entrapped by cartilaginous matrix (HE, 256X)

JPC Comment:
As the contributor mentions, primary intraocular osteosarcoma is rare in veterinary species and has been reported in dogs, cats, a cockatoo, and most recently, a rabbit. Makishima et al. described a primary intraocular osteosarcoma in an 8-year-old lop-eared rabbit. The animal had a history of cataracts, glaucoma, and posterior lens luxation in the left globe several years prior to the development of osteosarcoma. The neoplasm created significant exophthlamos and effaced almost the entire globe, extended beyond the sclera in the retrobulbar space, but did not invade the optic nerve or surrounding tissues. There was no evidence of metastasis, and the animal died due to respiratory failure caused by pulmonary neuroendocrine tumors.[8]

Primary appendicular osteosarcoma can be difficult to differentiate histologically from other primary bone tumors, including chondrosarcoma and fibrosarcoma. One key histologic feature considered diagnostic for osteosarcoma is the presence of osteoblasts producing osteoid. In equivocal cases, immunohistochemistry may be required to differentiate these bone tumors, and a recent study evaluated several markers with varying historical utility in osteosarcoma: alkaline phosphatase (ALP), runx2, osteonectin (ON), and osteopontin (OP). All four markers were found to be very sensitive for osteosarcoma, but specificity varied. The study authors

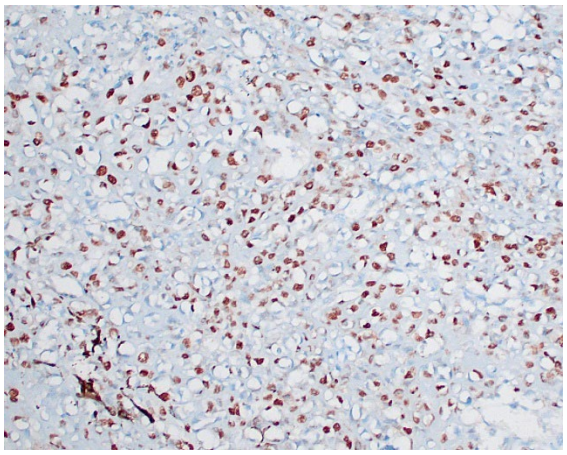

Figure 3-5. Globe, dog. Neoplastic cells, embedded in matrix, demonstrate immunopositivity for SABT2 (anti-SATB2, 400X)

found that ALP, which has the highest sensitivity but low specificity, is best used as an initial screening test, and runx2, an osteoblast transcription factor with highest specificity of the four markers, should be run on ALP-positive sections. Combining these two tests in parallel provided 87% sensitivity and 85% specificity in canine appendicular osteosarcomas.[2]

The moderator discussed how the presence of neoplastic cells within the scleral vessels adjacent to the trabecular meshwork is likely due to passive drainage and not necessarily indicative of primary vascular invasion. Additionally, differentiation between primary and metastatic neoplasia in this case cannot be done histologically and requires correlation with clinical findings. Historically, the lung has been the most common site for osteosarcoma metastasis, but the moderator also discussed that, anecdotally, oncologists are seeing an increased number of osteosarcoma metastasis to atypical sites. This may be due to prolonged survival times with advances in tumor treatment.

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CASE IV:

Signalment:
4.8-years-old, female, Limousin, (*Bos taurus*) bovine.

History:
Presented to the Institute of Veterinary Pathology with a history of thickened skin, hypotrichia, alopecia and signs of lameness.

Gross Pathology:
The skin displayed generalized thickening with folding and wrinkling. The peripheral lymph nodes were enlarged. Multiple white miliary foci were disseminated in the respiratory and vaginal mucosa, fasciae and scleral conjunctivae. The claws revealed rotation of the distal phalanges.

Laboratory Results:
No laboratory findings reported.

Microscopic Description:
Eye (anterior part): Multifocal expansion and bulging of the conjunctiva, iris and sclera by many interstitial mature protozoal cysts. The multi-layered protozoal tissue cysts are 150-350 µm in diameter with an outermost 15-30 µm pale eosinophilic, hyaline capsule. Next is a greenish intermediate layer (5 µm, not in all cysts), followed by a 5-15 µm rim of host cell cytoplasm containing 2-5 giant but flattened nuclei. The majority of the cyst is composed of the central parasitophorous vacuole containing numerous, tightly packed, crescent-shaped, 2x7 µm bradyzoites. Occasionally, a thin concentric layer of spindle-shaped cells with flattened nuclei (fibrocytes) and eosinophilic fibers (mature collagen) surrounds cysts. Some cysts are concentrically surrounded by small to moderate numbers of macrophages, multi-nucleated giant cells, lymphocytes and fewer plasma cells and eosinophils. Occasionally, cysts are directly beneath and bulging iridal anterior and posterior pigment layers and corneal endothelium (not on all slides).

Contributor's Morphologic Diagnosis:
Eye: Conjunctivitis and iritis, multifocal, granulomatous, mild, chronic with intralesional protozoal cysts.

Cause: *Besnoitia besnoiti*

Etiologic diagnosis: Protozoal conjunctivitis

Contributor's Comment:
Bovine besnoitiosis is a chronic debilitating disease of cattle, caused by the cyst-forming

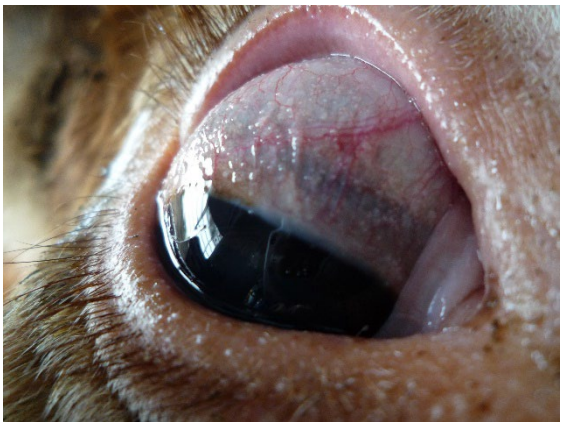

Figure 4-1. Globe, ox. Numerous Besnoitia cysts ae present within the sclera of the globe. (Photo courtesy of: Institute of Veterinary Pathology, Faculty of Veterinary Medicine, LMU Munich, Veterinaerstr. 13, 80539 Muenchen, http://www.patho.vetmed.uni-muenchen.de/index.html)

Figure 4-2. Globe, ox. Multiple sections of the anterior segment of the globe are submitted for examination. (HE, 3X)

apicomplexan parasite *Besnoitia besnoiti*. The disease is re-emerging in Europe and showed rapid spread to Germany, Italy, Switzerland, Hungary, Belgium, and Ireland within eight years.[11] The genus *Besnoitia* has 10 species (Table 4.1).[6] *B. besnoiti*, the type species mainly affects cattle, and wild ungulates may represent a natural reservoir of infection.[10] The life cycle of bovine besnoitiosis is incompletely understood. Cattle represent intermediate hosts, where cyst-formation takes place but the definitive hosts, where intestinal sexual reproduction is thought to occur are unknown. In analogy to the four species with complete knowledge of life cycle (*B. darling, B. neotomofelis, B. oryctofelisi, B. wallacei*) a heteroxenous life cycle is assumed.

In cattle, acute besnoitiosis is characterized by fast proliferation of tachyzoites in endothelial cells and cells of macrophage lineage. Clinical signs include pyrexia, anorexia, nasal and ocular discharge, peripheral lymphadenopathy, lameness and subcutaneous edema. Histological alterations in this stage consist of microcirculatory and vascular lesions like vasculitis, thrombosis, hemorrhage, and edema. Tachyzoites are few in naturally acquired bovine besnoitiosis and are difficult to identify in FFPE-tissues.[1,10]

In the chronic stage, pathognomonic parasitic cysts develop in various tissues. Preferentially affected are non-intestinal mucosa, scleral conjunctiva, skin and fasciae. For unknown reasons and in contrast to peripheral tissues, visceral organs harbor markedly reduced parasite load. Severe chronic bovine besnoitiosis leads to thickening and wrinkling of the skin, sole ulcers and – in bulls – orchitis and sterility.[3,6,13,14] Cysts consist of a central, cytoplasmic, single-membraned parasitophorous vacuole harboring numerous slow-replicating bradyzoites. The host cell cytoplasm surrounding the parasitophorous vacuole is usually thin and contains several enlarged, but flattened hots cell nuclei. The next layer (intermediate layer, inner cyst wall) is not present in every mature cyst but is readily visible in developing cysts. The outermost layer of the cyst is a pale-eosino philic, hyaline layer, consisting of interwoven collagen fibrils.13 The host cell of B. besnoiti is immunohistochemically positive for vimentin and smooth muscle actin suggesting myofibroblast origin.5 Usually, at

Table 4.1

Species	Target organs	Intermediate host	Definitive host
B. akodoni	Internal organs	Grass mouse	Unknown
B. bennetti	Non-intestinal mucosa, skin, SC	Donkey, burro, horse, mule	Unknown
B. besnoitii	Non-intestinal mucosa, skin, SC	Cattle, wild ungulates, antelopes	Unknown
B. caprae	Non-intestinal mucosa, skin, SC	Goat, sheep	Unknown
B. darlingi	Ear, internal organs, SC	Virginia opossum, (lizard)	Cat, bobcat
B. jellisoni	Internal organs	Deer mouse	Unknown
B. neotomofelis	Internal organs	Woodrat	Cat
B. oryctofelisi	Internal organs, muscle	Rabbit	Cat
B. tarandi	Non-intestinal mucosa, skin, SC	Caribou, reindeer, mule deer, muskox	Unknown
B. wallacei	Internal organs	Rat	Cat

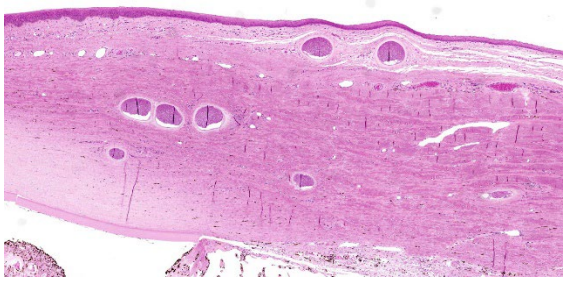

Figure 4-3. Sclera, ox. Cysts of Besnoitia benoitii are present within the sclera. (HE, 47X)

least some cysts are surrounded by pericystic inflammation, which mainly consists of T-cells, macrophages, multinucleated giant cells and eosinophils. Destruction of cysts is evident in subacute and chronic cases.[14]

In animals with obvious lesions, the diagnosis is made via clinical findings and histology. Because most of the chronic cases are mildly clinical or subclinical, diagnosis of bovine besnoitiosis on the herd level requires serology and/or PCR.[7,18,19] Epidemiology of bovine besnoitiosis is incompletely understood. Animals are infected via direct contact, biting arthropods or mechanical vectors. Transmission occurs mainly in the summer months when insect activity is high. In endemically infected herds, there is variable seasonal serological and clinical incidence and prevalence.[9,10]

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JPC Diagnosis:
Eye, conjunctiva, sclera, and iris: Multiple apicomplexan cysts.

JPC Comment:

This represents a classic case of ocular *Besnoitia* in a cow, and this protozoan produces characteristic cysts which can reach up to 5 mm in diameter and are visible on gross examination. Protozoal cysts appear as small white nodules on the haired skin of the face, ears, scrotum, and conjunctiva, where they are called scleral pearls.[2] Other classic signs of chronic *B. besnoitii* infection include alopecia, thickening, and wrinkling of the periocular skin, the scrotum, and other areas of the body due to scleroderma.[17] Gross differentials for the skin lesions include dermatophytosis and photosensitization, both of which can cause blepharitis, and *Moraxella bovis* and *Listeria monocytogenes*, both of which are important causes of conjunctivitis in cattle. None of these differentials would have pearlescent nodules.[3]

The acute phase of *Besnoitia* infection has less specific clinical signs (i.e. fever), lasts less than a month, and often goes undetected. The underlying lesion is vasculitis and thrombosis of small caliber vessels, which some authors have speculated is due to replication of tachyzoites within endothelial cells.[17] The acute phase may result in death due to nephrotic syndrome or respiratory distress.[17] A recent review article found that bulls are likely more susceptible than females to infection, and in the acute phase they can develop significant orchitis and infertility.[17]

This review article also found that infection of animals less than 6 months of age is rare,

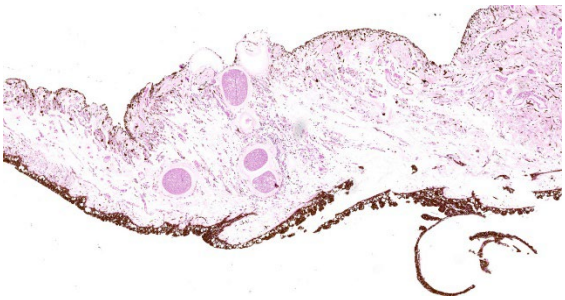

Figure 4-4. Iris, ox. Cysts of Besnoitia benoitii are present within the iris. (HE, 59X)

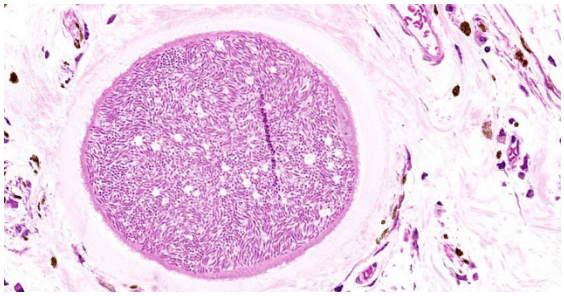

Figure 4-5. Iris, ox. High magnification of Besnoitia cysts demonstrating the thick hyaline wall and numerous enclosed bradyzoites. (HE, 562X)

and that the incidence of infection increased with advancing age.[17]

Besnoitia is an economically important disease in many areas of the world. Most cases are subclinical or have scleral cysts as the only clinical sign. When infection is introduced into a naïve herd, it is expected that 10% to 50% of animals will develop clinical signs and lose economic value within the next three years.[17] In endemically infected herds, the proportion of animals showing clinical signs is expected to increase with time.[17] A recently developed modified live vaccine controls clinical signs but does not appear to be effective in preventing subclinical infection.[17]

Some of the conference participants described the vacuolation of the corneal endothelium in this section as a pathologic change; the moderator, who has a special interest in ocular pathology, explained that this is a normal and expected finding. Unless coupled with attenuation or other corroborating evidence, it should not be considered abnormal.

The moderator discussed other protozoa which can be found in the eye, including *Trypanosoma, Toxoplasma, Leishmania* (25% of systemic cases involve the eye)*, and Neospora caninum; Encephalitozoon cuniculi* can also cause phaecoclastic uveitis in rabbits.

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