



MISTERY SLIDES SESSION - CYTOLOGY -

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Case #1

- Cat, DSH, female, 6-years-old
- Nodule in inguinal mammary gland
- 0.8 cm in diameter
- FNCS of the nodule























Diagnosis

- Cytological diagnosis: malignant spindle cells tumor with chondroid differentiation
- Histological diagnosis: mammary chondrosarcoma

WHO classification of feline mammary tumor

- Malignant tumor
 - Noninfiltrating (in situ) carcinoma
 - Tubulopapillary carcinoma
 - Solid carcinoma
 - Cribriform carcinoma
 - Squamous cell carcinoma
 - Mucinous carcinoma
 - Carcinosarcoma

– <u>Carcinoma or sarcoma in benign tumor</u>

Case #2

- Ferrett, Marshall, male, 6-years-old
- Fever and anorexia
- Ulcerated mass in axillary region
- FNCS of the mass

















Microbiology and PCR

Pseudomonas luteola











Diagnosis

- Cytological diagnosis: septic suppurative inflammation due to Pseudomonas luteola
- Histological diagnosis: severe deep bacterial suppurative and macrophagic dermatitis







INFECTIOUS DISEASE

Pyogranulomatous Pleuropneumonia and Mediastinitis in Ferrets (*Mustela putorius furo*) associated with *Pseudomonas luteola* Infection

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Martinez et al.

- Three pet ferrets in period 2008-2009
- Acute episodes of dyspnoea
- Pleural exudate
 - suppurative inflammation and rod shaped bacteria with a clear surrounding halo
- Necropsy: multifocal necrotizingpyogranulomatous pleuropneumonia and lymphoadenitis with aggregates of encapsulated microrganisms

Morphology of the microrganism

- TEM revealed multiple vesicular blebs of cell membrane (endotoxin release?)
- Polysaccharide capsule
 - Antinflammatory effect
 - Bacteria are able to elude the inflammatory response
- Flagella



Baum B, Richter B, Reifinger M, Klang A, Finnberg C, Loncaric I, Spergser J, Eisenberg T, Künzel F, Preis S, Pantchev N, Rütgen B, Guija de Arespacochaga A, Hewicker-Trautwein M. Pyogranulomatous panniculitis in ferrets (Mustela putorius furo) with intralesional demonstration of Pseudomonas luteola. J Comp Pathol. 2015 Feb-Apr;152(2-3):114-8

- One ferret (Mustela putorius furo) from Finland and two ferrets from Austria, aged 1-4.5 years and of both genders
- Pyogranulomatous subcutaneous inflammation affecting the inguinal, preputial and femoral regions, respectively.
- Histologically, microorganisms were detected within the lesions. The organisms had a capsule that stained positively by the periodic acid-Schiff reaction.
- Pseudomonas spp. were cultured from the lesions in two cases. In the third case, electron microscopy revealed a prokaryotic organism surrounded by an electron lucent matrix. 16S rRNA gene sequencing showed highest sequence homology to Pseudomonas luteola in all three cases.
- These cases might indicate a predisposition of ferrets for infection by these bacteria.

Follow-up

- Teraphy with antibiotics
 - Cefalexin
- Good response for some weeks
 - "The ferret eats and plays!!!"
- Sudden worsening of the conditions
 - Dyspnoea
 - Death
- Necropsy was denied by the owner

Case #3

- Dog, mongrel, 15-years-old
- Pericardial effusion

Test	Risultato	U.M.
Specific gravity	1021	
Total Protein	3.5	g/dL
Nucleated cells	13.83	10 ³ /μL
RBC	1.79	10 ⁶ /µL
Hgb	4.8	g/dL
Hct	15.5	%

Comparison of biochemical investigation

Pleural fluid

Plasma

Test	Risultato	Risultato	Min	Max	U.M.	Test
LDH	1116	65	50	150	IU/L	LDH
Bilirubina totale	1.18	0.40	0.11	0.31	mg/dL	Bilirubina totale
Proteine totali	3.5	6.6	5.7	7.1	g/dL	Proteine totali
Albumine	1.9	2.8	2.7	3.6	g/dL	Albumine
Globuline	1.6	3.8	2.6	3.9	g/dL	Globuline
Colesterolo	177	303	156	369	mg/dL	Colesterolo
Trigliceridi	5	56	30	112	mg/dL	Trigliceridi
Amilasi	703	1475	338	1101	IU/L	Amilasi
Lipasi	69	250	121	725	IU/L	Lipasi
Urea	108	60	16	49	mg/dL	Urea
Creatinina	1.66	1.58	0.83	1.42	mg/dL	Creatinina
Glucosio	1	88	88	119	mg/dL	Glucosio
Sodio	137	148	143	151	mEq/L	Sodio
Potassio	4.8	4.8	3.9	5.1	mEq/L	Potassio
Cloro	107	113	109	118	mEq/L	Cloro
Lattato	12.3	2.0	1.00	3.50	mmol/L	Lattato


















Diagnosis

• Cytological diagnosis: chronic hemorragic effusion with mesothelial reactivity

Bilirubin in urinary sediment











Brenner DS, Drachenberg CB, Papadimitriou JC. Structural similarities between hematoidin crystals and asteroid bodies: evidence of lipid composition. Exp Mol Pathol. 2001 Feb;70(1):37-42.

- Hematoidin crystals (HC) are found in tissues where extravasated erythrocytes undergo degradation.
- Previous studies have determined that hematoidin is composed, in part, of a bilirubin-like pigment
- By LM the HC demonstrated intense, golden-color, fine threads, both intracellularly and extracellularly, in small and large clusters, and in radiating, star-shape patterns ranging in size from 2 to 200 microm.
- By EM the HC were composed of a core of empty clefts, consistent with dissolved lipids, suggestive of cholesterol crystals, and were surrounded by myelinoid membrane aggregates. The AB showed by LM significant morphological similarities with the intracellular HC. By EM, the AB were composed of a core of dense phospholipid bilayer tubes surrounded by a halo of myelinoid membranes.
- We postulate that this shape is due to the physicochemical properties of <u>the</u> <u>accumulated lipids which originate from superfluous cell membranes created</u> <u>during cell fusion in the case of AB and after cellular (predominantly red cell)</u> <u>breakdown in the case of HC.</u> The golden color of the HC likely results from adsorption of hydrophobic bilirubin-like pigments left over from erythrocyte breakdown into the accumulated lipids.

Case #4

- Dog, Golden retriever, male, 8-years-old
- Previous diagnosis of atopy
- Cutaneous nodule on the chin
- FNCS of the nodule









Diagnosis

- Cytological diagnosis: plasma cell tumor with Mott cell differentiation
 - Differential: cutaneous lymphoma with Mott cell differentiation

LABORATORY MEDICINE YESTERDAY · TODAY · TOMORROW

The many faces of Mott cells

Contributed by Paola Cazzini, Victoria Elizabeth Watson, Holly Moore Brown Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA, United States

Mott cells are plasma cells characterized by cytoplasmic globules consisting of retained immunoglobulins, termed Russell bodies.¹ In 1890, Dr. Russell, a pathologist, described some of these intracellular "homogeneous hyaline-like structures" as "fuchsine bodies" based on their staining properties.² Dr. Russell was certain that they represented microorganisms or fungi, and that they were the cause of cancer. In 1905, a surgeon named Dr. Mott described "morular cells" as indicators of chronic inflammation while observing plasmacytic inflammation in lesions caused by trypanosomiasis in human and animal brains.3 They were later called "morula cells of Mott." Mott cells are typically seen after antigen-related stimulation or neoplastic proliferation of plasma cells.1 Russell bodies (RB) can vary greatly in shape, size, and staining properties, and may appear as multiple small spheres, single large inclusions displacing the nucleus, or needle-like inclusions, resulting in bulging of the cytoplasmic membrane (Figure 1). In cytologic specimens RB have a waxy or glassy appearance and generally stain basophilic with Romanowsky stains. In histologic sections RB appear eosinophilic with routine H&E staining. In transmission electron microscopy RB consist of cisternae of the endoplasmic reticulum (ER) that are filled with crystalline or amorphous inclusions.⁴ The accumulation of immunoglobulin chains in the ER is attributed to deficient immunoglobulin export from the ER due to deficient heavy and light chain linkage.^{5,6} which has been associated with the binding of immunoglobulin chains to the hsp70 BiP.6 or the absence of the CH1 domain due to genomic mutation.7

- HSP70: Heat Shock Protein 70
- BiP: Binding immunoglobulin Protein

CASE REPORT

B-cell lymphoma with plasmacytoid differentiation, atypical cytoplasmic inclusions, and secondary leukemia in a dog

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Veterinary Clinical Pathology ISSN 0275-6382

CASE REPORT

B-cell lymphoma with Mott cell differentiation in two young adult dogs

Nicole I. Stacy¹, Mary B. Nabity², Nicole Hackendahl³, Melanie Buote², Jennifer Ward⁴, Pamela E. Ginn⁴, William Vernau⁵, William L. Clapp⁶, John W. Harvey¹



Figure 2. Tissue imprints from 2 dogs with lymphoma. A predominant population of neoplastic intermediate to large lymphocytes is admixed with numerous Mott cells having mild anisokaryosis and anisocytosis. (A) Gastrointestinal mass from dog 1. Wright-Giemsa, × 100 objective. (B) Mesenteric lymph node from dog 2. Diff-Quik, × 100 objective.



Figure 1. (A) Peripheral blood smear. There is a markedly expanded population of intermediate-sized lymphocytes with clumped chromatin, inapparent or small nucleoli, and a moderate amount of pale blue cytoplasm. The lymphocytes lack the morphologic features of the lymphocytes in the inguinal lymph node. (B) Cytologic specimen of the inguinal lymph node. Note the bimorphic population of lymphocytes (arge immature cells mixed with smaller plasmacytoid cells with small dark nuclei) and the variable appearance of the cytoplasmic inclusions. Modified Wright's-Giemsa, x60 objective.

Follow-up

 The dog completely recovered despite a therapy with oclacitinib maleate (Apoquel[®]), an immunosoppressive drug

Case #5

- Dog, Boxer, 7-years-old, male neutered
- Subcutaneous mass on the left dorsum thought to be a cyst by the submitting veterinarian

Courtesy of dr. Kathy Freeman













Diagnosis

 Cytological diagnosis: 'Keloid' fibroma or fibrosarcoma

Cytologic appearance of a keloidal fibrosarcoma in a dog

Liz K. Little, Michael Goldschmidt

Abstract: A 5-year-old neutered male, mixed-breed dog was presented with a single 4-mm, nodular, firm, haired subcutaneous mass on the left flank that had been present for approximately 2 weeks. Cytologic preparations of the mass revealed many spindle cells, few mast cells, rare eosinophils, rare macrophages, abundant hyalinized collagen, and moderate numbers of erythrocytes. The spindle cells were oval to fusiform, with oval nuclei, finely stippled to lacy chromatin, 1–5 variably sized prominent nucleoli, and moderate to abundant cytoplasm with indistinct cell borders, wispy cytoplasmic extensions, and occasionally, fine magenta granulation. The cell population exhibited moderate anisocytosis, moderate anisokaryosis, and rare binucleation. The eosinophilic material occurred both in large angular aggregates with blunt ends and in amorphous aggregates with fine wispy projections. Histologic findings were consistent with a keloidal fibrosarcoma. To the authors' knowledge, this report is the first to describe the cytomorphologic characteristics of a keloidal fibrosarcoma in a dog. (*Vet Clin Pathol.* 2007;36:364–367)

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Figure 1. Fine needle aspirate of a subcutaneous mass in a dog. Note the bands of angular eosinophilic material and the spindle cell population. Wright's-Giemsa, \times 4 objective.



Figure 2. The spindle cell population is often associated with amorphous pink extracellular matrix. Wright's-Giemsa, \times 40 objective.

- Hyalinized collagen bundle fragments.
- More frequently see in mast cell tumour aspirates, but usually not present in large numbers.
- As far as I know no true 'keloid' formation in animals – close as we get is exuberant granulation tissue in horses! Presence does not change prognosis – dependent on histology and histologic grading, but most are not going to metastasize.

Case #6

- Dog, Labrador retriever, Male, 8-years-old
- Hypercalcemia (Ca: 15,7 mg/dl)
- Mass in mediastinum






















Diagnosis

- Cytological diagnosis: thymoma
 Mixed lymphoepithelial thymoma
- Histological diagnosis: thymic carcinoma

Thymic neoplasia

- Thymic lymphoma
 - Neoplasia of lymphoid cells
- Thymoma
 - Neoplasia of the thymic epithelial cells
 - Epithelial thymoma
 - Malignant type: "thymic carcinoma"
 - Mixed lymphoepithelial thymoma
 - Lymphocyte-predominant thymoma
- Thymolipoma/thymofibrolipoma
- Thymic carcinoid
- Squamous cell carcinoma
- Lymphangiosarcoma

Immunophenotype

- CD18 (beta integrin) 74.7%
- CD5 (T lymphocytes) 60.9%
- CD3 (T lymphocytes) 52.4%
- CD4 (T helper) 40.7%
- CD8 (T cytotoxic) 52.2%
- CD21 (B mature lymphocytes) 1.2%
- CD79 (pan B) 12.3%
- CD34 (blasts) 0.2%

Hypercalcemia

- <u>Atwater SW</u>, <u>Powers BE</u>, <u>Park RD</u>, <u>Straw RC</u>, <u>Ogilvie GK</u>, <u>Withrow SJ</u>.
 <u>Thymoma in dogs: 23 cases (1980-1991). J Am</u>
 <u>Vet Med Assoc.</u> 1994 Oct 1;205(7):1007-13.
 - Epithelial thype: 9/23
 - Lymphocytes-rich: 6/23

- Megaesophagus: 11 cases
 - Myasthenia gravis confirmed in 7 of 11
- Hypercalcemia: 2 cases

Case #7

- Dog, mongrel, 10-years-old, male
- Diabetes mellitus
- Liver enlargement
 - Hypoechoic mass within the liver



























Immunostaining: factor VIII

Diagnosis

- Cytological diagnosis: hepatocellular carcinoma
- Histological diagnosis: hepatocellular carcinoma with peritheliomatous differentiation

Vet. Pathol. 18: 427-438 (1981)

Canine Hepatocellular Carcinoma

A. K. PATNAIK, A. I. HURVITZ, P. H. LIEBERMAN and G. F. JOHNSON

Department of Pathology and Department of Medicine, The Animal Medical Center, and Memorial Sloan-Kettering Cancer Center, New York, N.Y.

Abstract. In a study of 110 primary hepatic neoplasms in dogs, 55 hepatocellular carcinomas and two combined hepatocellular and cholangiocarcinomas were diagnosed. These neoplasms were classified into the following 11 groups based on histo-architectural pattern: trabecular, peliod, cobblestone, peritheliomatous, anaplastic, pseudoglandular, pleomorphic, scirrhous, clear cell, solid, and combined hepatocellular and cholangiocarcinoma. The neoplastic hepatocytes varied from almost normal to highly anaplastic spindle cells. Pleomorphic and giant cells were common in some groups, rare or absent in others. Metastasis was found in 61% (35 of 57 dogs), with varied frequencies in the different groups. Cirrhosis was found in only 7% (4 of 57), in contrast to a much higher percent in man, indicating the possibility of a different pathogenesis in the dog.

Case #8

- Dog, Newfoundland, 2-years-old, female
- Used as Life-Guard Dog
 - Specialized in water rescue
 - Trained in small lakes
- Erosive-ulcerative plaque-like nodules on the back and flanks
- FNCS of the lesions



From: http://www.extraordinarydogs.org










Hypercalcemia associated with gastric pythiosis in a dog

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What is your diagnosis? Lymph node cytology from a dog

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Dual infection with Pythium insidiosum and Blastomyces dermatitidis in a dog

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Figure 1. Fine-needle aspirate of a gastric lymph node from a dog. Note parallel-walled, negative-staining hyphae consistent with *Pythium insidio-sum* or *Lagenidium* spp. Modified Wright–Giemsa, × 20 objective.



Figure 1. Popliteal lymph node aspirate from a dog. Wright-Giemsa.



Figure 1. Fine-needle aspirate of a markedly thickened gastric wall of a dog infected with *P. insidiosum*. Many poorly stained hyphae are surrounded by numerous neutrophils and fewer macrophages. Modified Wright's, bar = $20 \,\mu m$.





 In HE stained sections organisms may be seen as clear ghosts within eosinophilic degenerate foci or in other areas of necrosis and inflammation, but generally are not visible without special staining.

> TL Gross, PJ Ihrke, EJ Walder, VK Affolter. Infectious nodular and diffuse granulomatous and pyogranulomatous diseases of the dermis. In: Skin diseases of the dog and cat, 2005, Blackwell





Diagnosis

- Cytologic diagnosis: pyogranulomatous (suppurative-macrophagic?) inflammation due to fungal hyphae
- Histologic diagnosis: fungine pyogranulomatous deep dermatitis

Supplementary tests

- Colture of cutaneous biopsies: no growth
- PCR on cutaneous biopsies: Phytium spp

Phytiosis

- Pythium insidiosum is an oomycete pathogenic in mammals.
- Infection is acquired through small wounds via contact with water that contains motile zoospores or other propagules (zoospores or hyphae).
- Depending on the site of entry, infection can lead to different forms of pythiosis i.e. a cutaneous, vascular, ocular, gastrointestinal and a systemic form, which is rarely seen.
- The infection is not contagious; no animal-animal or animal-human transmission has been reported so far.
- Avoiding stagnant waters could be of help in prevention

Gaastra W et al. Pythium insidiosum: an overview. Vet Microbiol. 2010 Nov 20;146(1-2):1-16.

Case #9

- Cat, DSH, female, 7-years-old
- Firm cutaneous nodule, 2 cm.in diameter, on the left flank
- FNCS of the nodule



















Diagnosis

- Cytologic diagnosis: ossification process or remodelling of preexisting regional bone structures
- Histologic diagnosis: osteoma cutis

Osteoma cutis

- Very rare condition in dog and cat
- Definition:
 - "Osteoma cutis is the late stage of calcinosis cutis"

Thelma Lee Gross et al.

- Others definitions
 - Formation of bone in dermal or subcutaneous tissues
 - Primary: OC in normal skin without evidence of underlying lesion
 - Secundary: OC in damaged or disrupted skin

Multifocal osteoma cutis in a golden retriever

Dawn M. Martin, Jan Hall, Natalie Keirstead, Andrew Lowe

Abstract — A 10-year-old, spayed female, obese golden retriever, presented for immune-mediated thrombocytopenia, was successfully managed with the administration of vincristine and prednisone. However, 6 mo after discontinuing corticosteroid therapy because of suspected iatrogenic hyperglucocorticoidism, the patient was presented with multiple, firm, bilaterally symmetric, dermal masses composed histologically of differentiated cortical bone.

Résumé — Ostéome dermique multifocal chez un Golden retriever. Une Golden retriever âgée de 10 ans, stérilisée et obèse, a été présentée pour une thrombocytopénie à médiation immunitaire et traitée avec succès par administration de vincristine et de prednisone. Six mois après la suspension de la corticothérapie par crainte d'hyperglucocorticoïdisme iatrogène, l'animal présentait de multiples masses dermiques fermes à symétrie bilatérale, composées d'os cortical différentié.

(Traduit par Docteur André Blouin)

Can Vet J 2006;47:360-362

Case #10

- Cat, DSH, 10-years-old, male
- FIV positive
- Fever, generalized arthropathy with swelling of joints
- Flushing of synovial fluid









Diagnosis

- Cytological diagnosis: suppurative noninfectious arthritis with **ragocytes**
- Clinical diagnosis: probably Progressive Feline Polyarthritis

Ragocytes

- Ragocyte are described as being leukocytes with intracytoplasmic granules
 - <u>Davis MJ</u>, <u>Denton J</u>, <u>Freemont AJ</u>, <u>Holt PJ</u>. Comparison of serial synovial fluid cytology in rheumatoid arthritis: delineation of subgroups with prognostic implications. <u>Ann Rheum Dis.</u> 1988 Jul;47(7):559-62.
 - Hollander JE, Mc Carthy DJ, Astorga J et al. Studies on the pathogenesis of rheumatoid joint inflammation. Ann Intern Med. 1965;62: 271-80
 - Delbarre F, Kahan A, Amor B et al. Le ragocyte synovial. Presse med. 1964;72: 2192-32
- Immunofluorescence and others techinques have shown that the granules are the result of leukocytes taking up immune complexes from the fluid
 - Zwaifler NJ. The immunopathology of joint inflammation in rheumatoid arthritis. N Engl J Med. 1972;286: 141-7

Chronic Progressive Polyarthritis in a Female Cat

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ABSTRACT. Feline chronic progressive polyarthritis is a rare immune-mediated disease that has only previously been reported in male cats. A one-year-old female cat was presented with anorexia, lassitude and lameness. The tarsal, carpal and elbow joints revealed swelling, pain, stiffness, crepitus and regional lymphadenopathy, and fever was present. The cat was clinically diagnosed with chronic progressive polyarthritis based on the fever, swelling of joints, imaging of erosive proliferative periosteal polyarthritis, positivity for antinuclear antibody, synovial fluid analyses and urinalyses. Both feline leukemia virus antigen and feline immunodeficiency virus antibody were positive. Using hair root DNA, polymerase chain reaction amplification targeting the sex-determining region on the Y chromosome gene amplified the fragment of DNA from a normal male cat, but not amplified from a normal female cat or the present cat. Accordingly, the present cat was classified as genetically female. Cyclosporine treatment was started, and the general condition and movement quickly improved and continued for 8 months post-diagnosis. This is the first report of chronic progressive polyarthritis in a female cat. KEY WORDS: chronic progressive polyarthritis, cyclosporine, feline leukemia virus, female feline, immune-mediated disease.

- J. Vet. Med. Sci. 72(4): 511-514, 2010

<u>Pedersen NC</u>, <u>Pool RR</u>, <u>O'Brien T</u>. Feline chronic progressive polyarthritis. <u>Am J Vet Res.</u> 1980 Apr;41(4):522-35.

Twenty cats with a chronic progressive polyarthritis were studied. The disorder occurred exclusively in male cats, and all but six of the cats were between 1.5 and 5.0 years of age. There were two forms of the disease as determined by radiographic changes: joint instability and deformity, and clinical course. The most prevalent form of the disease was characterized by osteopenia and periosteal new bone formation surrounding affected joints. Marginal periarticular erosions and collapse of the joint spaces with fibrous ankylosis occurred with time, but joint instability and deformities were not seen. The second form of the disease was characterized by severe subchondral marginal erosions, joint instability, and deformities. The periosteal proliferative form resembled Reiter's arthritis of man, and the deforming type resembled human rheumatoid arthritis. The disease began as tenosynovitis and synovitis, with subsequent changes in the articular cartilage and periosteal bone. Histopathologic changes in these cats were similar to those occurring in both chronic Reiter's and rheumatoid arthritis of man. Chronic progressive polyarthritis of cats was not caused by identifiable bacteria or mycoplasma, but was etiologically linked to feline leukemia virus (FeLV) and feline syncytia-forming virus (FeSFV) infections. The FeSFV was isolated from the blood or was detected by a serologic test in all of the cats with the disease, whereas FeLV was isolated or identified by immunofluorescence technique in 60% of the cats. The arthritis could not be reproduced by inoculation of cell-free synovial tissue from diseased cats or with tissue culture fluid containing FeSFV and FeLV isolates. It was postulated that arthritis was an uncommon manifestation of FeSFV infection that occurred in predisposed male cats. Feline leukemia virus may not have been directly involved in the disease, but may have acted in some way to potentiate the pathogenic effects of FeSFV.