The Armed Forces Institute of Pathology
Department of Veterinary Pathology

Conference Coordinator
Matthew Wegner, DVM

WEDNESDAY SLIDE CONFERENCE 2010-2011

Conference 7

29 September 2010

Conference Moderator:
Thomas Lipscomb, DVM, Diplomate ACVP

CASE I: 598-10 (AFIP 3165072).

Signalment: 14-month-old , female, intact, Boxer dog (*Canis familiaris*).

History: Intestine and colon biopsies were submitted from a patient with chronic diarrhea.

Gross Pathology: Not reported.

Histopathologic Description: Colon: The small intestine is normal but the colonic submucosa is greatly expanded by swollen, foamy/granular histiocytes that occasionally contain a large clear vacuole. A few of these histiocytes are in the deep mucosal lamina propria as well, between the muscularis mucosa and the crypts. Many scattered small lymphocytes with plasma cells and neutrophils are also in the submucosa, and the histiocytic inflammation is also expanding into the inner muscular wall in some areas (may not be in submitted slide). The histiocytes sometimes contain many PAS-positive granules (showing PAS positive and negative histiocytes with Goblet cells in colonic mucosa), many do not, and no fungi or acid-fast bacteria are present.

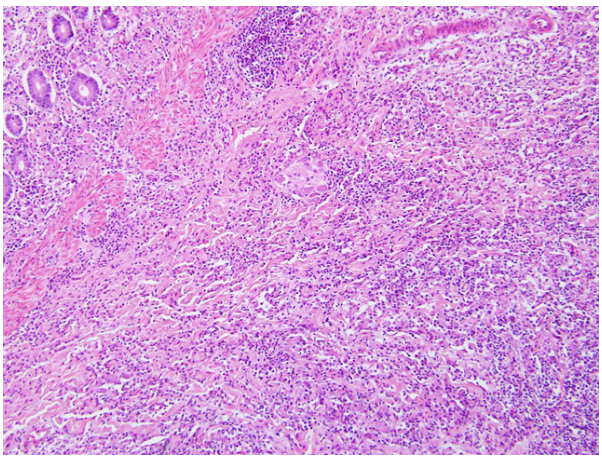
Contributor's Morphologic Diagnosis: Histiocytic ulcerative colitis of Boxer dogs.

Contributor's Comment: Boxers are prone to this condition, usually before two years of age, and an altered immunity is suspected.[1] The histiocytes sometimes contain many PAS-positive granules which are thought to be phagocytic debris and possibly phagocytized organisms that perhaps Boxers and French bulldogs are not able to process due to a genetic lysosomal defect.[1] In recent years, the condition has been successfully treated with enrofloxacin[2] and a new report indicates that this treatment correlates with eradication of intramucosal *Escherichia coli*, and the few cases that don't respond have an enrofloxacin-resistant strain of *E. coli*.[3]

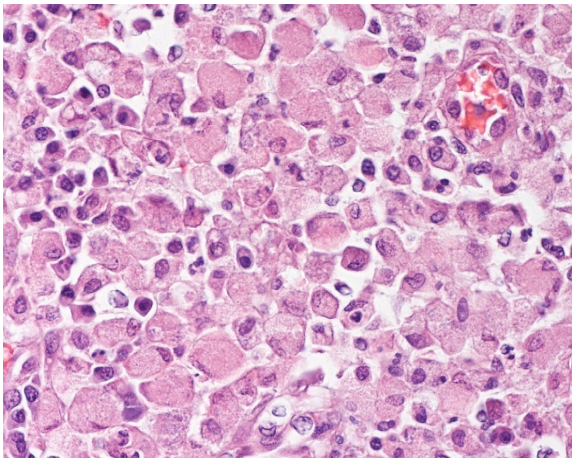
The histiocytic influx is reportedly centered in the submucosa and into the deep mucosa and may expand through the muscular wall to the serosa and adjacent lymph nodes.[1] Mucosal biopsies only may miss the lesions. Mucosal ulceration progresses with chronicity from superficial erosions to patchy ulcers that stop at the submucosa to only patchy intact islands of mucosa.

This dog was euthanized for this condition. A male littermate is normal. Interestingly, the clinician reported that he had an unrelated (unconfirmed) case in a young Boxer about this time and it did respond well to three weeks of enrofloxacin treatment.

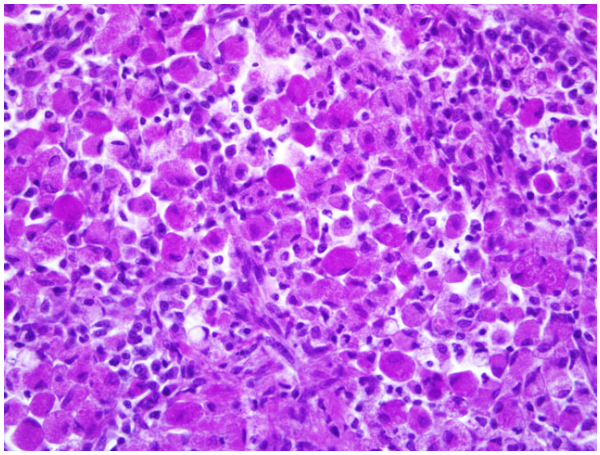
AFIP Diagnosis: 1. Colon: Colitis, histiocytic and lymphoplasmacytic, mucosal and submucosal, diffuse, severe with intrahistiocytic granular eosinophilic material.
2. Small intestine: Enteritis, histiocytic and lymphoplasmacytic, focally extensive, moderate with intrahistiocytic granular eosinophilic material.



1-1. Colon, dog. The mucosa and submucosa are markedly expanded by many mixed inflammatory cells that widely separate and replace colonic crypts. (HE 100X



1-2. Colon, dog. The cellular infiltrate is composed of many swollen, foamy to granular histiocytes. Photographs courtesy of AR Livestock and Poultry Commission Lab, Little Rock, AR 72215, jbritt@alpc.argov



1-3. Colon, dog. Histiocytes are filled with many granules that are demonstrated by the PAS stain. (Periodic-acid Schiff 400X)

Conference Comment: A number of studies over the years have noted bacteria within macrophages in histiocytic ulcerative colitis of Boxer dogs (HUC), but recognized pathogens such as *Salmonella*, *Campylobacter* and *Shigella* have not been detected. The very strong breed predisposition and the absence of an identified infectious agent resulted in the conclusion that the condition is a breed specific immune-mediated disease of unknown cause. However, some affected dogs were found to respond to treatment with chloramphenicol and, more recently, to enrofloxacin (a fluoroquinolone antibiotic). It has been noted that HUC has features that are similar to human forms of inflammatory bowel disease, such as Crohn's disease. Common features include granulomatous inflammation, bacteria within macrophages and responsiveness to fluoroquinolone antibiotics. HUC also has similarities to ulcerative colitis and Whipple's disease. Recent studies have shown that certain adherent and invasive strains of *Escherichia coli* are present in the lesional tissues of affected dogs. These strains have strong similarities to *E. coli* strains associated with some cases of Crohn's disease. HUC and Crohn's disease associated strains are more similar to *E. coli* associated with extraintestinal disease than to those causing diarrhea. These findings support the emerging concept that inflammatory bowel diseases result from an overly aggressive immune response to bacterial microflora in genetically susceptible individuals.[4]

In the sections of small intestine examined during conference, predominantly histiocytic inflammation similar to that present in the colon was found.

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http://www.arlpc.org

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1. Brown, CR, Baker, DC, Barker, IK. Alimentary System. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 5th ed., volume 2. Philadelphia, PA: Elsevier Ltd; 2007:112-113.
2. Davies DR, O'Hara, AJ, Irwin, PJ, Guilford, WG. Successful management of histiocytic ulcerative colitis with enrofloxacin in two Boxer dogs. *Australian Vet J*. 2004;82:58-61.
3. Mansfield, CS, Craven, JM, et al. Remission of histiocytic ulcerative colitis in Boxer dogs correlates with eradication of invasive intramucosal *Escherichia coli*. *J Vet Intern Med*. 2009;23:964-969.
4. Simpson, KW, Dogan, B, Rishniw, M et al. Adherent and invasive *Escherichia coli* is associated

with granulomatous colitis in Boxer dogs. *Infection and Immunity.* 2006;74:4778-4792.

CASE II: 9-1947 (AFIP 3164947).

Signalment: Adult, female, Pacific white-sided dolphin (*Lagenorhynchus obliquidens*).

History: An approximately 31-year-old, 126 kg, adult female, Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) maintained in a semi-closed, 3.8 million litre captive display pool with a long history of intermittent gastrointestinal problems was presented with sudden anorexia, abdominal pain, and vomiting. The aging dolphin had had multiple antibiotic treatments in response to inflammatory blood profiles and inappetence at several public display institutions and was known as an "old dolphin that often goes off". Although gastrointestinal disease had been suspected, the cause of the recurrent inflammatory changes in the peripheral blood was never definitively diagnosed. Starting in 2006, budding yeast and pseudohyphae were found on oral and gastric cytology in association with lethargy, inappetence and recurring inflammatory changes. Antifungal agents including oral itraconazole and nystatin were used and appeared to speed recovery and decrease the severity of the clinical signs. Repeated endoscopy of the esophagus and proximal stomach showed no significant lesions, although a thick koilin coating of the stomach occasionally hampered close examination of the gastric mucosa.

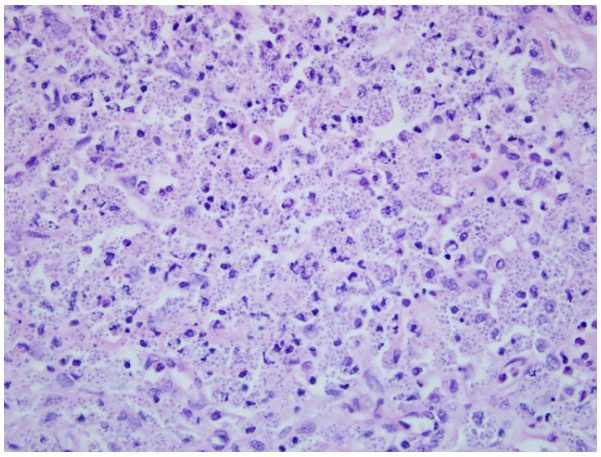
Gross Pathology: Necropsy showed an emaciated animal with moderate abdominal distension. On incision of the abdominal wall, there was approximately 2 L of serosanguineous ascites and an intestinal torsion within the craniodorsal aspect of the abdominal cavity with displacement of adjoining viscera. Extending from the duodenum caudally to the midlevel of ileum, there was multifocal to coalescing and occasional segmental yellow discoloration of the intestinal mucosa with variable amounts of submucosal edema and multifocal caseous to friable yellow white deposits. In more distal regions of the bowel, the serosa featured a fine cobblestone to granular texture, and was glistening and stippled to mottled dark red black.

Laboratory Results: Special culture on selective media identified *Candida krusei*.

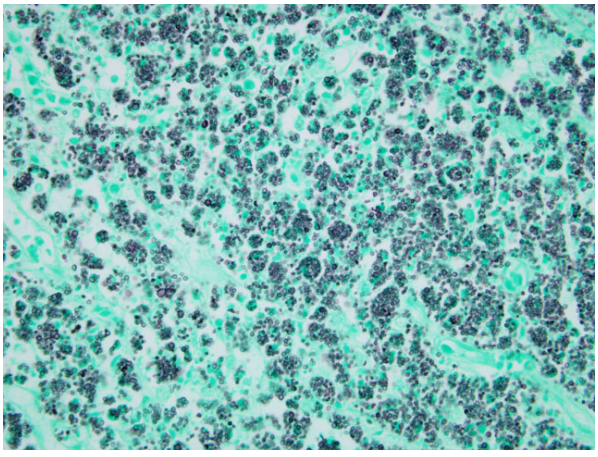
Histopathologic Description: Jejunum and small intestine: Microscopically, there was marked fibrinosuppurative and lymphohistiocytic enteritis with florid intralesional yeast.

Contributor's Morphologic Diagnosis: 1) Jejunum: Torsion, severe, segmental, acute with infarction and hemorrhage (Gross diagnosis)
2) Small intestine: Enteritis, marked, nodular to diffuse, lymphohistiocytic and fibrinosuppurative, with florid intrahistiocytic yeast morphologically consistent with *Candida* spp.

Contributor's Comment: Microscopic assessment of the grossly noted submucosal nodular proliferations in the multiple segments of small intestine disclosed florid, predominantly intrahistiocytic, yeast morphologically suggestive of *Histoplasma capsulatum, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Sporothrix schenckii, Torulopsis* (*Candida*) *glabrata*, and *Candida* spp. In-house culture yielded moderate growth of *Candida* spp. and submission of fresh tissue to a reference lab, the British Columbia Centre for Disease Control, for special culture on selective media identified *C. krusei*,



2-1. Intestine, Pacific white-sided dolphin. The inflammatory population is composed of many macrophages, lymphocytes, and neutrophils. Within histiocytes there are numerous yeast. (HE 400X)



2-2. Intestine, Pacific white-sided dolphin. The intrahistiocytic yeasts are highlighted by the GMS stain. (GMS 400X)

which is considered significant.[1] This organism is the conidal state of *Issatchenkia orientalilis* and is considered a commensal of the mucus membranes and skin of animals and humans. *Candida krusei* has been occasionally associated with bovine mastitis and there is a single case report of bronchopneumonia secondary to candidemia in a Holstein heifer. Fatal colonization of a gastrostomy tube has been reported in a cat. *Candida* spp. infections in humans are generally localized to the gastrointestinal, urogenital and respiratory tracts and have been associated with prolonged antibiotic administration, haemodialysis, chemotherapeutic agents, cancer or other severely debilitating disease, penetrating abdominal trauma, or patients with indwelling catheters. The pathogenesis of infection is characterized by initial colonization of mucocutaneous junctions of mucosa of the gastrointestinal tract, then proliferation, and then deeper tissue invasion. Candidiasis has been documented in a number of marine mammals, including bottlenose dolphins, killer whales, false killer whales, harbour seals, northern fur seals, California sea lions and a pygmy sperm whale. Infection may present as disseminated or more localized, such as dermatitis, blowhole erosions, glossitis, pharyngitis, pneumonia, nephritis, cystitis, or esophagitis.[4] To the best of our knowledge, *C. krusei* has not previously been reported in marine mammals. In this animal, there were no apparent pre-existing conditions within the examined tissues which may have predisposed or exacerbated infection.

AFIP Diagnosis: 1. Intestine: Enteritis, histiocytic, lymphocytic and neutrophilic, multifocally extensive, severe, with extensive ulceration and myriad intrahistiocytic yeast.
2. Intestine: Enteritis, mesenteritis, and peritonitis, fibrinosuppurative, multifocally extensive, severe, with necrosis, hemorrhage and myriad bacilli.

Conference Comment: Conference participants favored *Histoplasma capsulatum* as the etiology of the fungal infection. This highlights the potential problems associated with relying on morphology alone in the diagnosis of many infections. Correlation of histomorphology with culture and other specific techniques can avoid diagnostic errors. Intrahistiocytic clusters of the yeast-like forms of *Candida* can closely resemble *Histoplasma* in histologic sections. This is particularly true of *Candida glabrata*. Additionally, bacilli, which were demonstrated to be gram-negative, were present in areas of fibrinosuppurative inflammation and necrosis in the sections examined at conference.

Participants found this case to be a good opportunity to review the clinicopathologic features of candidiasis. As noted by the contributor, candidiasis typically presents clinically as a superficial mycosis of mucous membranes most often in young, debilitated, or immunocompromised animals, or those receiving prolonged courses of antibiotic therapy. Common anatomic locations of candidiasis include the mouth, esophagus, crop, and proventriculus in birds; the oral mucosa in mammals; and the stomach in piglets.[2] Birds are affected by *Candida* species more frequently than mammals. Grossly, infection by *Candida* results in a white pseudomembrane overlying mucous membranes. Histologically, pseudohyphae, blastoconidia, hyphae, and yeast-like organisms are present, and there is often necrosis or ulceration.

Conference participants also discussed specifics of virulence and immunity in candidiasis. *Candida* species have the ability to change phenotypes in a random and reversible manner in response to changes in the host environment resulting from antibiotic treatment, immune response, or altered host physiology. The phenotypic variants can exhibit changes in colony morphology, cell shape, antigenicity, and virulence. Virulence is related to the organism's ability to adhere to cells, and adherence to host cell is mediated by several classes of adhesins. One class of adhesions is an integrin-like protein which binds to arginine-glycine-aspartic acid groups on fibrinogen, fibronectin, and laminin. A second adhesins class, resembling transglutaminase substrates, binds to epithelial cells; and a third group of agglutinins binds to endothelial cells or fibronectin. Several secreted enzymes, such as aspartyl proteinases, aid in tissue degradation, facilitating organism invasion. *Candida* species also secrete adenosine to block neutrophil degranulation, thus preventing the production of free oxygen radicals which would be damaging to the organisms.[2]

Both innate and cell-mediated immunity are necessary for clearing infections. Neutrophil and macrophage phagocytosis and subsequent oxidative destruction of the yeast are important in preventing establishment of infection. The filamentous form of this organism escapes the phagolysosome and replicates in the cytoplasm of infected cells. *Candida* yeasts stimulate dendritic cell production of IL-12 to a greater degree than the filamentous form, resulting in a protective T_H1 response; in contrast, the filamentous form induces a non-protective T_H2 response.[3] Similar to other fungi, this organism elicits a T_H17 response, resulting in recruitment of neutrophils and monocytes to the site of infection. For a thorough review of the general pathology involved in the various T-cell responses, readers are encouraged to review WSC 2008-2009, Conference 16, Case 4.

Contributor: Animal Health Center, British Columbia Ministry of Agriculture and Lands, 1767 Angus Campbell Road, Abbotsford, BC, V3G 2M3

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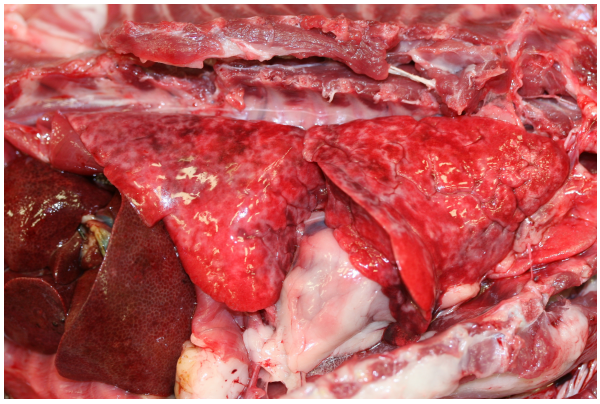
CASE III: TAMU-02 2010 (AFIP 3167479).

Signalment: 13-year-old, female, domestic long hair, feline (*Felis catus*).

History: The cat had a 5-day history of increased respiration, with 3-4 days of rapid shallow breathing and 2 weeks of weight loss. The owners had noticed a recent change in the cat's vocalization. Thoracic radiographs revealed a diffuse bronchointerstitial pattern throughout the lungs. There was no response to treatment with bronchodilators or glucocorticoids.

Gross Pathology: All lung lobes are diffusely firm, fail to collapse, and have an irregular surface that is mottled pink and dark red. The lungs ooze red fluid on cut section (edema).



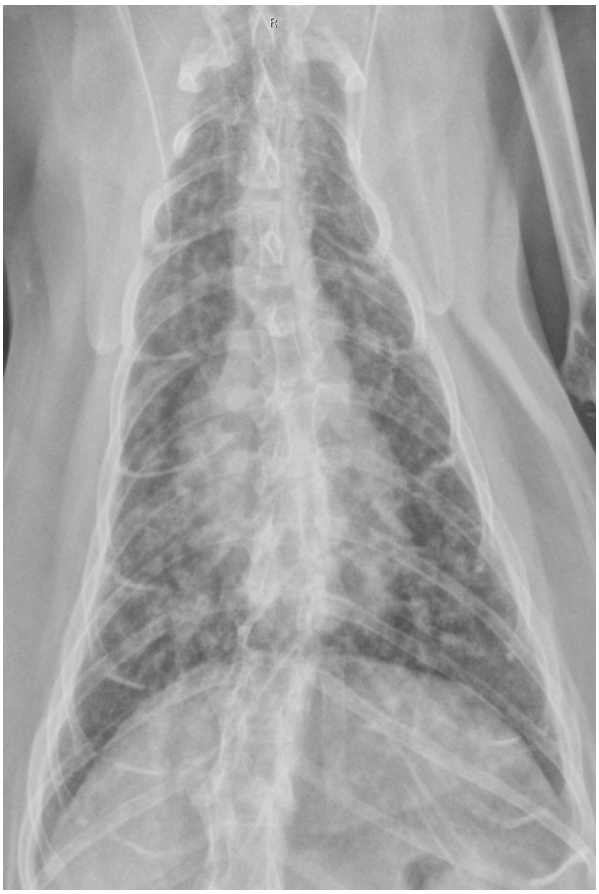
3-1. Lung, cat. Lung lobes are firm, fail to collapse, and have an irregular surface that is mottled pink to dark red. Photograph courtesy of Department of Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, wcorapi@cvm.tamu.edu

Laboratory Results: Histoplasmosis antigen detection using an enzyme immunoassay was negative.

Histopathologic Description: Lung: Throughout the section of lung, large numbers of alveoli and alveolar ducts contain densely packed, round to slightly spindle-shaped cells with variably distinct cell borders that completely fill the alveolar and ductal lumens. The cells form dense rounded clusters within alveoli and are often arranged in a lightly streaming pattern. The infiltrating cells have a histiocytic appearance, characterized by a moderate amount of lightly eosinophilic to pale basophilic cytoplasm, which is sometimes lightly vacuolated, and cell nuclei that are round to oval, often eccentrically placed and slightly indented, and which contain variably condensed basophilic chromatin. Mitotic figures are rare (<1 per 400x field). Some alveoli also contain individual or small central clusters of macrophages with abundant, highly vacuolated, foamy cytoplasm and small condensed nuclei. Many alveoli are segmentally lined by prominent, cuboidal epithelial cells (type II pneumocyte hyperplasia). The smooth muscle within alveolar septae is markedly thickened in many areas (smooth muscle hyperplasia). Peribronchiolar and peribronchial lymphocytes are prominent, and clusters of densely packed lymphocytes with lesser numbers of plasma cells are also scattered throughout the section. Many alveoli contain abundant eosinophilic proteinaceous fluid (edema). In some areas the normal alveolar architecture is replaced by thin interlacing bands of collagenous tissue (interstitial fibrosis), with moderate numbers of lymphocytes and plasma cells and multifocal areas of mild hemorrhage. Alveolar septae are lost in many areas resulting in enlarged, confluent air spaces (emphysema).

Contributor's Morphologic Diagnosis: Severe, diffuse, proliferative, alveolar histiocytosis with smooth muscle hyperplasia, interstitial fibrosis and type II pneumocyte hyperplasia.

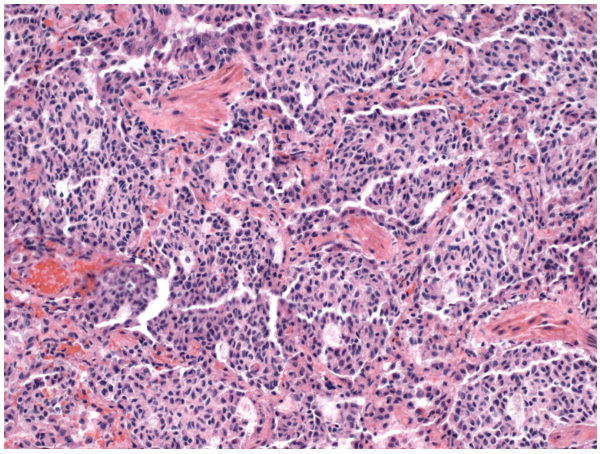
Contributor's Comment: The marked pulmonary histiocytic disease in the lungs of this cat is representative of a recently described histiocytic proliferative disorder in cats, pulmonary Langerhans cell histiocytosis (PLCH), that targets the lungs but can variably affect other organs.[3] The severe bronchial pattern with a moderate interstitial component observed throughout the lungs in thoracic radiographs of this cat was clinically suggestive of histoplasmosis, severe asthma, or inflammatory airway disease. However, histoplasmosis antigen detection using an enzyme immunoassay to detect antigenuria was negative, and the cat did not respond to treatment with bronchodilators or glucocorticoids. Gross necropsy findings, which included impression smears showing a uniform population of what was presumed to be



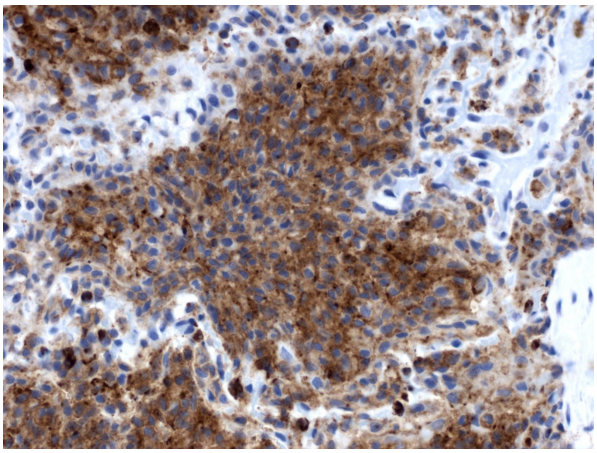
3-2. Lung, cat. Thoracic radiographs demonstrate a bronchointerstitial pattern throughout the lungs. Photograph courtesy of Department of Pathobiology, College of Veterinary Medicine and Biomedical sciences, Texas A&M University, College Station, Texas, wcorapi@cvm.tamu.edu

spindle cells, were more suggestive of pulmonary neoplasia or fibrosis. The presence of a uniform population of cohesive and streaming histiocytic cells within many alveoli and alveolar ducts throughout the lung of this cat is consistent with PLCH. The infiltrating cells were strongly positive for vimentin, CD18, and E-cadherin, which supports a Langerhans cell phenotype as previously described.[3]

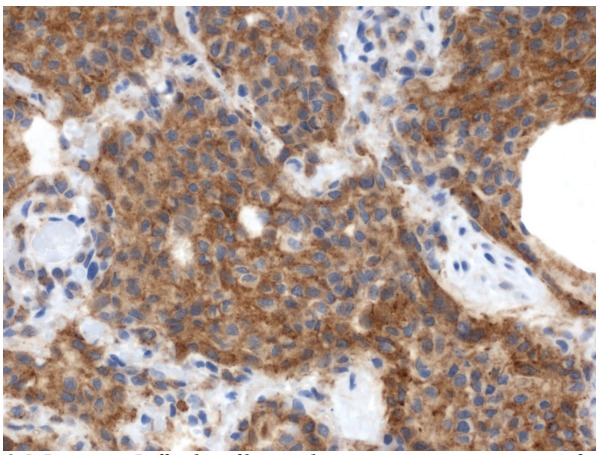
In the corresponding human interstitial lung disease, which occurs primarily in young adult cigarette smokers, PLCH was found to be the result of mixed clonal and nonclonal expansion of nonmalignant Langerhans cells that arises in a setting of Langerhans cell hyperplasia.[11] This, along with the fact that it is frequently associated with clinical regression after steroid therapy and cessation of smoking, supports the view that PLCH represents a reactive disorder rather than a neoplastic process. It occurs in a slightly higher percentage in women and is frequently found only in the lungs, although multiorgan involvement may also occur.[9,10] PLCH represents one of a spectrum of Langerhans cell proliferative diseases (Langerhans cell



3-3. Lung, cat. Multifocally, many alveoli and alveolar ducts contain a uniform population of cohesive and streaming histiocytic cells with abundant foamy cytoplasm. Photograph courtesy of Department of Pathobiology, College of Veterinary Medicine and Biomedical sciences, Texas A&M University, College Station, Texas, wcorapi@cvm.tamu.edu



3-4. Lung, cat. Diffusely, infiltrating histiocytes are immunopositive for CD-18. Photograph courtesy of Department of Pathobiology, College of Veterinary Medicine and Biomedical sciences, Texas A&M University, College Station, Texas, wcorapi@cvm.tamu.edu



3-5. Lung, cat. Diffusely, infiltrating histiocytes are immunopositive for E-cadherin. Photograph courtesy of Department of Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, wcorapi@cvm.tamu.edu

histiocytosis), which occur more frequently in children and are marked by proliferation and infiltration of various organs by Langerhans cells.[8,10] In addition to the lung, the organs most commonly affected by Langerhans cell histiocytosis include bone and skin, although any organ can be affected. In the recent report of PLCH in 3 cats, affected organs included the lung, pancreas, kidney, liver, and various lymph nodes.[3] Extrapulmonary involvement was not observed in the present case. A characteristic feature of PLCH, in addition to the marked proliferation of alveolar histiocytes, is the presence of rod-shaped Birbeck granules, the hallmark organelle of the Langerhans cell, within the cytoplasm of lesional histiocytes when viewed with transmission electron microscopy.

AFIP Diagnosis: Lung: Histiocytosis, atypical, intrabronchiolar and intra-alveolar, multifocal, marked, with extensive alveolar edema, moderate lymphoplasmacytic and histiocytic inflammation, and hyperplasia of bronchiolar smooth muscle, consistent with pulmonary Langerhans cell histiocytosis.

Conference Comment: Conference participants readily identified the overwhelming infiltrate of histiocytic cells in the lung described by the contributor, but most experienced difficulty with histologic interpretation of the underlying pathologic process; some favored a neoplastic condition, while others interpreted the lesion as granulomatous inflammation. The conference moderator emphasized that the infiltrative cell type consists almost exclusively of histiocytic cells with mildly atypical morphology, including mild anisokaryosis and hyperchromatic nuclei, supporting a histiocytic proliferative lesion versus granulomatous inflammation. The striking similarity to the cases reported by Busch *et al*. strongly supports pulmonary Langerhans cell histiocytosis (PLCH). The immunohistochemical findings reported by the contributor provide further confirmation.

Reports of histiocytic diseases of the cat are few and limited to feline progressive histiocytosis,[2] feline pulmonary Langerhans histiocytosis,[3] histiocytic sarcoma[7] and hemophagocytic histiocytic sarcoma;[4] the cell type in the first two conditions is of Langerhans cell lineage, dendritic cell origin for the third, while the findings in the last entity are most consistent with macrophage origin. In the feline progressive histiocytosis and feline pulmonary Langerhans histiocytosis, it remains uncertain whether the conditions represent a reactive or neoplastic process. In contrast to cats, histiocytic diseases in the dog are much more common, and the nature of the conditions as reactive or neoplastic are better characterized. The classifications and corresponding cell of origin are as follows: reactive cutaneous/ systemic histiocytosis (interstitial dendritic cell); cutaneous histiocytoma (Langerhans cell); local and disseminated histiocytic sarcoma (myeloid dendritic cell); and hemophagocytic histiocytic sarcoma (macrophage).[1,5,7]

Development of dendritic cells, Langerhans cells and macrophages begins with CD34+ progenitor cells in the bone marrow, and further differentiation produces three subsets of cells: CD34+/CLA+ (cutaneous lymphocyte antigen); CD34+/CLA-; and CD34+/ IL-3Rα-rich cells.[1] The CD34+/CLA+ and CD34+/ CLA- cells, under the influence of stem cell factors or GM-CSF and TNF-α differentiate into CD1+/CD14- and CD1a-/CD14+ cells, respectively. Again, under the influence of GM-CSF and TNF-α, CD1a-/CD14+ cells differentiate into interstitial dendritic cells, or, if stimulated by M-CSF, undergo differentiation to macrophages. The CD1+/CD14- subtypes differentiate into Langerhans cells under the influence of GM-CSF, TNF-α, and TGF-α. The CD1a/CD14+ subtypes can also arise from CD14+ blood monocytes under the influence of GM-CSF and IL-4. Myeloid dendritic cells arise from CD34+/IL-3Rα-rich cells when stimulated by IL-3 and GM-CSF.[1]

The immunophenotyping of the cells comprising the histiocytic diseases varies based on the reference text or journal consulted, and reflects the continuous information explosion in this very active field of research. After review of the literature and the veterinary reference text of *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*, the following list outlines the most consistent and commonly cited immunophenotypes:[1-7]

- Langerhans cells: MHC II; CD1a, c; CD11c; CD18; langerin; ICAM-1; and E-cadherin positive
- Interstitial dendritic cells: MHC II; CD1c; CD4; CD11b, c; CD18; CD90 (Thy-1) positive
- Myeloid dendritic cells: MHC II; CD1; CD11c; ICAM-1; ± CD90 positive
- Macrophage: MHC II; CD11d/CD18; α2-integrin; ± CD11c/CD18 and CD1c positive

Readers are encouraged to review Wednesday Slide Conference 24, Case IV, 2008-2009, for a thorough review of canine histiocytic diseases.

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http://www.cvm.tamu.edu/vtpb/

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CASE IV: UFSM-1 (AFIP 3065818).

Signalment: 5-year-old, male, mongrel, dog (*Canis familiaris*).

History: The dog presented with apathy, anorexia, vomiting, and diarrhea with blood, icterus, fever (40.8°C), mild dehydration, tachycardia, dyspnea and subcutaneous edema in the pelvic limbs and generalized enlargement of the lymph nodes.

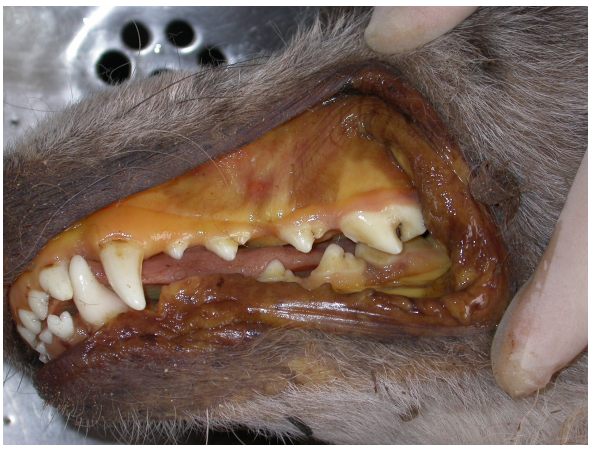
Laboratory Results: A CBC performed at presentation revealed hypochromic macrocytic regenerative anemia, leucocytosis due to regenerative left shift and lymphocytosis, and regenerative thrombocytopenia (Table 1). Blood smears revealed marked anisocytosis and polychromasia, several RBC's with Howell-Jolly bodies, and large numbers of nucleated RBC's, mainly metarubricytes (23/100 leucocytes), but also lesser numbers of rubricytes (2/100 leucocytes). Marked spherocytosis and large platelets (macroplatelets) were additional findings in the blood smear. No blood parasites were found either within blood cells or free in the plasma.

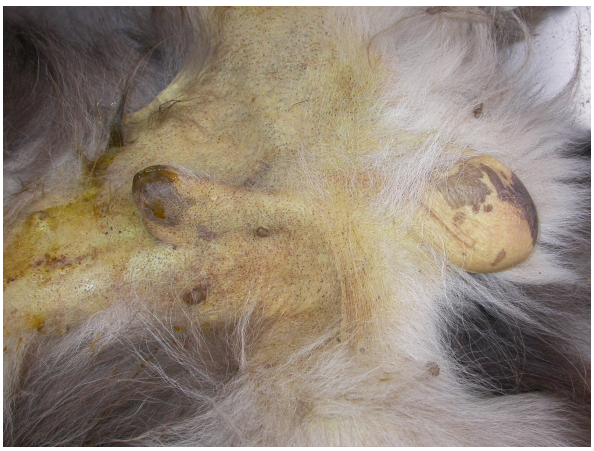
The biochemistry panels showed mild increase in total plasma proteins due to increase in albumin, moderate increase in serum activity of alanine aminotransferase and marked increase in the total serum bilirubin (Table 2). Urinalysis revealed marked bilirubinuria.

Based on the laboratory results described above, a clinical diagnosis of extravascular hemolytic anemia was established. The marked spherocytosis suggested an immune mediated origin. The dog was treated with 1 mg/kg prednisone but died the following day.

Gross Pathology: There was marked yellow discoloration (icterus) of mucous membranes, skin, subcutaneous tissues and intima of large arteries. The spleen was markedly (about 5x) enlarged and had a dry (no blood oozing) fleshy texture to the cut surface. All lymph nodes were moderately enlarged, soft, light brown and wet at cut surfaces. The mucosa of the entire small intestine was dark red (hemorrhagic) and the contents were admixed with blood. The lungs were red and wet and did not collapse when the thoracic cavity was opened. Large amounts of fluid oozed from the cut surface of the lungs. A large amount of whitish-pink foam could be observed within the trachea and main bronchi (pulmonary edema). Additional findings included serous atrophy in the coronary adipose tissue of the heart, hydropericardium, petechiae and paint brush hemorrhages in the endocardium of the left ventricle. The bone marrow of the long bones was markedly red and filled the whole marrow space.

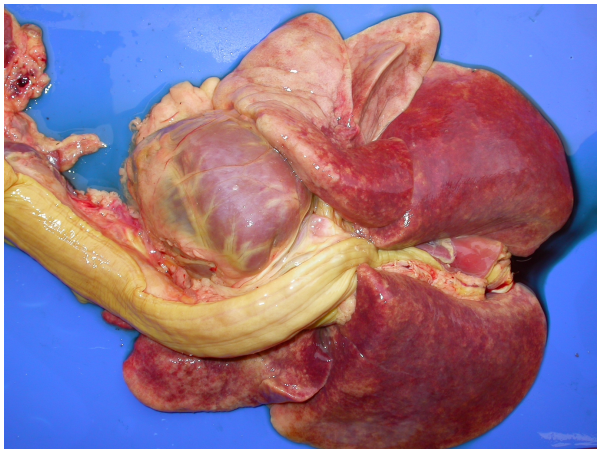
Histopathologic Description: Spleen and heart: In the spleen there is a marked inflammatory infiltrate consisting of some lymphocytes and large numbers of plasma cells. The cellular infiltrate obliterates a large part of the splenic red pulp. There are few plasmablasts and Mott cells within the inflammatory infiltrate; however, the majority of the inflammatory cells consists of mature plasma cells. Multiple aggregates of histiocytes are seen throughout the spleen and compress the adjacent splenic tissue. Oval to round, 2µm protozoal organisms can be observed within the cytoplasm of endothelial cells of the splenic capillaries, but not in venules, arterioles, veins or arteries. There are 5-20 organisms per parasitized cell. In the liver (not submitted), there was paracentral coagulative zonal necrosis; the cholangioles and bile



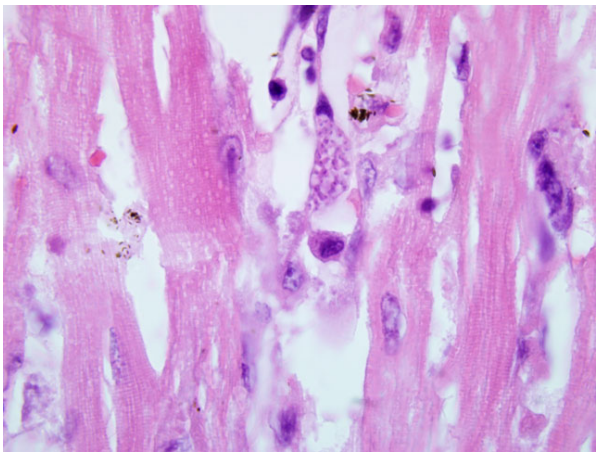


4-1, 4-2. Oral mucosa and skin, dog. Diffusely there is yellow discoloration (icterus) of mucous membranes and skin. Photographs courtesy of Departamento de Pathologia, Universidade Federal de Santa Maria, Santa Maria Brazil, claudioslbarros@uol.com.br

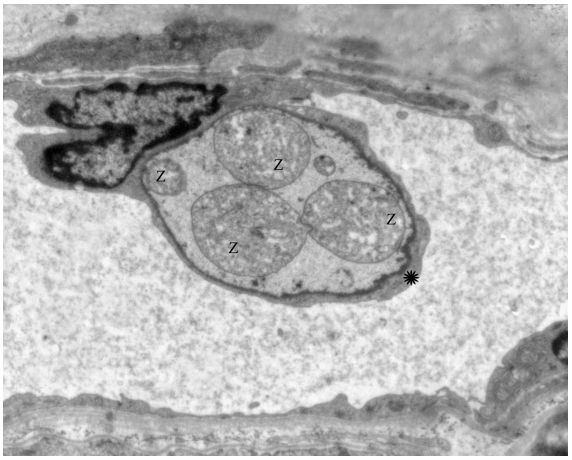
4-3. Spleen, dog. The spleen is enlarged and has a dry, fleshy texture on cut surface. Photograph courtesy of Departamento de Pathologia, Universidade Federal de Santa Maria, Santa Maria Brazil, claudioslbarros@uol.com.br



4-4. Heart and lung, dog. The lungs are red and wet and oozed large amounts of fluid from the cut surface. Photograph courtesy of Departamento de Pathologia, Universidade Federal de Santa Maria, Santa Maria Brazil, claudioslbarros@uol.com.br



4-5. Heart, dog. Intraendothelial protozoal zoites measuring 5-20um are found in the vicinity of areas of mild inflammation and degenerate cardiomyocytes. (HE 1000X)



4-6. Choroid plexus, endothelial cell, dog. The cytoplasm contains several protozoal zoites (Z). (TEM) Photograph courtesy of Departamento de Pathologia, Universidade Federal de Santa Maria, Santa Maria Brazil, claudioslbarros@uol.com.br

Table 1 – CBC (reference values are within parentheses)

WBCs (/mm^3)	32,200	(6,000-17.000)	RBC's (x10^6/mm^3)	1.3	(5.5-8.5)
Neutrophils (%)	72	(60%-77%)			
Neutrophils (abs.)	23,184	(3,000-11,500)			
Bands (%)	6	(0%-3%)	Hemoglobin (g/dL)	3.8	(12.0-18.0)
Bands (abs.)	1,932	(0-300)			
Metamyelocytes (%)	2	(0%)			
Metamyelocytes (abs.)	644	0	Hematocrit (%)	12	(37-55)
Myelocytes (%)	-	(0%)			
Myelocytes (abs.)	-	0			
Lymphocytes (%)	17	(12%-30%)	MCV (fl)	92	(60.0-77.0)
Lymphocytes (abs.)	5,474	(1,000-4,800)			
Monocytes (%)	2	(3%-10%)			
Monocytes (abs.)	644	(150-1,350)	MCHC (%)	32	(32.0-36.0)
Eosinophils (%)	1	(2%-10%)			
Eosinophils (abs.)	322	(100-1,250)			
Basophils (%)	-	(rare)	Platelets (x10^3/mm^3)	98	(200-500)
Basophils (abs.)	-	(rare)			

Table 2 – Biochemistry panel

Parameter	Unit	Result	Reference values
Alanine aminotransferase	U/L	180	4.0-24.0
Albumin	g/dL	4.4	2.6-3.3
Creatinine	mg/dL	1.2	0.5-1.5
Total bilirubin	mg/dL	6.5	0.1-0.5
Fibrinogen	mg/dL	200	200-400
Globulins	g/dL	4.2	2.7-4.4
Total plasma proteins	g/dL	8.8	6.0-8.0
BUN	mg/dL	56	21.0-60.0

ductules were distended by bile pigment and there was a lymphoplasmacytic inflammatory infiltrate in the portal triads. The same inflammatory infiltrate is observed in the myocardium, renal interstitium, and pulmonary interalveolar septa. In the myocardium, the inflammatory infiltrate is associated with mild degeneration of cardiomyocytes. Large numbers of nucleated RBC's occurred within hepatic sinusoids. The protozoal organisms described in the spleen and myocardium can also be observed parasitizing endothelial cells of capillaries in the kidney, lymph nodes, liver, bone marrow and choroid plexus of the fourth ventricle. Transmission electron microscopy (TEM) of the choroid plexus shows zoites (z) within the cytoplasm (asterisk) of an endothelial cell. Zoites (z) can also be visualized by TEM within endothelial cells of a lymph node.

Contributor's Morphologic Diagnosis: 1) Spleen, reactive hyperplasia, lymphoplasmacytic, associated with intraendothelial zoites, morphology consistent with *Rangelia vitalii*. 2) Myocardium, myocarditis, lymphoplasmacytic, mild to moderate, with mild fiber degeneration, associated with intraendothelial zoites, morphology consistent with *Rangelia vitalii*.

Contributor's Comment: Based upon the clinicopathological findings, a diagnosis of hemolytic anemia associated with infection by *Rangelia vitalii* (rangeliosis) was made. Rangeliosis, colloquially known as nambi-uvú (in native Brazilian Indian idiom meaning literally "bleeding ear"), "peste de sangue" and "febre amarela dos cães" (Portuguese for "blood ill" and "yellow fever of dogs," respectively) is an extravascular hemolytic disorder affecting dogs from southern Brazil. For several reasons this disease remained almost forgotten for the last 50 years, and during this period it was variably mistakenly diagnosed as other canine infectious diseases (hemolytic or otherwise), such as babesiosis, erlichiosis and leishmaniasis. The true nature of the disease surfaced again in 2001 when a group of Brazilian researchers put forth an effort to elucidate its cause, pathogenesis and etiology, and thus established it as a distinct disease entity of dogs. [3,4,6] Although the precise taxonomic classification of the causative organism of rangeliosis is still uncertain, the agent was established as a protozoal organism of the phylum Apicomplexa, order Piroplasmorida based on ultrastructural, immunohistochemical and *in situ* hybridization studies.[5] Until definitive nomenclature is established, *Rangelia vitalii* (named after two Brazilian researchers – from the first half of the 20th Century - Rangel and Vital) is maintained as the parasite's designation.

It is currently accepted that *R. vitalii* is transmitted by tick vectors (*Rhipicephalus sanguineus* and *Amblyomma aureolatum*) to dogs and several wild mammal species in the State of Rio Grande do Sul (RS) in southern Brazil. The life cycle of *R. vitalii* is unknown, but it has been speculated that the vectors *R. sanguineus* and *A. aureolatum* circulate the protozoan between wild mammals and domestic dogs, the latter probably being an aberrant host.[3] Experimental transmission to susceptible dogs using blood from spontaneously affected dogs was achieved recently in two independent studies.[4,5] The clinical and laboratory aspects of experimental disease differ somewhat from natural disease, suggesting that the parasite needs to replicate in the tick in order to acquire some aspects of virulence. Experimental disease is also fatal if left untreated.

The great majority of dogs with spontaneous rangeliosis develop clinical signs of extravascular hemolysis: pallor of mucous membranes; icterus; and hepatosplenomegaly.[3,4] Other clinical signs include apathy, anorexia, fever, vomiting, diarrhea, mucopurulent oculonasal discharge, tachypnea, tachycardia, subcutaneous edema of the pelvic limbs, and petechiae and ecchymosis on the mucous membranes.[3]

Hematologic findings are typical of extravascular hemolysis and include hypochromic macrocytic anemia with excessive regeneration. Anisocytosis, polychromasia, Howell-Jolly bodies and several nucleated red blood cell precursors (metarubricytes and rubricytes) are observed on peripheral blood smears.[3,4] Most of the affected dogs also present with varying degrees of spherocytosis,[3,4] a hematological finding highly suggestive of immune mediated hemolytic anemia.[1] The numbers of circulating reticulocytes are high (5%-28%, average 12.5%). Erythrophagocytosis is occasionally observed, particularly in those cases in which there is marked associated spherocytosis. Normochromic normocytic anemia is observed on occasion due to the extreme contrast between the small and falsely hypochromic-appearing spherocytes and the large polychromatophils recently released from the bone marrow. Plasma from affected dogs is bright yellow. WBC counts frequently reveal leucocytosis due to regenerative left shift resulting from long-standing and non-specific stimulation on the bone marrow. In some of the cases the leucocytosis may present as a leukemoid reaction. Other common hematological findings include lymphocytosis and monocytosis.[3]

In a smaller percentage of the cases of spontaneous canine rangeliosis, affected dogs develop a bleeding disorder similar to disseminated intravascular coagulation (DIC) characterized by extensive hemorrhage from the tips, margins, and outer surface of the pinnae.[5] Dogs affected in this manner have a moderate decrease in platelet numbers and numerous large platelets are observed in the circulation, indicating regenerative thrombocytopenia. However,

due to its moderate intensity, it is apparent that thrombocytopenia itself is insufficient to induce the hemorrhage in these dogs, and some other disorder(s) of hemostasis may be in place.[3,4]

There is no specific biochemical test for the diagnosis of rangeliosis, but serum alanine aminotransferase (ALT) is increased in most cases. This elevation most likely results from anemia, hypoxia of centrolobular hepatocytes, and death of these cells. Bilirubin levels are consistently increased due to the extravascular hemolysis and the urine is darkened by the excretion of large amounts of bilirubin and urobilinogen; hemoglobinuria is never part of the clinical picture.[3]

In contrast to most infectious hemolytic anemias, the clinical diagnosis of rangeliosis is generally made on response to therapy, since the parasite is seldom found in the blood smears from affected dogs (only in approximately 4% of the cases).[3] Currently, there are no commercial or in-house tests to detect antibodies or antigens associated with *R. vitalii*. Fine needle aspiration (FNA) or excisional biopsy of lymph nodes, spleen and bone marrow with subsequent cytological evaluation can also be important aids in the clinical diagnosis. Due to the intraendothelial location of the parasite, fine needle aspiration may yield false negative results.

Necropsy findings in dogs dying from rangeliosis are consistent with those seen in other causes of extravascular hemolysis. The mucous membranes, subcutaneous tissue, muscle fascia, serosal surfaces and arterial intima are markedly icteric and peripheral blood is thin and watery. The liver is enlarged and has a red-orange hue or, in more severe cases, a greenish discoloration. Accentuation of the hepatic lobular pattern is common. The spleen is enlarged and fleshy and all lymph nodes are swollen, moist, and red,[3] and may have multifocal to coalescing white areas.[5] Hyperplastic bone marrow is markedly red and fills the entire marrow space.

Microscopic evaluation of lymph nodes reveals marked erythrophagocytosis and, depending on the stage of the disease, there is hemosiderosis and severe paracortical lymphoid hyperplasia. In the spleen, there is lymphoid hyperplasia characterized by marked plasmacytosis which, in some cases, resembles the plasma cell proliferation pattern observed in myelomas; however, in rangeliosis, most plasma cells are mature and there are few plasmablasts, Mott cells and/or "flame cells".[3] In non-lymphoid organs, the same lymphoplasmacytic infiltrates are observed, and occasionally there is a granulomatous infiltrate which includes multinucleate giant cells that sometimes phagocytize parasitized endothelial cells.[4] In the liver, there is centrolibular coagulative necrosis and accumulation of bile pigment. In the bone marrow, there is marked erythroid and megakaryocytic hyperplasia characterized by replacement of the marrow adipose tissue by hematopoietic cells with high mitotic index, a drop in the myeloid to erythroid ratio, and increased numbers of megakaryocytes and megakaryoblasts. Foci of extramedullary hematopoiesis can be observed frequently in the spleen and liver and less frequently in the lymph nodes and adrenal glands.[3]

The parasite is found within the cytoplasm of endothelial cells in several organs and consists of 2.0-2.5 µm round to oval, basophilic organism when stained with hematoxylin and eosin; its cytoplasm is pale and inconspicuous and the nucleus is prominent, basophilic and eccentrically located.[3,5] The frequency in which the parasite was observed in several organs was reported from the study of 11 cases of canine rangeliosis as: kidneys (7/11), lymph nodes (7/11), liver (6/11), bone marrow (4/11), spleen (3/11), tonsils (2/11), lung, brain, stomach and gallbladder (1/11).[4] The authors pointed out that not all organs were histologically examined in each of the 11 cases. Other reports indicate lymph nodes, bone marrow, kidneys and choroid plexus are the organs with the highest parasite load.[4] About 20-30 organisms can be found within the cytoplasm of each parasitized endothelial cell. The organisms usually can be seen in smears from bone marrow sampled during the necropsy and stained with Giemsa and Panoptic.[4] In various reports, immunostaining for *Leishmania chagasi*, *Neospora caninum* and *Toxoplasma gondii* was consistently negative;[4,5] however, the parasite reacted positively with an anti-*Babesia microti* antibody.[5] *Rangelia vitalii* was also positive on *in situ* hybridization for *B. microti*[4] indicating that the organism belongs to the same group as *Babesia*. However, unlike babesia, *R. vitalii* is characterized by an intraendothelial stage. Parasitized cells are immunopositive for Von Willebrand factor, indicating their endothelial nature. The ultrastructural characteristics of *R. vitalii* were reported by Loretti & Barros 2005 (these authors mistakenly spelled the parasite's name as *R. vitalli*) as having an apical complex that includes a polar ring and rhoptries but no conoid; the parasite is contained within a parasitophorous vacuole that had a trilaminar membrane with villar protrusions and was located within the cytoplasm of capillary endothelial cells.[6]

Necropsy finding of dogs dying from rangeliosis, coupled with the hematological findings, suggest the diagnosis of hemolytic anemia; the finding of the causative agent in the cytoplasm of endothelial cells confirms a presumptive diagnosis.

Differentials for this case should include infection by *R. vitalii*, *Histoplasma capsulatum*, *Trypanosoma cruzi*, *Leishmania* spp., *Toxoplasma gondii*, and

Neospora caninum. *Histoplasma* and *Leishmania* organisms are typically found in macrophages and may elicit an intense histiocytic to granulomatous response. *Histoplasma capsulatum* yeasts stain with PAS and GMS stains. *Leishmania* and *T. cruzi* have a kinetoplast, which is absent in *R. vitalii.* Additionally, the amastigotes of *T. cruzi* form pseudocysts within the sarcoplasm of cardiomyocytes and not within endothelial cells. The zoites of *T. gondii* and *N. caninum* form 2-5 µm and 4-7 µm tachyzoites, respectively, and have no kinetoplast. *N. caninum* can parasitize endothelial cells but usually parasitize a large spectrum of host cells and are PAS positive. Similarly, *T. gondii* affects a large spectrum of host cells, although usually not endothelial cells, and induces a whole different set of lesions.

AFIP Diagnosis: 1. Heart: Myocarditis, lymphoplasmacytic and histiocytic, multifocal, mild to moderate with cardiomyocyte degeneration, capillary fibrin thrombi and numerous intraendothelial protozoa.

2. Spleen: Plasmacytosis, diffuse, marked with multifocal reticuloendothelial cell hyperplasia and scattered intraendothelial protozoa.

Conference Comment: The contributor provides an excellent, thorough discussion of this unique and poorly-known infection. Conference participants were unfamiliar with this condition, and many favored *T. cruzi.* The key observation necessary to reach the correct diagnosis is the intraendothelial location of the organism. Once that is recognized, literature searches could lead to articles on this fascinating disease.

While reviewing the submitted CBC, biochemistry panel and cytology, conference participants discussed the clinicopathologic differentiation between extravascular and intravascular hemolysis. The diagnostic features for each are briefly summarized in the chart below.[2]

Certain breeds of dogs and cats possess hereditary biochemical deficiencies or predispositions to extravascular hemolysis.[2] In dogs,

	Extravascular Hemolysis	**Intravascular Hemolysis**
Cause (general categories)	1. Antibody and/or complement mediated 2. Decreased RBC deformability 3. Premature RBC aging (decreased glycolysis and [ATP]) 4. Increased macrophage phagocytosis	1. Complement-mediated lysis 2. Physical damage 3. Oxidative damage 4. Osmotic lysis 5. Membrane alterations
Onset	Usually chronic course with insidious onset	Peracute to acute
CBC	Neutrophilia, monocytosis, thrombocytosis	No significant findings
Biochemistry	Hyperbilirubinemia with the unconjugated form dominating early	1. Hemoglobinemia: plasma discolored red; increased MCHC and MCH; decreased serum haptoglobin 2. Hyperbilirubinemia with unconjugated dominating early
Urinalysis	Bilirubinuria	Hemoglobinuria; hemosiderinuria; ± Bilirubinuria
Erythrocyte morphology	Spherocytes, schistocytes, keratocytes; if a regenerative response, reticulocytes, Howell-Jolly bodies, polychromasia and macrocytosis	Schistocytes, keratocytes, Heinz bodies, eccentrocytes

phosphofructokinase deficiency is seen in cocker spaniels, English springer spaniels and some mixed breeds. Pyruvate kinase deficiency is noted to occur in the Basenji, beagle, Chihuahua, dachshund, pug, miniature poodle, West Highland White terrier, and Cairn terriers as well as Abyssinian, Somali and domestic short-haired cats. A hereditary predisposition to hemolytic anemia is suspected in the border collie, cocker spaniel, English springer spaniel, German shepherd dog, Irish setter, Old English sheepdog, poodle and the whippet.

Contributor: Departamento de Patologia, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil. http://www.ufsm.br/lpv

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