Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2018-2019

C o n f e r e n c e 3

12 September 2018

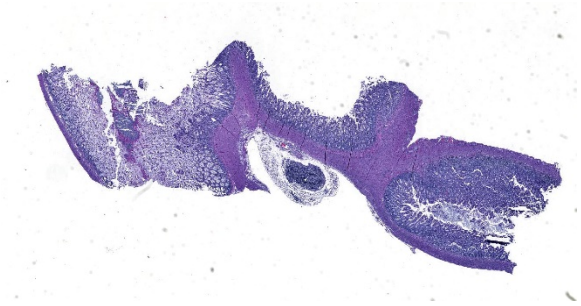
CASE I: AFIP 15-13332 (JPC 4067411-00).

Signalment: 3-week-old male domestic shorthair kitten

History: A 3-week-old male domestic short hair kitten was one of 8 kittens found before taken to a cat rescue. The litter was split into two different foster homes. All kittens developed diarrhea with lethargy and inappetence. The kitten in this case was euthanized after a week of developing symptoms and two more died subsequently (exact timing unknown). The remaining two surviving kittens are alive and thriving.

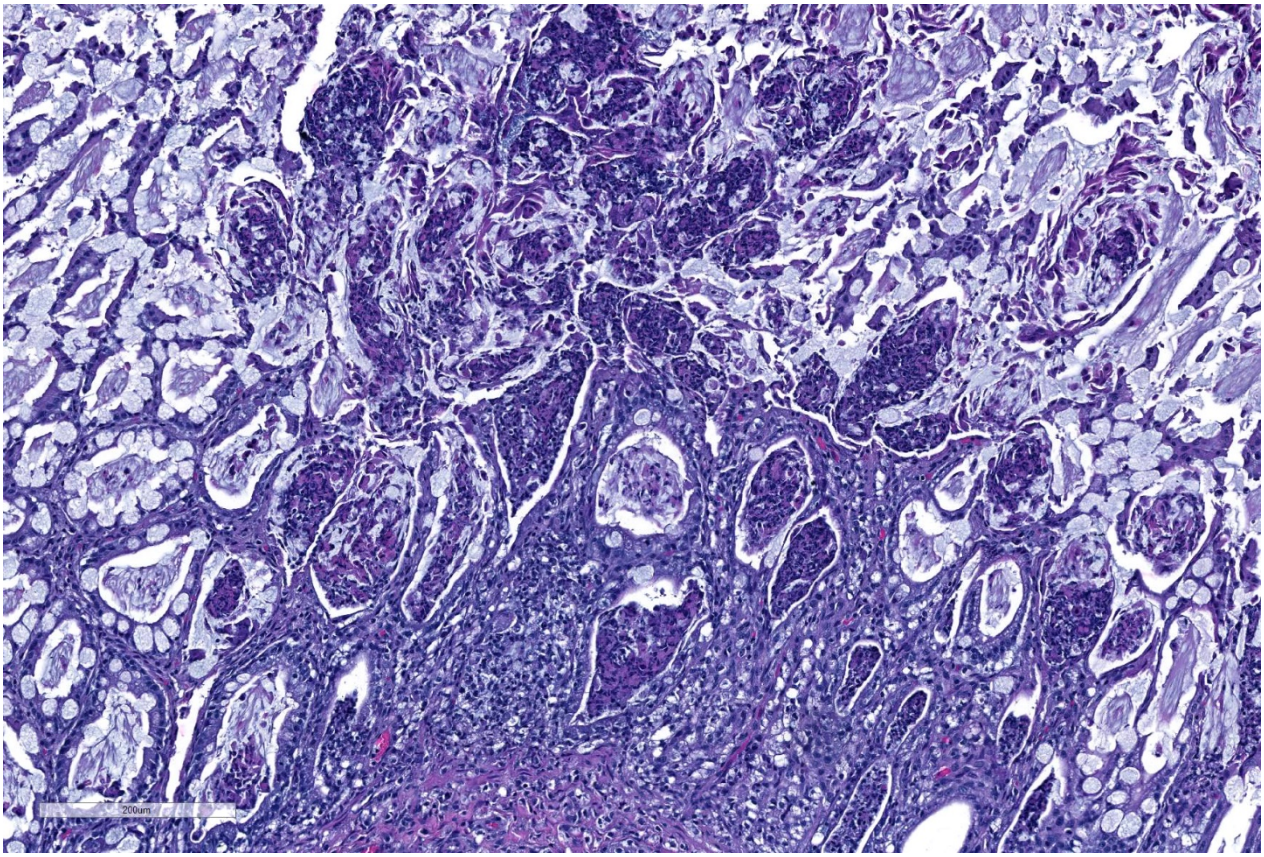
Gross Pathology: A male kitten (0.11 kg) in fair body and postmortem condition was presented for autopsy. The umbilicus has no lesions. The stomach is expanded by ~ 5 ml of curdled milk. The rectum and distal colon contain yellow-green dry string-like fecal material. Both lungs are wet and mottled dark and pale pink with areas of hyperinflation. No other significant gross lesions identified.

Laboratory results: Hemolytic *E. coli* and group G beta-hemolytic *Streptococcus* were both cultured from pulmonary tissue.



Ileocolic junction, kitten. A section of the ileocolic junction with ileum on the left and the colon on the right is submitted. (HE 12X)

Microscopic Description: Ileum and colon. The section of ileum contains frequent crypt necrosis and ~50% intestinal villi blunted with occasional complete villous loss. There are large numbers of cocci and rod bacteria within the lumen and crypts of the tunica mucosa, both extracellularly and intra-cellularly, throughout the majority of stained sections. Slender rod-shaped bacteria are arranged in one or more parallel stacks within the cytoplasm of numerous enterocytes. The lumen of the ileum is heavily colonized by a mixed population of gram-negative rods and cocci, with moderate numbers of cocci directly associated with the surface of mucosal epithelial cells. The tunica muscularis and serosa appear unaffected. The section of intact colon is included to



Colon, kitten. There is necrosis of colonic glands with replacement by neutrophils and macrophages. Remaining colonic glands are dilated and filled with variable combinations and concentrations of necrotic glandular epithelium, degenerate neutrophils, cellular debris, and mucin. (HE, 137X)

demonstrate that the changes to the ileum are not likely due to autolysis. The colon contains few luminal bacteria, but moderate numbers of enterocytes contain slender rod bacteria as described above.

Contributor's Morphologic Diagnoses: Ileum and colon: Marked multifocal to coalescing necrotizing enterocolitis with extracellular, adherent and intracellular mixed bacteria.

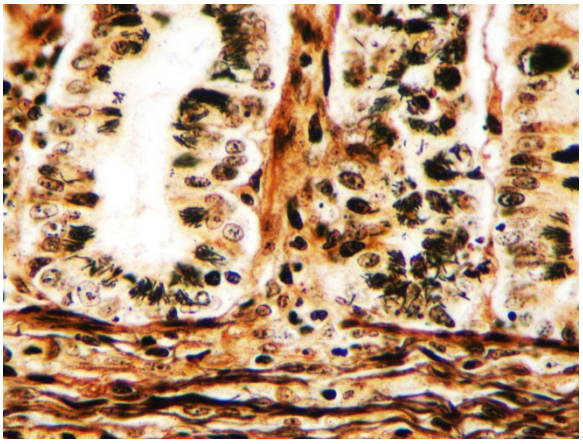
Contributor's Comment: Differentials to consider in a case of enterocolitis in neonatal kittens include: bacteria (*E. coli, Salmonella*), primary viral enteritis (parvovirus, enteric coronavirus), protozoa (*Isospora, Giardia*) or severe systemic disease, such as caused by feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV). The two surviving kittens were 8 weeksold at the time of this submission and both had tested negative for FIV and FeLV the week prior. Culture of fecal samples from both kittens yielded *E. coli*, however only one harbored the hemolytic phenotype. Salmonellae were not recovered using routine media.

In the case presented here, there are concurrent intestinal bacterial infections by *C. piliforme* and *E. coli*. *E.coli* had primarily colonized the small intestine while *C. piliforme* was associated with crypt necrosis in the large intestine. An area of overlap within the ileum, as highlighted in the submitted slide, shows the co-existence of both bacteria. There have been few published feline cases of Tyzzer's disease, and a

bacterial co-infection in a cat has not yet been reported. A recent publication, however, describes dual infection by *C. piliforme* and enteropathic and effacing *E. coli* causing enterotyphlocolitis in a Syrian hamster.[1]

Tyzzer's disease in cats has been associated with viral co-infection with FIP and FeLV.[2,5] Coronaviral protein was not detected in the small intestine or colon by immunohistochemistry in this kitten (a quantity of fecal material sufficient for testing could not be obtained during necropsy); thus, a concurrent or underlying and predisposing viral infection, while unlikely, cannot be completely ruled out. Other factors that are more likely to have predisposed this kitten to Tyzzer's disease are failure of passive transfer, environmental stress (exposure, relocation), immune compromise, or concurrent disease.

Suboptimal immune competency of the host has been associated with the dissemination of extraintestinal pathogenic *E. coli*.[4] Colonization of the small intestine with *E. coli* and subsequent sepsis could have been a primary event in this case. Bacterial culture identified moderate numbers of hemolytic *E. coli* and low numbers of group G beta-hemolytic *Streptococcus* in the lungs. Histopathology of the lung identified mild, diffuse, interstitial pneumonia consistent with sepsis.



Colon, kitten. Glandular epithelial cells contain numerous slender bacilli in haphazard stacks within their cytoplasm, consistent with C. piliforme. (Warthin-Starry 4.0, 400X) (Photo courtesy of: Oregon State University Diagnostic Laboratory, http://vetmed.oregonstate.edu/diagnostic)

E. coli is a constituent of the normal feline intestinal microflora and does not normally produce disease. When provided with a compliment of virulence factors, however, *E. coli* may become an opportunistic pathogen resulting in local and systemic infection and even death. At least seven distinct pathotypes have been identified, including entero-pathogenic (EPEC), enterotoxigenic (ETEC), and shiga-toxin producing *E. coli* (STEC; also referred to as enterohemorrhagic *E. coli* or EHEC).[8] Shiga toxin-producing Escherichia coli (STEC) causes hemolytic uremic syndrome (HUS) in humans and has zoonotic potential. Most shiga-toxin producing *E. coli* belong to various serotypes including O26, O103, O111, O145, and O157, the latter being the most commonly identified.[7] A study of cats and dogs in Buenos Aires, Argentina, where STEC is an endemic pathogen, identified O157 in 4/149 (2.7%) cats and 34/450 (7.5%) dogs (3). Testing of the *E. coli* isolate did not detect O157:H7 antigen in this case.

Group G beta-hemolytic *Streptococcus* isolates include: *Streptococcus dysgalactiae* subspecies *equisimilis*, *S. milleri*, *S. canis*, and *S. intestinalis*. Both *S. dysgalactiae* subspecies *equisimilis* and *S. milleri* are associated with human infection, while dogs and pigs are reservoirs for *S. canis* and *S. intestinalis*, respectively. *S canis* is the most likely strain to be identified in this case and is typically considered a commensal and extracellular pathogen in cats, however it has been shown to cause severe feline disease

such as necrotizing fasciitis, sinusitis, bacteremia, and toxic shock–like syndrome.[9] Because of the low numbers of *Streptococcus* organisms isolated from the lungs, and absence of pathogenic changes consistent with primary *Streptococcus* infection, we consider this an opportunistic colonization in this cat.

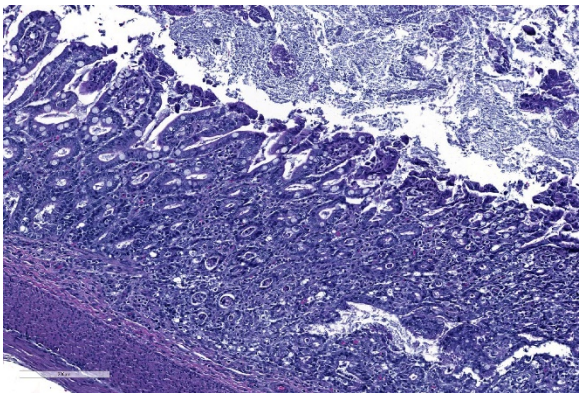
The clinical presentation of Tyzzer's disease can range from subclinical infection and incidental finding to mild to marked mucoid diarrhea or death without premonitory signs. The mechanism of host cell attachment and entry by *C. piliforme* is not fully understood. Following colonization of the distal ileum, cecum, and colon, bacteria can penetrate into the portal vasculature to seed the liver.[7] Neither necrotizing inflammation nor clostridial forms were identified within the liver in this kitten. *C. piliforme* infection limited to the intestinal tract could reflect an early stage of infection, prior to hematogenous spread, and would suggest that *C. piliforme* colonization occurred secondary to *E. coli* infection.

JPC Diagnosis: 1. Colon: Colitis, necrotizing, diffuse, marked with numerous crypt abscesses, crypt hyperplasia and intracytoplasmic bacilli within crypt epithelium.

2. Ileum: Ileitis, necrotizing, diffuse, mild to moderate with numerous surface-adherent bacilli.

Conference Comment:

Ernest Edward Tyzzer (1895-1965) was an American pathologist whose investigations far exceeded the disease which today bears his name. A graduate of Harvard medical school in 1902, many of his discoveries impacted veterinary science far more than human medicine. In 1901, he received a



Ileum, kitten. Crypt abscesses are also present within the ileum as well, indicating a necrotizing enteritis. (HE 137X)

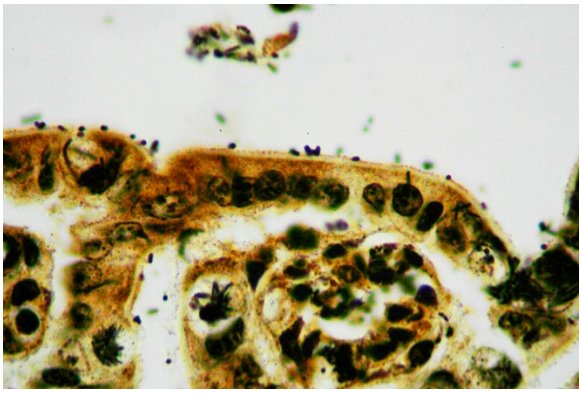
master's degree studying myxosporidian infections in menhaden. Between 1905 and 1916, he was research director for the Harvard Cancer Commission, investigating spontaneous and transplanted tumors in mice, but continuing his interest in infectious diseases as well.[10] In 1910, he identified the genus *Cryptosporidium* sp. for the first time. He was responsible for the development of the DBA inbred strain of mouse during this time, as well as the identification of Leishmania sp. as the cause of Ooroya fever.[10]

In 1916, Dr. Tyzzer became the head of the Department of Comparative Pathology at Harvard, a chair he held until his retirement in 1942.[10] In 1917, he first identified the disease that now bears his name in Japanese waltzing mice, a strain he was utilizing in cancer research. In this position, he correctly identified the flagellate *Histomonas meleagridis* as the causative agent of blackhead, as well as detailing its life cycle. (The principles which he used to raise turkeys on his experimental farm were subsequently published by the Massachusetts Department of Agriculture and he was given a citation by the Governor of Massachusetts for "saving the turkey industry of Massachusetts).[10] Additional investigations during his career provided seminal work in equine

encephalomyelitis as well as coccidiosis in gallinaceous birds.

Over a century following its identification, many questions remain about *C. piliforme*. A common commensal in rodents, disease has been difficult to reproduce in healthy dogs and cats. Concomitant disease, especially infection with immunosuppressive viruses such as FELV, FIV, feline panleukopenia, and the mutated coronavirus of feline infectious peritonitis have been incriminated in the majority of feline infections, and experimental immunosuppression by glucocorticoids or cyclophosphamide has also been used to enhance experimental infections.[6] Transmission from the feces of rodents has been suggested as a source of infection of dogs and cats, but has not been proven. Basic information about the pathogenesis of C. piliforme infection, such as the mechanisms of attachment and entry into cells by *C. piliforme*, have not yet been identified, and inability to culture the organism on artificial media further hampers laboratory research.[6]

Attaching and effacing *E. coli (*AEEC*)*, which has been reported in a wide variety of species (and is the only pathotype reported in the rabbit), employs a unique procedure for colonization of the intestine. The classic "cup and pedestal" attachment to the luminal surface results from the bacteria's ability to manipulate the host cytoskeleton, in particular the recruitment of actin filaments beneath adherent bacteria.[8] An additional virulence factor of AEEC is intimin and its receptor-translocated intimin receptor (TIR). TIR is secreted directly by AEEC into the cytoplasm of intestinal epithelium via injectisomes (aka, needle complexes or T3SS apparatus), whereupon intimin expressed on the bacterial surface.[8]



Ileum, kitten. Bacilli are adhered to the luminal surface of ileal enterocytes. (Warthin-Starry 4.0, 400X) (Photo courtesy of: Oregon State University Diagnostic Laboratory, http://vetmed.oregonstate.edu/diagnostic)

Conference participants reviewed the pathogenesis of Tyzzer's disease in a variety of animal species, as well as reviewed the various common pathotypes of E. coli which produce disease in domestic species. A Giemsa stain run on this slide demonstrated both the mucosal-adherent *E.coli* as well as the intracellular *C. pilforme* extremely well.

Contributing Institution:
Oregon State University Diagnostic Laboratory
http://vetmed.oregonstate.edu/diagnostic

References:

1. Aboellail TA, Hk N, Mahapatra D. A Naturally Occurring Enterotyphlocolitis Associated with Dual Infection by Clostridium piliforme and Enteropathogenic Attaching and Effacing Escherichia coli in Syrian Hamsters. J Vet Sci Med 2013;1.
2. Bennett AM, Huxtable CR, Love D. Tyzzer's Disease in Cats Experimentally Infected with Feline Leukaemia Virua. Vet Microbiol 1977;2:49–56.
3. Bentancor A, Rumi MV, Gentilini MV, Sardoy C, Irino K, Agostini A et

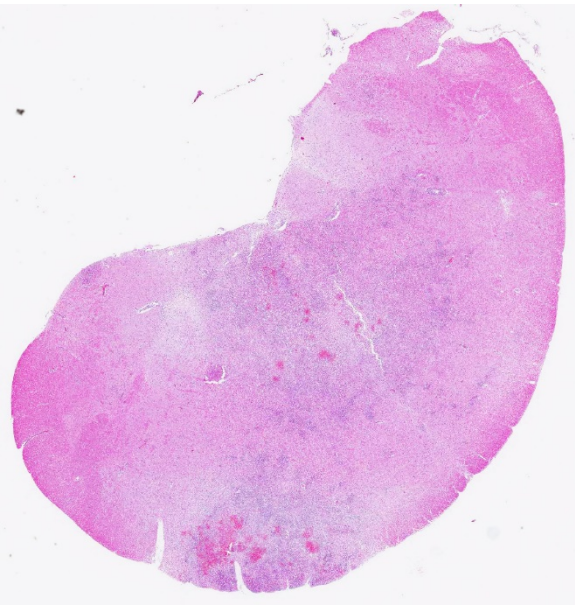
al. Shiga toxin-producing and attaching and effacing Escherichia coli in cats and dogs in a high hemolytic uremic syndrome incidence region in Argentina. FEMS Microbiol Lett 2007;267:251–256.
4. Bleibtreu A, Gros P-A, Laouénan C, Clermont O, Le Nagard H, Picard B et al. Fitness, stress resistance, and extraintestinal virulence in Escherichia coli. Infect Immun 2013;81:2733–2742.
5. Ikegami T, Shirota K, Goto K, Takakura A, Itoh T, Kawamura S et al. Enterocolitis associated with dual infection by Clostridium piliforme and feline panleukopenia virus in three kittens. Vet Pathol 1999;36:613–615.
6. Jones BR, Greene CE. Tyzzers Disease. In Greene, CE, ed. *Infectious Diseases of the Dog and Cat, 4th edition.* St. Louis, MO. Elsevier Saunders, 2012 pp. 391393.
7. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran's Pathologic Basis of Disease, Professional Edition: Expert Consult-Online.
8. Marks SL, Rankin SC, Byrne BA, Weese JS. Enteropathogenic Bacteria in Dogs and Cats: Diagnosis, Epidemiology, Treatment, and Control. J Vet Intern Med 2011;25:1195–1208.
9. Pesavento PA, Bannasch MJ, Bachmann R, Byrne BA, Hurley KF. Fatal Streptococcus canis infections in intensively housed shelter cats. Vet Pathol 2007;44:218–221.
10. Weller TH. Ernest Edward Tyzzer 1875-1965, A biographical memoir. *Nat Acad Sci* 1978; 49(14):351-373.

CASE II: AFIP 15-13332 (JPC 4067411-00).

Signalment: 8-month-old Romney ewe lamb (*Ovis aries*)

History: The ewe lamb was found recumbent and unable to stand. Paddling movements and spasms were observed prior to euthanasia.

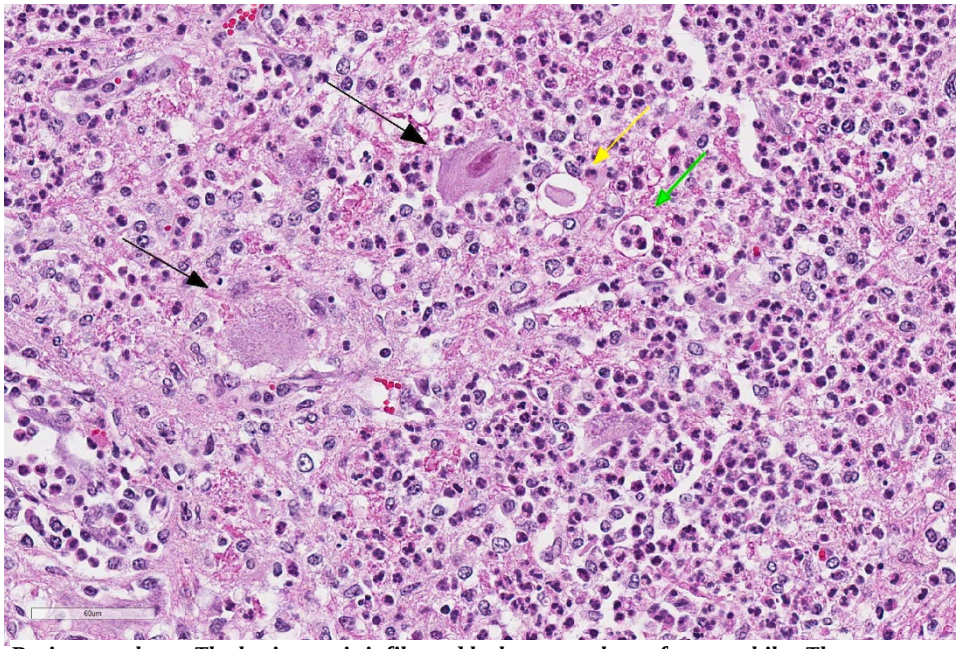
Gross Pathology: The 8-month-old Romney ewe lamb was in good body condition (BCS 3/5), with good muscle mass, fat reserves, and adequately hydrated. Numerous variably-sized (2 mm-5mm) areas of hemorrhage were present in the medulla oblongata and extended caudally to the level of the obex and cranial cervical spinal cord. No other gross lesions were present elsewhere.



Brainstem, sheep. There is rarefaction, hemorrhage and a cellular infiltrate in up to 50% of the section. (HE, 5X)

Laboratory results: None.

Microscopic Description: Within the brainstem and affecting both gray and white matter, are multifocal to coalescing, variably-sized aggregates of numerous intact and degenerate neutrophils and lesser numbers of macrophages and gitter cells (microabscesses) surrounded by small areas of gliosis. Within and adjacent to the areas of inflammation, numerous axons are swollen and eosinophilic (spheroids) and rare hypereosinophilic, shrunken neurons with pyknotic nuclei surrounded by neutrophils are present (neuronal necrosis and neuronophagia). Multifocally, the white matter is edematous and rarefied. Within the parenchyma, there are numerous densely cellular perivascular cuffs mainly composed of lymphocytes and plasma cells, with lesser number of macrophages, neutrophils and rare eosinophils. A Gram stain reveals moderate numbers of predominantly intracellular, short, plump gram-positive coccobacilli. Additionally, there are multifocal areas of hemorrhage in the parenchyma and locally extensive regions of leptomeningeal hemorrhage. Multifocally, lymphocytes and plasma cells, and lesser numbers of neutrophils and macrophages, surround and disrupt meningeal blood vessels.



Brainstem, sheep. The brainstem is infiltrated by large numbers of neutrophils. There are swollen, degenerating axons (black arrows), dilated myelin sheaths with swollen axons (spheroids – yellow arrow), and a myelin sheath which contains neutrophils and debris (green arrow). (HE, 379X)

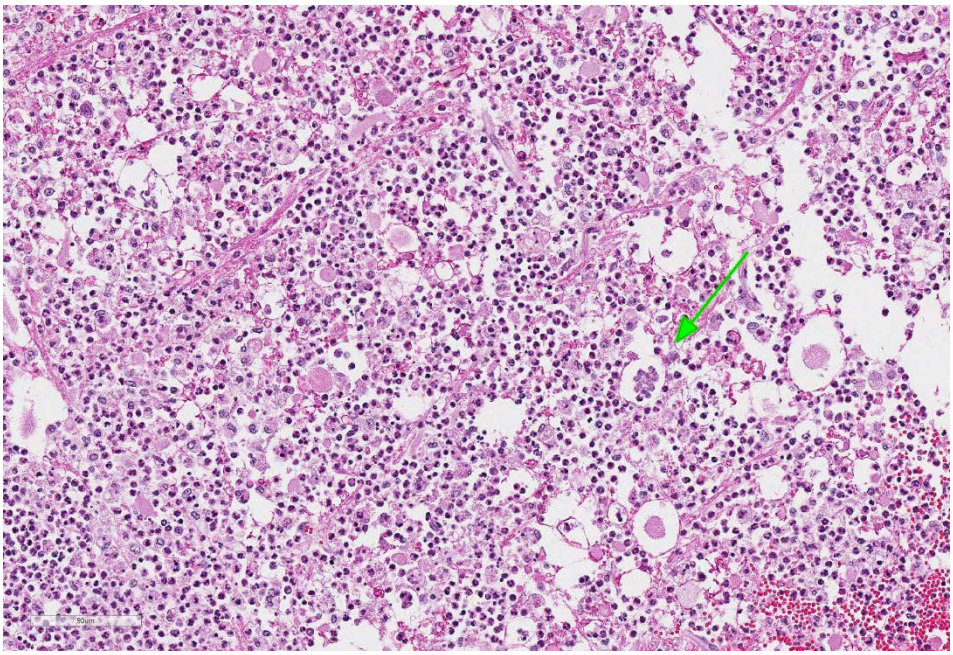
Contributor's Morphologic Diagnoses: Brainstem. Meningoencephalitis, necrosuppurative, subacute, multifocal to coalescing with gram-positive coccobacilli (Listeric encephalitis)

Contributor's Comment: The clinical signs and the microscopic findings in the brain of this ewe lamb are consistent with listeric encephalitis.[1-6,9] *Listeria monocytogenes* belongs to the bacterial genus *Listeria*, which are gram-positive, non-spore-forming, facultative anaerobic, intracellular coccobacilli ubiquitously found in the environment and commonly present in the faeces of healthy animals.[1,3,5,6] It has been suggested that domesticated ruminants play a key role in the maintenance of *Listeria* spp. in the environment through a continuous faecal–oral enrichment cycle.[1] There are currently six species in the genus Listeria, but only *L. monocytogenes* and *L. ivanovii* are considered pathogenic for domestic animals.[5-7]

Virulent strains possess a group of virulence genes such as the positive regulatory factor A, internalins, hemolysins (such as

listeriolysin O), phospholipases, a hexose phosphate transporter and others, which allow them to replicate within and spread between eukaryotic cells.[1,4-8] Critical to the virulence of *Listeria*, is the ability to escape intracellular killing within macrophages by lysis of the phagosomal membrane and escape into the cytoplasm, actions mediated by the secretion of listeriolysin O.[5-7] The importance and role of these genes in neurovirulence and neuroinvasion in natural infections, whether by hematogenous infection or axonal migration, is yet to be determined.[6-7]

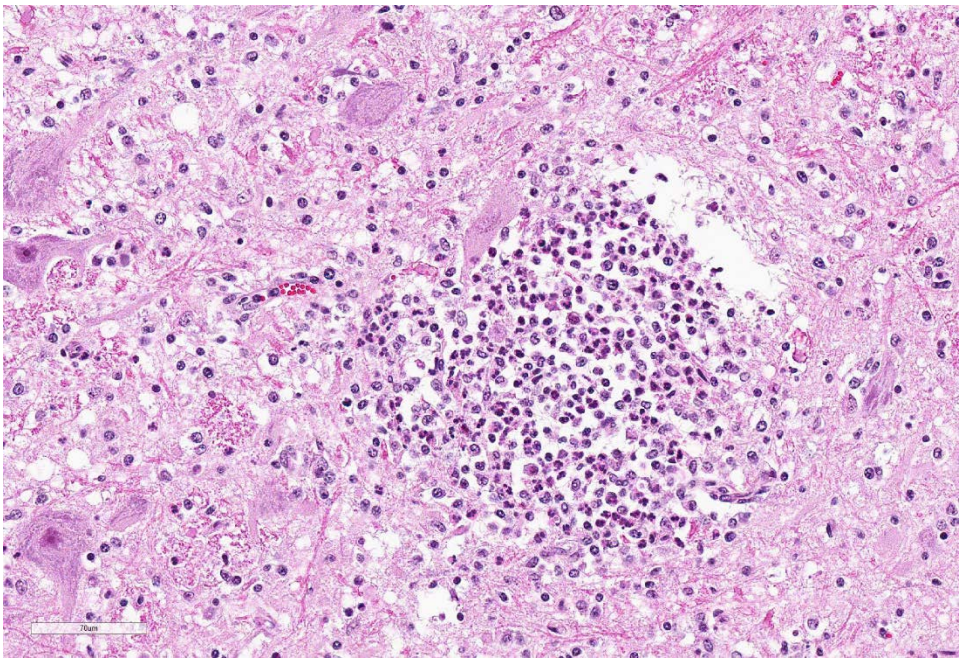
In ruminants, the main clinical features of *Listeria* infection are encephalitis, septicaemia, abortion, mastitis and gastroenteritis.[1-6,9] Listeriosis in ruminants occurs primarily in sporadic cases and is considered to be non-contagious.[1,5] In general, the disease is more frequent in winter and early spring and outbreaks of disease are known to occur when animals are exposed to a single contaminated source such as silage.[1,3-6] However, outbreaks of listeric encephalitis have been described unrelated to silage feeding.[8-9] Pathogenesis of listeric encephalitis in sheep, goats and cattle suggests that *L. monocytogenes* gains access to the brainstem via migration through axons of various cranial nerves (most commonly the trigeminal nerve), using the oropharyngeal mucosae as the port of entry.[6-8] Once the bacteria has accessed the CNS it spreads further from the brainstem into other brain regions probably by intracerebral axonal migration.[7-8] Clinical signs of listeric encephalitis are similar in ruminants and vary depending on the topography of the CNS lesions.[1-3] The neurologic signs of listeriosis in sheep reflect dysfunction of caudal brainstem, cerebellar peduncles and/or cranial nerves III through XII.[1-3] Gross lesions of the brain are generally absent, or limited to mild meningeal congestion, unilateral or bilateral areas of malacia in the brainstem or spinal cord, and mild clouding of the cerebrospinal fluid.[1,3,5-8] In this case the gross lesions consisted of variably-sized areas of hemorrhage in the medulla oblongata that extended caudally to the level of the obex and cranial cervical spinal cord. Histologically, microabscesses, focal gliosis with adjacent perivascular cuffs consisting predominantly of lymphocytes with histiocytes, plasma cells and occasional neutrophils characterize listerial



Brainstem, sheep. Large numbers of neutrophils infiltrate areas of white matter, resulting in necrosis and formation of large numbers of spheroids. Several dilated myelin sheaths (HE, 226X)

encephalitis.[1,6-8] In severe cases, such as the present case, lesions may coalesce to affect large areas of brain tissue.[7] Meningitis is often present, and thought to develop secondary to parenchymal lesions.[5-7] Species differences can be observed in lesion distribution throughout the CNS, and in the extent and cellular composition of the inflammatory reactions.[7] Although the characteristic lesion is the presence of microabscesss both in cattle and small ruminants, cattle tend to have relatively more macrophages and more prominent mononuclear perivascular cuffs than small ruminants. Neuronal necrosis appears to be more frequent in small ruminants than in cattle.[7]

In this case, bacterial culture was not attempted since the clinical presentation, together with the gross and histological lesions, and the demonstration of gram-positive, intracellular coccobacilli in histological sections, were consistent with *Listeria* infection.



Brainstem, sheep. Focal areas of lytic necrosis (microabscesses) are scattered throughout the brainstem. (HE, 379X)

JPC Diagnosis: Brainstem: Rhomb-encephalitis, necrotizing and suppurative, multifocal to coalescing, severe, with microabscesses and mild lymphohistiocytic meningitis.

Conference Comment: The contributor has none an excellent job in describing the general aspects, virulence factors, and pathogenesis of the encephalitic form of listeriosis in small ruminants. A number of other syndromes have been identified in small ruminants infected with *Listeria monocytogenes*, to include enteritis, septicemia, abortion, mastitis, and ophthalmitis. In addition, the contributor has correctly utilized the various, often non-intuitive spellings of the tissue Gram stain, named after Dr. Hans Christian Gram, who developed the method in 1884. When describing the particular stain, the upper case "Gram" is used – "She performed a Gram stain on each section". When describing the stain's positivity or negativity, the lowercase version is used – "The bacteria were consistent gram-positive in every section."

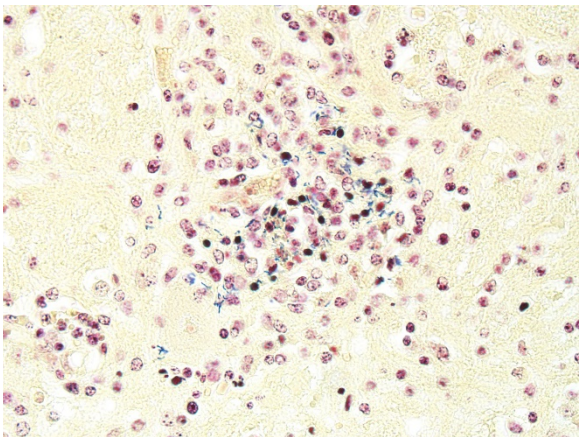
While the portal of entry for pathogenic *Listeria* sp. in cases of encephalitis is presumed to be the buccal mucosa, all other forms of disease cause by this pathogen in ruminants are presumed to develop through the lower gastrointestinal tract. Enteritis, the most common presentation in humans, has been

rarely identified in sheep.[5] Adult sheep may develop a marked enteritis with extensive necrosis of Peyer's patches as well as necrohemorrhagic enteritis and abomasitis.[5]

Within the gut, bacteria will enter macrophages, often via M cells, whereupon they escape from phagolysosomes and are transported throughout the body. This bacteremia may develop into a listerial septicemia - septicemia occurs most often in immunosuppressed animals and pregnant animals, and its characteristic lesions are most often seen in the fetus. In the fetus, multiple foci of necrosis are seen in the liver and spleen and the organism is readily demonstrated within these lesions. Occasional massive outbreaks of septicemia involving pregnant mucinous have been described with clinically affected animals exhibiting fever and profuse diarrhea.[5]

The gravid uterus appears to be highly susceptible to infection during listerial septicemia in ruminants and many other species. Another pathogenic form of *Listeria*, *L. ivanovii*, has been recorded as a cause of abortion in cattle and sheep, albeit with less frequency than *L. monocytogenes*. In cases of listerial abortion, there are necrotic lesions within cotyledons as well as a diffuse intercondylar urinary placentitis characterized by a reddish brown exudate.[5] The fetus is usually autolytic with evidence of septicemia as discussed previously.

Less common but documented listerial syndromes include iritis and kerato-conjunctivitis in both cattle in sheep (likely complications of cranial nerve infection), as well as subclinical or clinical mastitis, both seen in silage-fed animals during winter.



Brainstem, sheep. Microabscesses contain numerous gram-positive bacilli, characteristic of L. monocytogenes. (HE, 379X)

Conference participants discussed the various virulence factors associated with *Listeria*, including internalin (initiates E-cadherin-facilitated entry into the cell), listeriolysin (a perforin which lyses the phagosomes of neutrophils and macrophages), and act A protein (which co-opts actin within the cytoskeleton to facilitate cell-to-cell transfer). Major differences between *L.* monocytogenes and *H. somni*, another neurotropic bacillus in ruminants, were identified as vasculitis in cases of *H. somni*, microabscess formation by *L. monocytogenes*, and *L. monocytogenes'* preference for the brainstem as a result of transaxonal migration from the oral cavity along the cranial nerves.

Contributing Institution:
Massey University
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http://ivabs.massey.ac.nz
http://vet-school.massey.ac.nz

References:

1. Brugere-Picoux. Ovine listeriosis. *Small Ruminant Res.* 2008;**76**:12–20.

2. Constable PD. Clinical examination of the ruminant nervous system. *Vet Clin North Am Food Anim*. 2004;**20**:185–214.
3. Fentahun T, Fresebehat A. Listeriosis in small ruminants: A review. *Advances in Biological Research*. 2012;**6**:202-209.
4. Hamon MA, Ribet D, Starvu F et al. Listeriolysin O: the Swiss army knife of Listeria. *Trends Microbiol*. 2012;**20**:36-368.
5. Low JC, Donachie W. A review on *Listeria monocytogenes* and Listeriosis. *Vet J*. 1997;**153**:9-29.
6. Maxie MG, Youssef S. Nervous system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 5th ed. Vol. **1**. Philadelphia, PA: Elsevier; 2007:282–457.
7. Oevermann A, Di Palma S, Doherr MG, et al. Neuropathogenesis of naturally occurring encephalitis caused by *Listeria monocytogenes* in ruminants. *Brain Pathol***.** 2010**;20:**378–390.
8. Oevermann A, Zurbriggen A, Vandevelde M. Rhombencephalitis Caused by Listeria monocytogenes in Humans and Ruminants: A Zoonosis on the Rise? *Interdiscip Perspect Infect Dis*. 2010:**2010**:1-22
9. Reuter R, Bowden M, Palmer M. Ovine listeriosis in south coastal Western Australia. *Aust Vet J*. 1989;**66**:223–224.

CASE III: 12L-15812 (JPC 4067411-00).

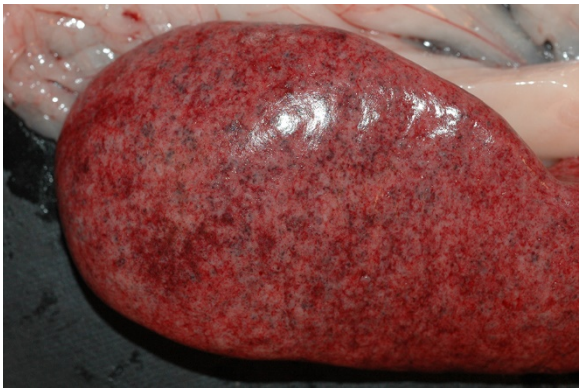
Signalment: 12-year-old, female, Ragdoll cat, (*Felis catus*)

History: Three days period of nausea and vomiting, followed by unconsciousness, convulsions and finally death.

Gross Pathology: Spleen: Splenomegaly, with pale pink / white marbled parenchyme. The liver showed disseminated, 2-5 mm sized, partly confluent pale white subcapsular and parenchymal areas. Within the gastrointestinal tract, a focal acute ulcerative gastritis, and a multifocal chronic ulcerative duodenitis was present. The duodenal lymph nodes showed mild enlargement and a homogeneous pale cream cut surface.

Laboratory results: None.

Microscopic Description: Section of spleen with mild mesothelial hyperplasia and mild capsular fibrosis. The splenic architecture (red and white pulp) is widely effaced by infiltration of a population of monomorphic large, approximately 20-40µm sized, plump, round cells with fine granular eosinophilic cytoplasm and round, small, hypochromatic, eccentric nuclei with one nucleolus. Mitotic figures are rare, focal apoptotic cells, mild diffuse hemosiderosis and hyperaemia is present.

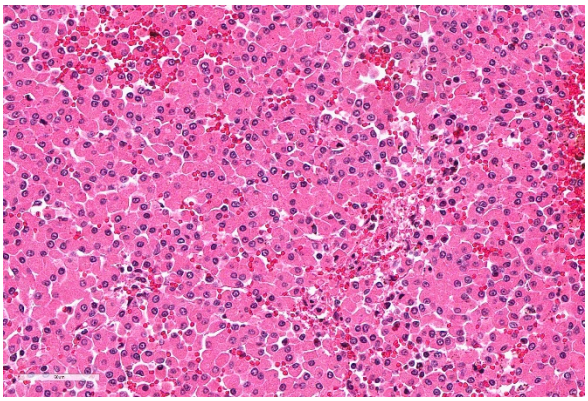


Spleen, cat: The spleen was diffusely enlarged with a pink-white marbled surface. (Photo courtesy of: University of Liverpool, http://www.liv.ac.uk/vetpathology/index.htm)

Special stains: Toluidine blue stain: Neoplastic cells reveal variable amounts of metachromatic fine cytoplasmic granula.

Immunohistochemistry:

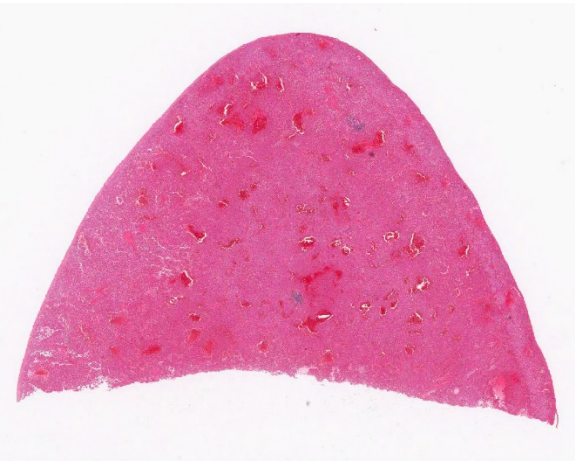
- Anti-Mast Cell Tryptase: Neoplastic cells exhibit variable degrees of weak positive reaction
- CD3: Neoplastic cells: negative reaction
- CD45R: Neoplastic cells: negative reaction



Spleen, cat: Neoplastic cells have abundant granulated eosinophilic cytoplasm and nuclei are often excentric. (HE, 400X)

Contributor's Morphologic Diagnoses: Diffuse visceral (splenic) mast cell tumor with mild mesothelial hyperplasia and capsular fibrosis, Ragdoll, *Felis catus*.

Contributor's Comment: According to the information sheet of Veterinary Society of Surgical Oncology (VSSO, http://www.vsso.org/Splenic_MCT.html), about 50% of feline visceral mast cell tumours are located in the spleen. Other sites of visceral mast cell tumours are the mediastinum, lymph nodes, and intestines. Visceral mast cell tumours present in three forms, smooth, diffuse, and nodular. These neoplasms often metastasize to liver (90%), visceral lymph nodes (73%), bone marrow (23%-40%), lung (20%), and intestine (17%), often accompanied by pleural and peritoneal effusions. The cutaneous involvement of mast cell tumours in cats with primary visceral MCT is less frequent (18%).

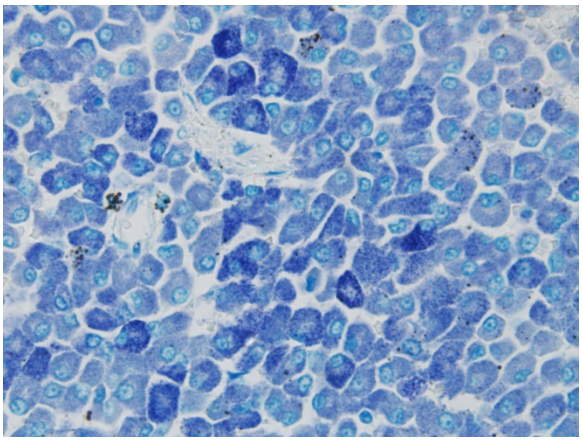


Spleen, cat: The splenic parenchyma is replaced by sheets of round cells with multifocal hemorrhage. (HE, 9X)

In the present case, metastases in mesenteric lymph nodes and bone marrow was observed. Within sections of stomach and duodenal ulcerations, no neoplastic mast cells could be demonstrated. large numbers of neoplastic mast cells as observed in the spleen, were observed within hepatic sinuses.

JPC Diagnosis: Spleen: Mast cell tumor.

Conference Comment: Review of the recent literature reveals a number of terms which are interchangeably used to describe non-cutaneous mast cell tumors in the cat. Visceral mast cell tumor, systemic mastocytosis, and mast cell leukemia are all in common usage; the use of the term mast cell leukemia implies mastocytemia. Mastocytemia is not uncommon in cats with visceral mast cell tumors; rates of up to 68% have been reported.[4]

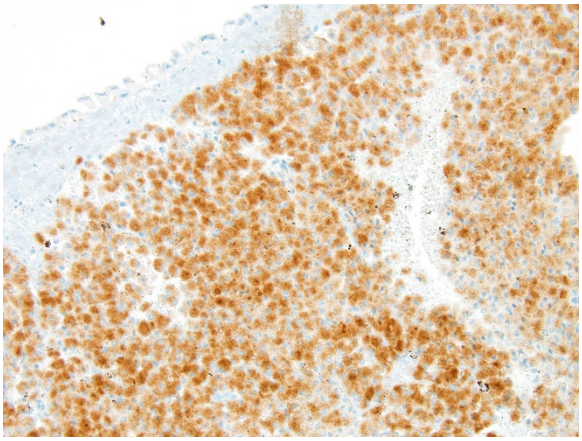


Spleen, cat: Neoplastic cells have numerous cytoplasmic metachromatic granules. (Toluidine blue, 400X)

Splenic involvement of mast cell tumors ranges from 15-50% in various reviews.[1,5] The liver and intestine are also common sites, and multiorgan disease is also seen. Although cats with visceral tumors may have concomitant cutaneous tumors as well, visceral mast cell tumors are considered a separate disease from cutaneous mast cell tumor; evidence that visceral tumors arise from a cutaneous primary is lacking in the veterinary literature.

Grossly, visceral mast cell tumors often cause diffuse organ enlargement, but may sometimes appear nodular.[1] Histologically, neoplastic mast cells have a finely granulated poorly stained cytoplasm and granules may not be recognizable on hematoxylin and eosin stained sections. Moreover, cytoplasmic granules may not stain with Toluidine blue or Giemsa. This is thought to be the case when neoplastic mast cells arise from the intestinal mucosa; special fixation in media other than formalin may be required for their cytoplasmic granules to be metachromatic.[1]

Immunohistochemical of evaluation of feline mast cell tumors may be frustrating. Reported rates of cytoplasmic expression of c-Kit ranges from 35[3] to 85%[5]. In the submitted case, Toluidine blue and Giemsa stains run at the JPC were strongly positive, and the tumors showed diffuse strong cytoplasmic expression of c-kit (toluidine blue and c-Kit illustrated in images 3-4 and 3-5, respectively.) One study demonstrated 14 missense c-kit mutations in 13 of 20 tumors; with 11 mutations located in exon 8, and 3 in exon 9.[5] No correlation was observed between c-kit mutations and tumor differentiation, mitotic activity, or survival time.[5]



Spleen, cat: Neoplastic cells have are strongly immunopositive for c-kit. (200X)

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http://www.liv.ac.uk/vetpathology/index.htm

References:

1. Kiupel, M. Mast Cell Tumors. In: Meuten DJ, ed. *Tumors in Domestic Animals, 5th ed.* Ames IA, Wiley and Sons 2017. pp 195-199.
2. Lamm CG, Stern AW, Smith AJ, Cooper EJ, Ullom SW, Campbell GA. Disseminated cutaneous mast cell tumors with epitheliotropism and systemic mastocytosis in a domestic cat. *J Vet Diagn Invest* 2009; 21:710-715.

3. Mallett CL, Northrup NC, Saba CF, Rodriguez CO, Rassnick KM, Gieger TL, Childress MO, Howerth EW. Immunohistochemical characterization of feline mast cell tumors. *Vet Pathol* 2012; 50(1): 106-109.
4. Piviani M, Walton RM. Significance of mastocytemia in cats. *Vet Clin Pathol* 2013; 42:4-10.
5. Sabattini S, Barzon G, Giantin M, Lopparelli RM, Dacasto M, Prata D, Bettini G. Kit receptor tyrosine kinase dysregulations in feline splenic mast cell tumors. *Vet Comp Oncol* 2017; 15(3):1051-1056.
6. Veterinary Society of Surgical Oncology. http://www.vsso.org/Splenic_MCT.html

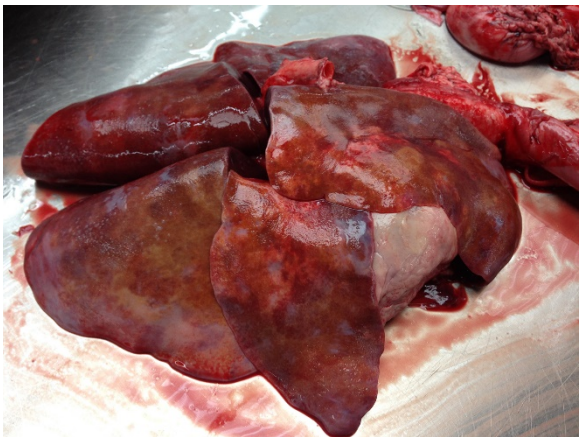
CASE IV: AFIP Canine 315010212_lungs (JPC 4067411-00).

Signalment: 1 year-old female Weimeraner dog, *Canis lupis familiaris.*

History: After undergoing anesthesia for laminectomy of T10 this dog failed to regain independent breathing and was euthanized during recovery.

Gross Pathology: The lungs have a mottled appearance composed of small areas of hemorrhage intermixed with coalescing areas of pale grey to tan tissue with multifocally slightly raised whitish nodules of a few millimeters in diameter. Both lungs are markedly oedematous, have an increased consistency and are firm to touch. There is a small amount of fibrin on the pleura of the cranioventral lobes.

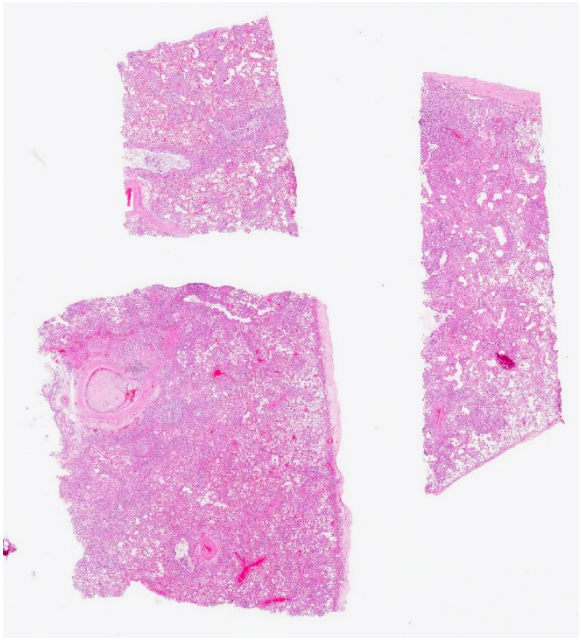
Laboratory results: None.



Lung, dog. The lungs have a mottled appearance due to areas of hemorrhage, collapse, consolidation, and emphysema. (Photo courtesy of: Utrecht University, Faculty of Veterinary Medicine, Department of Pathobiology, www.uu.nl/faculty/veterinarymedicine/EN/labs_services/vpdc)

Microscopic Description: Lung: The normal architecture of the lung is severely distorted by the presence of a predominantly granulomatous interstitial infiltrate associated with the presence of numerous *Angiostrongylus vasorum* larvae and eggs.

In both the alveolar interstitium and free within the alveolar spaces are moderate to large numbers of transverse or longitudinal sections of nematode larvae measuring approximately 20x80 µm and fewer multinuclear (embryonated) and uninuclear (non-embryonated) eggs, recognizable as approximately 60x90 µm, oval-shaped accumulations of coarsely granular eosinophilic material. These are typically surrounded by moderate numbers of extravascular erythrocytes, epithelioid macrophages (which occasionally show erythrophagy and contain cytoplasmic hemosiderin granules) and scattered multinucleated giant cells. The alveolar septa are often indistinct and are markedly expanded by moderate numbers of macrophages, fewer plasma cells and lymphocytes, and occasional eosinophils as



Haired skin, dog. Several section of lung are submitted for examination. At subgross magnification, a large arteriolar thrombus is visible as well as hypercellularity and exudation within a large airway. (HE, 5X)

well as moderate edema, and parasites. Alveoli are frequently lined by plump cuboidal epithelial cells (type 2 pneumocyte hyperplasia).

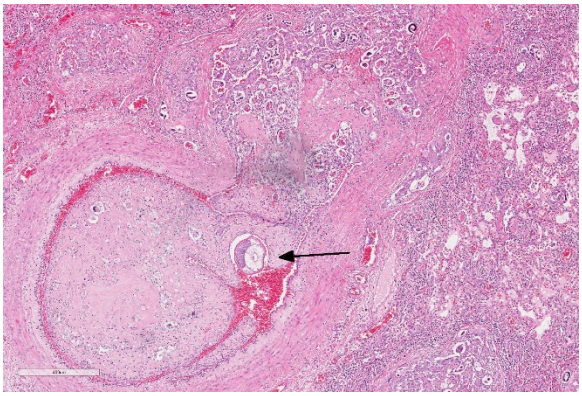
Throughout the section, there are mild to moderate vascular changes including moderate hypertrophy of the lamina media, separation of the collagen fibers of the tunica adventitia by fine reticular eosinophilic material (edema), and the presence of parasites within the vascular lumen. In the adventitia of some vessels, there are increased numbers of small-caliber blood vessels.

In some sections, adult nematodes are present within the lumen of large blood vessels; these are approximately 300µm in diameter and are characterized by a thin eosinophilic cuticle surrounding coelomyarian musculature and a pseudocoelom containing digestive and reproductive tracts. In some cases this is associated with partial occlusion of the lumen by an organizing thrombus composed of haphazardly arranged collagen bundles and fibroblasts, with endothelial cells forming small vascular channels (recanalisation) and scattered parasite eggs. Throughout the thrombotic material are moderate numbers of mixed inflammatory cells, predominantly plasma cells and macrophages. The lamina intima is markedly expanded by edema and moderate numbers of plasma cells and macrophages with fewer lymphocytes and occasional neutrophils. There is intimal hyperplasia characterized by the formation of multiple, endothelium-lined small, rounded, projections into the vessel lumen (proliferative endoarteritis).

Also in the less-severely affected areas, alveolar spaces and bronchiolar lumens contain moderate numbers of epithelioid macrophages, erythrocytes and protein-rich material. Bronchial epithelium shows mild hypertrophy and there are scattered intramucosal neutrophils.

The pleura is moderately expanded by mild fibrosis and edema and in some sections there are scattered groups of parasite eggs and larvae surrounded by macrophages, plasma cells and lymphocytes.

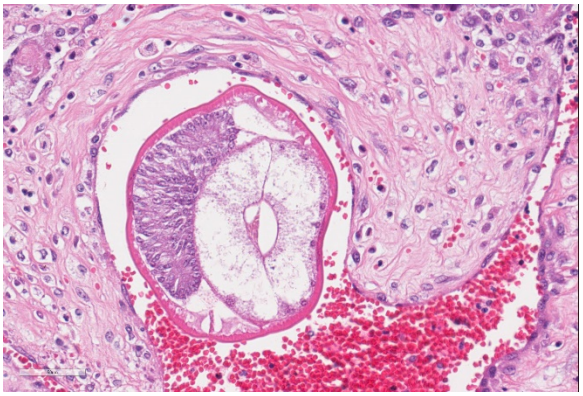
Contributor's Morphologic Diagnoses: Lung: Severe, chronic, multifocal to



Lung, dog. An arteriole is thrombosed and recanalized at the periphery. There is a cross section of an adult helminth within the thrombus (arrow) (HE, 54X)

coalescing interstitial granulomatous pneumonia with *Angiostrongylus vasorum* in different stages of development, interarterial thrombosis and marked pleural fibrosis.

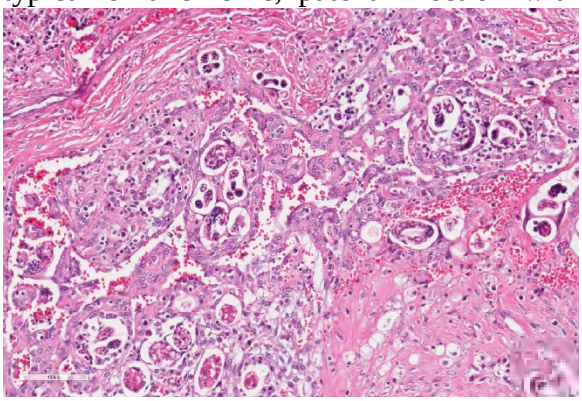
Contributor's Comment: *Angiostrongylus vasorum*, or French heartworm, is endemic in the Americas, parts of Africa and some European countries but until the end of the last decade was not considered endemic in the Netherlands.[5] The few cases reported before this time were typically seen in dogs which had been travelled outside of the country. Following the emergence of the disease in dogs which had never been outside the Netherlands, research was carried out to determine the prevalence of this parasite in Dutch foxes and intermediate hosts. Interestingly, concurrent to the increase in cases of *A. vasorum* seen in domestic dogs there has been an expansion of the range and population of foxes reported in the Netherlands. Combined with the wet Dutch environment that is conducive to the survival of the gastropod intermediate hosts, establishment of *A. vasorum* in the Netherlands is not surprising.[5] Of particular importance is the finding that L3 or the infective stage can be released from the intermediate host in fresh water meaning that infection may occur not only by ingestion of slugs and snails but by drinking water from the many canals and ditches that are prominent features of the Dutch countryside.[5]



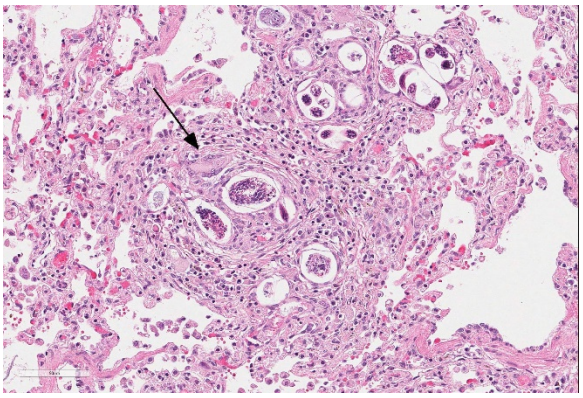
Lung, dog. The adult helminth as a thin hyaline cuticle, low polymyarian-coelomyarian musculature, lateral cords, a pseudocoelom, and cross sections of a gonad and intestine. (HE, 400X)

There was no history available for this young dog which underwent surgery for suspected lymphoma of the spinal cord from which it did not recover. The severe parasitic pneumonia may explain the post-surgical failure to resume independent breathing.

The histological lesions in this case are typical of a chronic, patent infection with multiple stages of parasite present and organizing thrombosis seen in the pulmonary vessels. The adults inhabit the right ventricle and pulmonary arteries releasing eggs after a pre-patency period of 35-60 days.[2] These are transported to the pulmonary capillaries from which L1 larvae hatch and penetrate into the alveolar spaces before being coughed up and excreted in the faeces. L1 are taken up by intermediate hosts (including snails, slugs and frogs) in which larval development continues to the infectious L3 stage and infection occurs in definitive hosts when these are ingested and mature larvae and young adult worms migrate via the circulatory system ultimately inhabiting the right ventricle. The presence of adult worms



Lung, dog. There is a plexiform area of recanalization in the adjacent artery which contains numerous cross-sections of embyronated and larvated metastrongyle eggs. (HE, 210X)



Lung, dog. There are numerous granulomas scattered throughout the pulmonary parenchyma centered on helminth larvae and eggs. Rare multinucleated macrophages (arrows) are seen. (HE, 280X)

in the circulatory system is thought to activate both intrinsic and extrinsic coagulation pathways resulting in intravascular consumptive coagulopathy and bleeding tendencies.[4] This may explain the persistent bleeding at the that was noted incision site during the operation preceding the death of this dog.

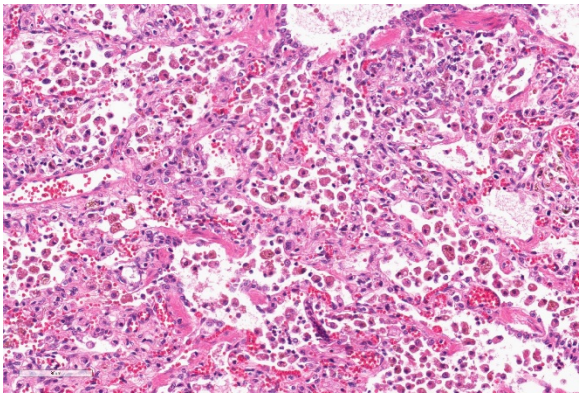
Whilst infection with *A. vasorum* can cause a range of other clinical signs the majority of cases develop cardiorespiratory problems, typically culminating in heart failure.[2,3] Other clinical presentations may reflect aberrant larval migration to organs such as the eye and brain.[2,3,5]

JPC Diagnosis: Lung: Pneumonia, interstitial, granulomatous, diffuse, severe, with proliferative endarteritis, thrombosis, and recanalization, and rare adult and numerous metastrongyle larvae and eggs.

Conference Comment:

Angiostrongylus vasorum, termed French heartworm because it was reported first in France in the 1800s[1], is a metastrongyle nematode considered to be the most pathogenic lungworm of dogs. *A. vasorum* has a worldwide distribution and the infection seems to be increasing in recent decades. Red foxes are the natural definitive hosts and are important reservoirs of infection for domestic dogs, however, natural infection has also been reported in other species including wolves, coyotes, and Eurasian badgers.[1]

Adults reside in the pulmonary artery and right heart ventricle in canids. Clinical signs run a gamut from mild coughing and exercise intolerance to fatal cardiopulmonary disease which may manifest as sudden death.
A. vasorum may cause right heart failure and extensive pulmonary lesions as a consequence of egg embolization. Prepatent lesions are mild with adult worms in the pulmonary arteries and a few 1-2 mm diameter, red, firm, multinodular to confluent areas of hemorrhage and edema at the lung periphery.[2] Female adults have a "barber pole" appearance due to their intertwined red intestine and white ovaries. At patency, lesions are more severe including proliferative endarteritis and interstitial granulomatous pneumonia, as displayed in this case. Vascular lesions are characterized by proliferative endarteritis, including thrombosis, thickening of tunica intima by

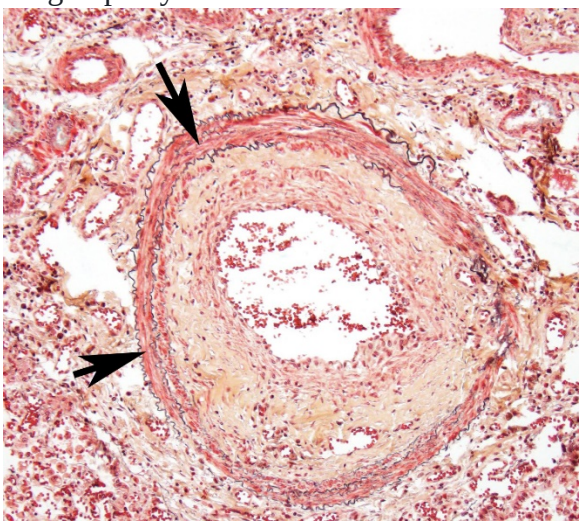


Lung, dog. Throughout the remainder of the section, alveolar septa are expanded by intraseptal macrophages, collagen, and patchy Type II pneumocyte hyperplasia. Alveoli contain numerous macrophages (often hemosiderin-laden), hemorrhage, edema, and rare migrating helminth larvae. (HE, 250X)

fibromuscular tissue, and medial hypertrophy with infiltration of eosinophils, lymphocytes and plasma cells.[2]

Coagulation abnormalities are commonly seen in association with *A. vasorum* infection. A prolonged activated partial thromboplastin or prothrombin times, thrombocytopenia, and increases in circulating D-diameters and fibrin degradation products suggests disseminated intravascular coagulation.[2,4] Affected dogs may have petechiae and ecchymoses in multiple tissues. The cause of this coagulopathy is unknown but may represent thrombosis triggered by parasite proteins or endothelial damage from adult and larval nematodes.[2]

Cerebral lesions to include mass lesions and areas of hemorrhage are noted as a combination of aberrant larval migration as well as the previously described coagulopathy.



Lung, dog. Pulmonary arterioles exhibit marked myointimal proliferation – within the tunica intima, there is proliferation of smooth muscle cells on the luminal side, with deposition of collage centrifugally. The internal elastic lamina is marked by black arrows. (Movat pentachrome. 200X)

The moderator reviewed a Movat's pentachrome stain and demonstrated a mural change in the branches of the pulmonary arteries which the moderator interpreted as myointimal proliferation. In this condition, vascular smooth muscle cells migrate into the tunica intima, undergoing both proliferation and secretion of extracellular matrix and collagen. This non-specific change results from endothelial activation and is often seen in cases of heartworm infection.

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References:

1. Bourque AC, Conboy G, Miller LM, et al. Pathological findings in dogs naturally infected with Angiostrongylus vasorum in Newfoundland and Labrador, Canada. *J Vet Diagn Invest*. 2008;**20**(1):11–20.
2. Caswell JL, Williams KG: In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., pp. 648. Elsevier Limited, Philadelphia, PA, 2007.
3. Gallagher B, Brennan SF, Zarelli M, et al. Geographical, clinical, clinicopathological and radiographic features of canine angiostrongylosis in Irish dogs: a retrospective study. *Ir Vet J*. 2012;**65**(1):5.
4. Ramsey IK, Littlewood JD, Dunn JK, et al. Role of chronic disseminated

intravascular coagulation in a case of canine angiostrongylosis. *Vet Rec*. 1996;**138**:360–363.
5. van Doorn DCK, van de Sande AH, Nijsse ER, et al. Autochthonous Angiostrongylus vasorum infection in dogs in The Netherlands. *Vet Parasitol*. 2009;**162:**163–166.