Joint Pathology Center
Veterinary Pathology Services

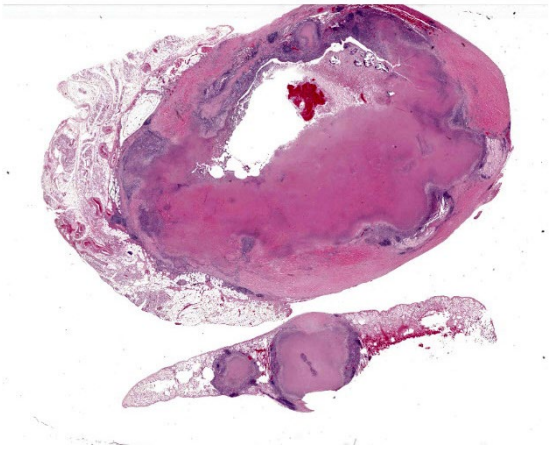
WEDNESDAY SLIDE CONFERENCE 2026-2027

Conference #10 — 28 October 2026

CASE I:

Signalment:
8.5-year-old cynomolgus macaque *(Macaca fasicularis)*

History:
This 8.5-year-old cynomolgus macaque was housed in the same room as a confirmed *Mycobacterium tuberculosis* complex positive macaque. This animal arrived at the National Institutes of Health quarantine facility in August 2016 and was transferred to USAMRIID in November 2016. Prior to its transfer to USAMRIID,[8] tuberculin skin tests had been administered and all were negative. Physical examination and cage side observation were unremarkable. On 7 March 2018, the animal was sedated for thoracic radiographs and serology testing for *Mycobacterium tuberculosis*. Thoracic radiographs revealed a diffuse nodular pattern and the submitted serum was positive for M. Tb complex. The animal was subsequently euthanized.

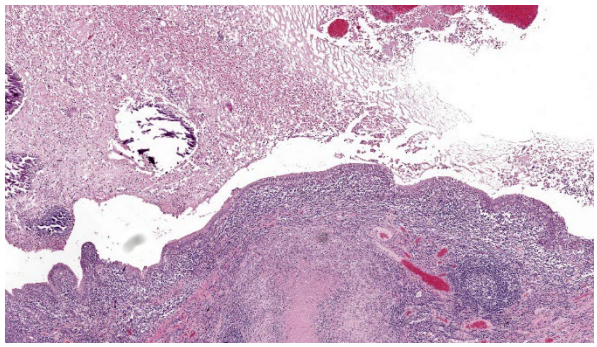


1-1. Lung, cynomolgus macaque: Two sections of lung are submitted for examination. Large granulomas are centered on airways within these sections. The larger granuloma at top has a cystic center, which corresponds to the lumen of an airway (endobronchial granuloma). (HE, 10X)

Gross Pathology:
This cynomolgus macaque had a body condition score of 4 of 5 with adequate subcutaneous and internal adipose tissue. All lung lobes contained white, multifocal to coalescing nodules that contained yellow, caseous material when incised. There were multiple fibrous adhesions that extended from the lungs to the thoracic body wall.

Laboratory Results:
Serum was positive for Mycobacterium tuberculosis complex antibodies and an immunofluorescence assay performed on lung detected Mycobacterium tuberculosis complex in a pulmonary granuloma.

Microscopic Description:
Lung (two sections): Multifocally, there are granulomas that measure up to 1.5 cm in greatest diameter that efface and compress pulmonary parenchyma. Granulomas are composed of a large, central area of eosinophilic necrotic debris with multifocal areas



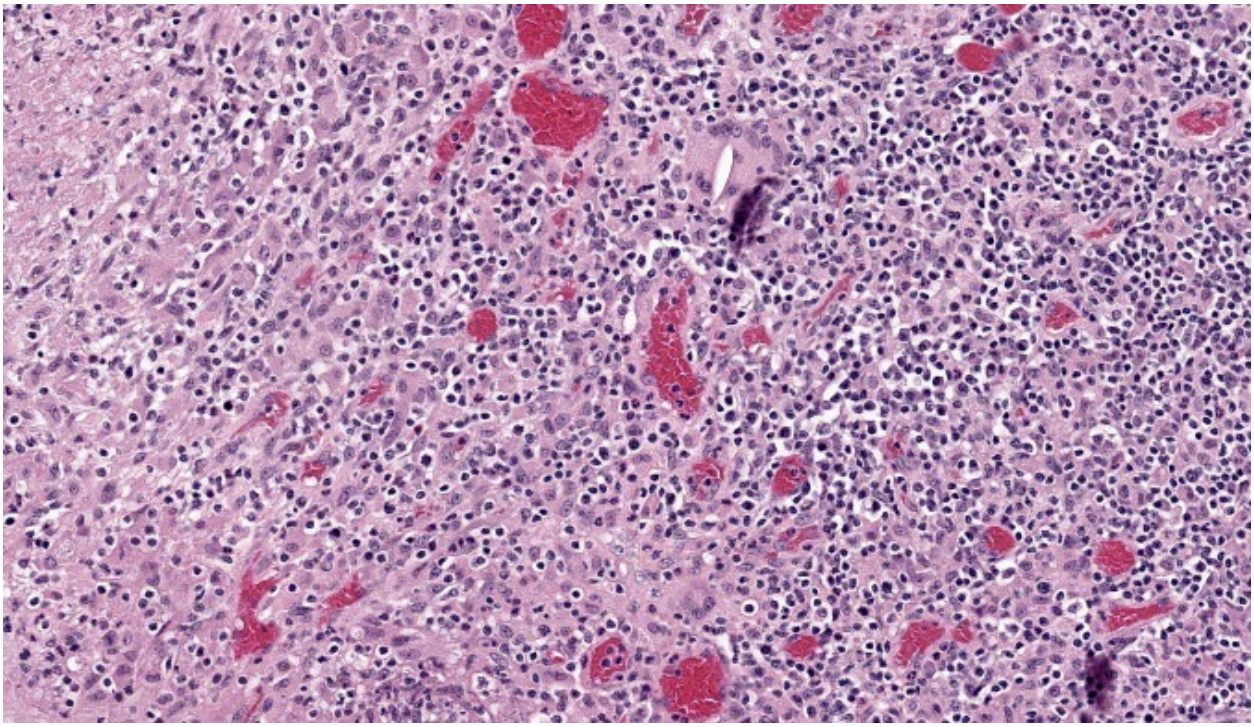
1-2. Lung, cynomolgus macaque: The largest granulomas extends into a bronchiole, with a layer of remnant airway epithelium and abundant and multifocally mineralized granular debris in its lumen. (HE, 70X)

of deeply basophilic, granular material (mineral). At the periphery of the central necrosis, there is a rim of low numbers of mostly degenerate neutrophils and low numbers of foamy macrophages and rare Langhans giant cell macrophages. This is surrounded by fibroblasts and abundant collagen and many lymphocytes and plasma cells. The largest granuloma obliterates a bronchus with degeneration, necrosis, and loss of lining epithelium with replacement by large numbers of neutrophils and foamy macrophages, fewer eosinophils, necrotic debris, fibrin, and edema. Subjacent to the remaining epithelium and inflammation, there is extensive fibrosis and the formation of trabeculae of acellular brightly eosinophilic material that are lined by plump spindle cells (reactive bone formation). Alveolar septa are compressed and multifocally, alveolar spaces contain edema. Multifocally, the pleura is expanded by abundant fibrous connective tissue.

Contributor's Morphologic Diagnoses:
Lung: Granulomas, multifocal, marked with fibrosis, edema, and reactive bone formation.

Contributor's Comment:
This case was one of a few cynomolgus macaques in our institution that tested positive to M. tuberculosis (Mtb). M. tuberculosis is a non-motile, nonsporulating, weakly gram-positive, acid-fast positive bacillus that appears microscopically as a straight or slightly-curvedlod.[15] It is a member of the M. tuberculosis complex that also includes M. bovis, M. africanum, M. canetti, and M. microti.[15] It is estimated a third of the world's population is infected and approximately 1.7 million people die from TB every year.11 Infection with Mtb is compounded by emergence of multi-drug resistant strains and co-infection with HIV and an estimated 11 million people are co-infected annually.[11 16]

Macaques can be infected by exposure with infected humans and vice versa and common primate housing and breeding strategies that are used to enhance socialization and welfare may promote transmission of Mtb.[1] Climate change and habitat encroachment by humans have led to overlapping of home ranges of wildlife such as nonhuman primates with human settlements.[10] Both New World and Old World species are susceptible, but most reports of mycobacteriosis have been in cynomolgus and rhesus macaques.[1]
After infection with the bacteria, pulmonary macrophages are attracted to the cell wall components of the bacteria. Within the macrophage, mycobacteria survive phagocytosis by phagosome escape and inhibition of phagosome-lysosome fusion. Containment of infection is dependent on the host's ability to mount an appropriate Th1 response and sequester the bacteria within granulomas. If the Th1 response is adequate, the infection is contained, and low numbers of mycobacterial bacilli are sequestered within



1-3. Lung, cynomolgus macaque: The wall of the granuloma contains abundant eosinophilic debris (far left), which is surrounded by large numbers of epithelioid macrophages, lymphocytes, plasma cells, and few Langhans-type multinucleated macrophages. (HE, 377X)

walled-off granulomas in the lungs. However, if the Th1 response is not adequate to the control the infection, bacilli-containing macrophages may spread to distant organs and seed granulomas in those locations.[1]

When exposed to Mtb, cynomolgus macaques generally follow three different disease progressions: rapid progression to disease, active-chronic infection, and no apparent disease (latent infection).[2] As early as two weeks after infection, some monkeys have evidence of bronchopneumonia by thoracic radiography and elevation of erythrocytic sedimentation rate (ESR) by 4 weeks. At 4 weeks, these animals begin to exhibit clinical signs of disease including anorexia that is often accompanied by progressive weight loss.[19] Granulomas are observed in the lungs as early as 4 weeks postinfection with enlargement of hilar lymph nodes with granulomas by 5 weeks postinfection.[8] By 6 weeks, Mtb can be cultured from bronchoalveolar lavage (BAL) and gastric aspirates. As time progresses, anorexia and weight loss worsen with cachexia and the appearance of respiratory symptoms such as tachypnea and dyspnea. At necropsy, there are granulomas disseminated throughout the lungs with hilar and anterior mediastinal lymphadenopathy and extrapulmonary lesions in the liver, spleen, mesentery, kidneys, and other sites.[14] Granulomas in macaques are like those found in human TB disease and may be solid, necrotic and/or caseous.[19],

Active-chronic infection in cynomolgus macaques can be defined as persistent disease with evidence radiographic involvement of the lungs/thoracic cavity, positive bacterial cultures and/or other clinical signs of active disease.[2] Compared to macaques with rapid,

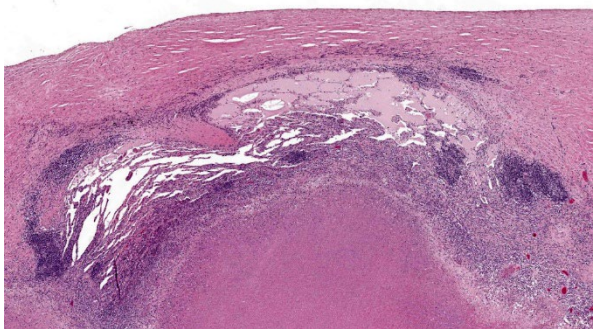
acute disease that rapidly progresses to euthanasia/death, animals with active-chronic infection may survive for months, apparently controlling their Mtb disease, and then succumbing to re-activated acute disease.

Finally, cynomolgus macaques with no apparent disease or latent infection have no radiographic involvement after 4 weeks of infection and no clinical signs of disease for at least 6 months.[2] When necropsied, these and possible hilar and/or mediastinal lymph node enlargement and caseation without evidence of spread outside the thoracic cavity. These monkeys appear to be controlling the infection and maintaining low numbers of bacteria within the granulomas, like latent disease in humans. Some monkeys can control an experimental infection with no apparent signs of disease for >21 months.[2]

Accurate clinical diagnosis of infected macaques can be difficult and even positive cases of Mtb have been found in so-called closed colonies of macaques.[12] The most common procedure for Mtb screening in macaques is tuberculin skin testing (TST) performed via intradermal eyelid injection of old mammalian tuberculin. The palpebra is observed 24, 48 and 72 hours after injection and reactions are graded according to a standard protocol.[12] Unfortunately, false-negative or false-positive results are common and the TST continues to remain inadequate in detecting Mtb-infected NHPs.[6] False-positive tests occur because some of the antigenic components in the tuberculin are shared between pathogenic and nonpathogenic mycobacteria.[5] False-negative tests occur when a NHP has a less than robust DTH response.

Detection of *Mycobacterium tuberculosis* complex(MTBC)-specific antibodies can be performed using different serologic assays, but results have been found to be suboptimal with the generation of false-positives and false-negatives. Seventeen newer diagnostic tests have been developed including the **PRIMIGAM** test which measures the interferon-gamma response to purified protein derivatives of M. bovisand M. avium.[5] This test is commercially available and is reported to have higher specificity, but lower sensitivity for detection of Mtb than the standard TST. Use of both assays or the other in parallel with the TST could increase overall sensitivity in a TB screening program for rhesus and cynomolgus macaques.[18]

The gold standard of detection of active Mtb in humans and NHP remains the sputum culture. Unfortunately, sputum culture is highly insensitive and requires biosafety level-3 laboratory conditions for testing.[17] Molecular diagnosis of Mtb using polymerase chain reaction testing to amplify the 1S6110 repetitive nucleic acid sequence characteristic of MTBC can be



1-4. Lung, cynomolgus macaque: Granulomas occasionally entrap remnant pulmonary parenchyma, with marked dilation of alveolar spaces and filling with abundant edema. The granuloma extends into and incorporates the overlying pleura (HE, 70X)

used. However, this testing cannot differentiate between Mtb complex species.[17]

The sole available vaccine against tuberculosis, *Mycobacterium bovis* Bacille Calmette Guerin (BCG) vaccine, protects children from developing severe tuberculosis (TB), but it mostly fails to protect against pulmonary TB in adults and is unsuitable for use in immunocompromised individuals. A recent study showed that when vaccinated intravenously vs. the standard intradermal route and then challenged with M. tuberculosis, rhesus macaques had decreased bacterial burden in the lungs and higher lgM titers. Another study found that immunizing rhesus macaques with BCG by the IV route and then challenging them with Mtb resulted in marked increases in antigen-responsive T cells and unprecedented protection against the challenge.

Recently, an experimental vaccine with a CMV vector expressing six or nine Mtb antigens was developed and evaluated against a challenge with Mtb in rhesus macaques. A major focus of new vaccines is to elicit large numbers of memory T cells that recognize and activate infected cells before Mtb can establish a niche in which to evade host immunity.[3] Nearly a year after the rhesus macaques were vaccinated and boosted, these novel vaccines reduced the burden of TB and prevented detectable infection in >40% of animals after Mtb challenge.[3]

Despite their large size and high cost, non-human primates (NHP) have been shown to be excellent animal models of Mtb. NHPs can be readily infected through instillation of bacilli into the bronchi via bronchoscope. About 50% will develop primary TB, while the other half will develop latent disease.[15] Rhesus or cynomolgus macaques are most used to study Mtb and to test and evaluate vaccines. Multiple studies have shown that rhesus macaques are more susceptible to TB infection and disease than cynomolgus macaques; therefore, rhesus macaques tend to be utilized for studying active TB, while cynomolgus macaques are used more to study latent or chronic TB.[13,19]

NHPs can also be used to model the effect of HIV and Mtb coinfection using coinfection with simian immunodeficiency virus and Mtb. When rhesus macaques were infected with a strain and dose of Mtb to induce a latent infection, later infection with SIV, reactivated the TB infection as assessed by clinical, pathological, and microbiological measures including increases in serum C-reactive protein, pneumonia by chest radiography, decreased survival, bacterial load in bronchoalveolar lavage fluid and higher numbers of lesions at necropsy.[11]

Mice have been used to study TB, but there are some issues that make mice a less than optimal animal model. First, mice are relatively resistant to infection with Mtb and can harbor high numbers of Mtb in the lungs without showing clinical signs.[15] When mice do develop Mtb lesions in the lung, they do not develop in a manner like humans, and they do not form cavitary lesions or exhibit fibrous capsules. The lesions in their lungs may be "inverted"' granulomas where the lymphocytes and plasma cells are centrally located with the infected macrophages located peripherally. The centrally located, IFN-v-producing T cells have greater access to the infected macrophages

and may thus allow for better control of infection and reduced necrosis.[15] Despite these shortcomings as an animal model for Mtb, mice are widely used as a small animal model because of the low cost, availability of immunological reagents and the availability of inbred and genetically engineered strains that are well-characterized.[4]

Guinea pigs develop Mtb lesions similar to humans including robust delayed type hypersensitivity (DTH) responses to mycobacterial antigens, development of caseous and mineralized granulomas, primary and hematogenous pulmonary lesions, fibrous capsule formation and dissemination.[15] Some of the downsides of the guinea pig Mtb model include a high susceptibility to Mtb infection which makes them unsuitable for studies of latency, the pulmonary lesions contain primarily granulocytes, many of which are eosinophils which is not seen in human Mtb, poorly characterized inbred guinea pig strains and the availability of fewer reagents.[15]

Rabbits are relatively resistant to Mtb, but can be infected by challenge doses of larger numbers of bacteria than is required to infect mice or guinea pigs. Once challenged, rabbits develop a typical DTH response with caseous and cavitary granulomas that resolve quickly with very few recoverable bacilli.[15] Since they clear disease successfully and quickly, they are not good models for latent TB and their larger size makes them more costly to use than mice or guinea pigs. Finally, the availability of only minimal numbers of immune reagents makes them less desirable as a model.

Contributing Institution:
Pathology Division, USAMRIID, 1425 Porter Street, Fort Detrick, MD 21702
https://usamriid.health.mil/

JPC Diagnoses:
Lung and pleura: Granulomas, multiple, chronic, with mineralization and reactive bone formation.

JPC Comment:
This week's moderator is an alumnus of the DoD Veterinary Residency Program at the Armed Forces Institute of Pathology, who ultimately became its Director, following its move to the Joint Pathology Center. He currently is the chief of the Directorate of Infectious Disease Pathogenesis at the National Institute of Allergy and Infectious Disease in Rockville, MD.

Kudos and bravo to this longstanding contributor for an outstanding write-up that has done absolutely all of the heavy lifting in this case. Tuberculosis in non-human primates has appeared in the stacks of the Wednesday Slide Conference five times in the last 30 years, with additional cases in a dog (WSC 2024, Conf 17, Case 4) and an experimentally infected guinea pig which manifested the disease in its mammary gland (WSC 2008, Conf 10, Case 4).

While gross images of the lesion in the lung were not submitted, the cystic nature of the large airway-centered granuloma in this case is perhaps unfamiliar to people who have not encountered tuberculosis. Tuberculoid granulomas that infiltrate the airway wall (a.k.a. endobronchial granulomas) may spill their necrotic centers into the airway, resembling an abscess more than a traditional solid granuloma and delaying diagnosis.[20] To further confuse this issue, an uncommon variant of tubercular granulomas is referred to as a "cold abscess," in which the inflammatory cells in the

wall are typically those of acute inflammation, and epithelioid macrophages and Langhans giant cells are uncommon.[21]

In his lab at the NIAID, Dr. Alves was able to confirm the etiologic diagnosis in this section with an immunomarker for *M. tuberculosis*.

References:

1. Bailey C and Mansfield K. Emerging and reemerging infectious diseases of nonhuman primates in the laboratory setting. *Veterinary Pathol.* 2010;47(3):462-481.
2. Capuano Ill SV, Croix DA, Pawar S, et al. Experimental *Mycobacterial tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human *M. tuberculosis* infection. *Infect Immun.* 2003;71(10):5831-5844.
3. Carpenter SM and Behar SM. A new vaccine for tuberculosis in rhesus macaques. *Nature Med.* 2018;24(2):124-126.
4. Chan ED, Verma D and Ordway DJ. Animal models of mycobacterial infection. *Current Protocols in Immunology e98.* 2020;129:1-52.
5. Garcia MA, Yee J, Bouley DM, et al. Diagnosis of tuberculosis in macaques, using whole-blood in vitro interferon-gamma (PRIMAGAM) testing. *Comp Med* 2004;54(1):86-92.
6. Gong W, Yang Y, Luo Y, et al. An alert of *Mycobacterium tuberculosis* infection of rhesus macaques in a wild zoo in China. *Exp. Anim.* 2017;66(4):357-365.
7. Irvine EB, O'Neil AO, Darrah PA, et al. Robust lgM responses following intravenous vaccination with Bacille Calmette-Guérin associated with prevention of *Mycobacterium tuberculosis* infection in macaques. *Nature lmmun.* 2021;22:1515-1523.
8. Lin PL, Pawar S, Myers A, et al. Early events in *Mycobacterium tuberculosis* infection in cynomolgus macaques. *Infect and lmmun.* 2006;74(7):3790-3803.
9. Maiello P, DiFazio RM, Cadena AM, et al. Rhesus macaques are more susceptible to progressive tuberculosis than cynomolgus macaques; a quantitative comparison. *Infect and lmmun.* 2018;86(2):1-14.
10. Meesawat S, Warit S, Hamada Y, et al. Prevalence of *Mycobacterium tuberculosis* complex among wild rhesus macaques and 2 subspecies of long-tailed macaques, Thailand, 2018-2022. *Emerging Infectious Diseases.* 2023;29(3):551-560.
11. Mehra S, Golden NA, Dutta NK, et al. Reactivation of latent tuberculosis in rhesus macaques by co-infection with Simian Immunodeficiency virus. *J Med Primatol.* 2011;40(4):233-243.
12. Payne KS, Novak JJ, Jongsakul K, et al. *Mycobacterium tuberculosis* infection in a closed colony of rhesus macaques *(Macaca mulatta). J of AALAS.* 2011;50(1):105-108.
13. Pena JC and Ho WZ. Non-human primate models of tuberculosis. *Microbial Spectrum.* 2016;4(4):TBTB2-0007-2016.
14. Pereira AHB, Lopes CAA, Pissinatti TA, et al. Pulmonary granuloma is not always the tuberculosis hallmark: pathology of tuberculosis stages in new world and old-world monkeys naturally

infected with the *Mycobacterium tuberculosis* complex. *J Comp Path.* 2022;199:55-74.

15. Sakamoto K. Pathology of Mycobacterium tuberculosis infection. *Vet Pathol* 2012;49(3):423-439.

16. Sharpe S, White A, Gleeson F, et al. Ultra low dose aerosol challenge with *Mycobacterium tuberculosis* leads to divergent outcomes in rhesus and cynomolgus macaques. *Tuberculosis.* 2016;96:1-12.

17. Wilbur AK, Engel G, Rompis A, et al. From the mouths of monkeys: detection of *Mycobacterium tuberculosis complex* DNA from buccal swabs of synanthropic macaques. *Am J Primatol.* 2012;74(7):676-686.

18. Yee JL, Prongay K, Van Rompay KKA, et al. Tuberculosis detection in nonhuman primates is enhanced by using testing algorithms that include an interferon-γ release assay. *Am J Vet Res.* 2022;83(1):15-22.

19. Zhang J, Xian 0, Guo M, et al. *Mycobacterium tuberculosis* Erdman infection of rhesus macaques of Chinese origin. *Tuberculosis.* 2014;94:634-643.

20. Patel VR, Viswam D, Rajesh V, Nagrajan A, Seema K, Babu S. Tissue is issue: Not all masses are tumors. Am J Case Rep. 2012;13:51-4.

21. Kabiri EH, Alassane EA, Kamdem MK, Bhairis M, Amraoui M, El Oueriachi F, El Hammoumi M. Tuberculous cold abscess of the chest wall: A clinical and surgical experience. Report of 16 cases(Case series). Ann Med Surg (Lond). 2020 Feb 13;51:54-58. doi: 10.1016/j.amsu.2020.02.001. PMID: 32099646; PMCID: PMC7029049.

The opinions, interpretations, conclusions, and recommendations presented are those of the author and are not necessarily endorsed by the U.S. Army or Department of Defense.

USAMRIID is fully accredited by the AAALAC International, and all animal research conducted at USAMRIID complies with the Animal Welfare Act and other federal statutes and regulations relating to animals in research and adheres to The Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.

CASE II:

Signalment:
11-month old, female, texel, ovine

History:
A group of 16, 11 month-old sheep were turned out onto 10 acres of mountain pasture



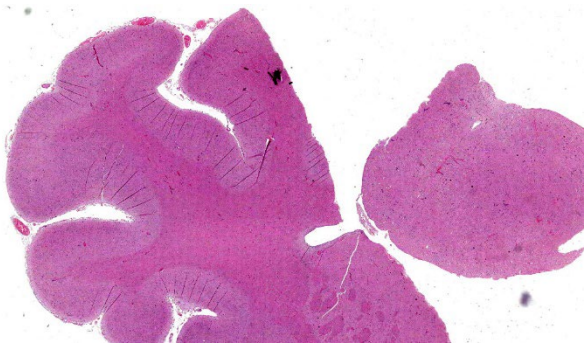
2-1. Presentation, sheep: Abundant ticks (*Ixodes ricinus*) were identified in the skin folds of the axilla and perineum. (*Photo courtesy of:* Veterinary Sciences Centre, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland, http://www.ucd.ie/vetmed/).

on an organic farm located on the west coast of Ireland in December. The sheep were home bred but had not grazed this pasture previously. The sheep were treated with deltamethrin and avermectin prior to release to pasture. 14 days after turnout the farmer found 9 of the sheep dead. Three of the surviving animals presented with lethargy, trembling, frothing at the mouth and degrees of recumbency; all subsequently died despite treatment. The farmer described a previous occurrence of undiagnosed 'sudden' death in two sheep grazing the same pasture the previous August.

Gross Pathology:
The nine carcases submitted were in variable states of preservation: some had been partially scavenged by wildlife. Splenomegaly was present in 6 of the 9 carcases. In the most well preserved bodies, there was fibrinous pericardial exudate, wet heavy lungs and liquid intestinal contents. Abundant ticks (*Ixodes ricinus*) were identified in the skin folds of the axilla and perineum. No toxic plants were found in the rumen. Brains appeared normal.

Laboratory Results:
PCR and whole genome sequencing positive for flavivirus. PCR positive for *Anaplasma phagocytophilum*. Blood samples from surviving sheep microscopic agglutination test (MAT) positive for Louping ill virus and ELISA positive for *Anaplasma phagocytophilum*

Microscopic Description:
Diffuse, variably severe perivascular cuffing (comprising predominantly lymphocytes with lesser numbers of plasma cells and histiocytes) in white and grey matter areas with meningeal involvement multifocally. Similar leukocytes (some degenerate) variously infiltrate vascular walls and endothelial cell swelling is



2-2. Cerebrum, sheep: One section of cerebrum and one section of thalamus is submitted for examination. (HE, 12X)

observed. Perivascular haemorrhage associated with a proportion of blood vessels. Nodular to diffuse gliosis in grey matter. Occasionally and multifocally, neurons are hypereosinophilic, shrunken and angulated (necrotic) and are closely surrounded by glial cells (satellitosis).

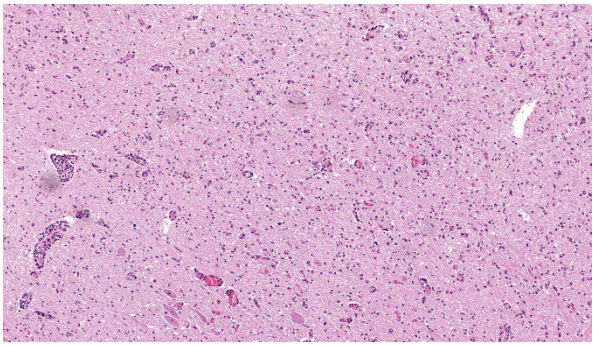
Contributor's Morphologic Diagnoses:
Brain: lymphoplasmacytic meningoencephalitis, multifocal, moderate with neuronal necrosis and satellitosis.

Contributor's Comment:
Louping ill is an arboviral encephalomyelitis of sheep caused by louping ill virus (LIV), a tick-borne neurotropic flavivirus of the genus *Flavivirus* in the family *Flaviviridae*[3]. The name originates from affected animals' characteristic bounding gait ('louping' is Scottish vernacular for 'leaping' or 'bounding') caused by virus-mediated CNS and in particular cerebellar injury and inflammation. The disease is enzootic in upland regions of Ireland and the United Kingdom, and is associated with transmission by the three-host tick *Ixodes ricinus*, which is widespread in these areas. Louping ill primarily affects sheep, but can also infect goats, cattle, horses, deer, red grouse, and occasionally humans [2,3,8]. A recent LIV-associated meningoencephalomyelitis in a foxhound

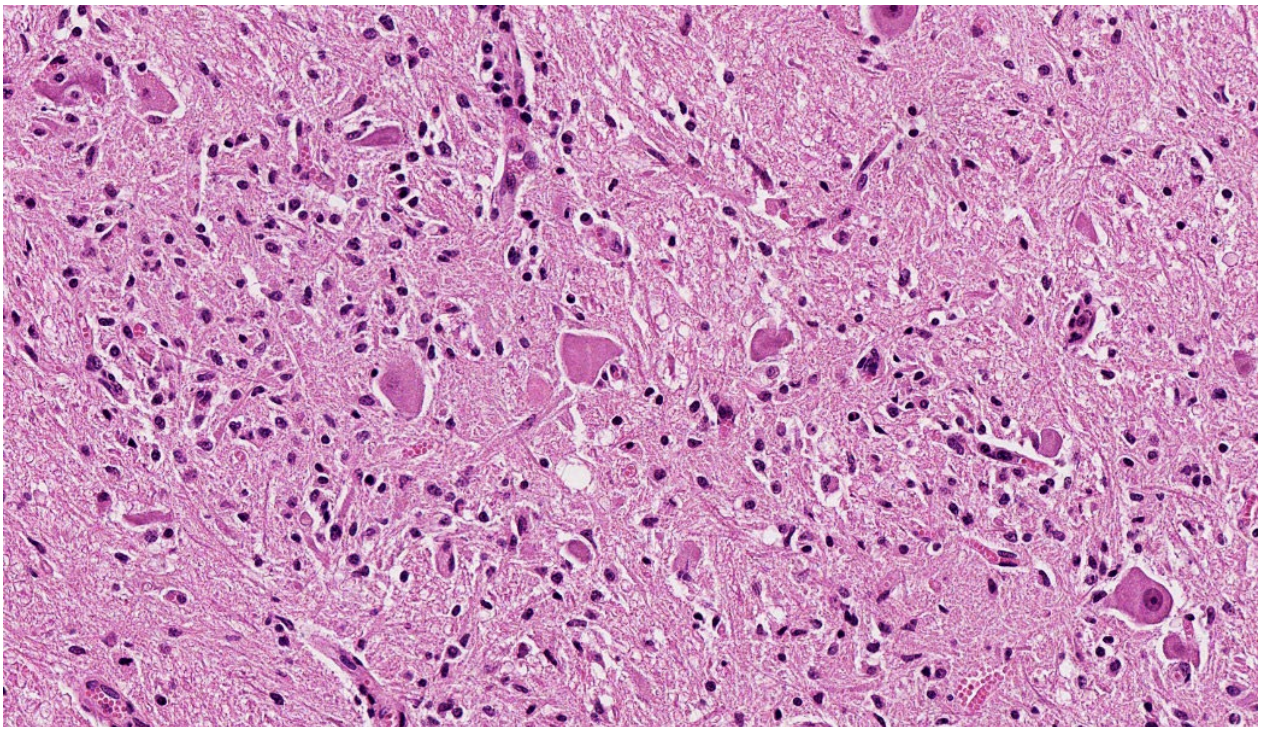
with histopathological and molecular confirmation further expands the known host range and underscores the need for broader surveillance of the virus as a potential cause of encephalitis in multiple species[4].

Ixodes ricinus also serves as the vector for *Anaplasma phagocytophilum*, the agent of tick-borne fever (TBF), a synergistic co-infection that increases the risk and clinical severity of louping ill through immunosuppression[8,10]. Such co-infection in the described case likely contributed to the severe mortality. Splenomegaly, as reported in this case, is a common gross postmortem finding in TBF infection in sheep. Following inoculation by tick bite, LIV initially replicates in draining lymph nodes before disseminating to other lymphoid organs and the CNS via viraemia. The incubation period is typically five to six days, with viraemia preceding the onset of neurological signs [3,10] which follow invasion of the central nervous system (CNS), via the blood–brain barrier or indirectly via the olfactory nerves following viral localisation in nasal structures[3]. Within the CNS, LIV displays tropism for neurons, particularly cerebellar Purkinje cells and motor neurons of the spinal cord [3,8]. Histological features include gliosis, neuronal necrosis, neuronophagia, and mononuclear perivascular cuffing, often accompanied by variable leptomeningitis. Lesions typically localise to the brainstem, cerebellum, and cervical cord[3,8]. Experimental studies confirm that pre-existing infection with *A. phagocytophilum* increases the susceptibility of the CNS to invasion with LIV and increases lesion severity, likely through impaired neutrophil function and suppression of innate immunity[8,10]. Recent data indicates that *A. phagocytophilum* genotypes are widespread in *I. ricinus* ticks in Ireland[5].

Clinically, louping ill may present as sudden death or acute-onset progressive neurological signs such as ataxia, tremors, hyperesthesia, nystagmus, or recumbency, with death occurring within hours to days. Clinical signs usually occur following turnout to areas with suitable tick habitats indicating tick exposure as a significant risk factor. In naïve flocks, mortality can exceed 60%, particularly in lambs with waning maternal immunity or no prior exposure, but can occur at any age[3,10]. The sheep in the current case were likely to be immunologically naïve as they, or their dams, had not grazed the pasture previously. In the authors' experience, the initial febrile phase is rarely described in the clinical history provided at postmortem examination. This likely reflects the disease's occurrence in extensive grazing systems, such as mountain pastures, where close monitoring of individual animals is not feasible. Animals exhibiting advanced neurological signs rarely recover, and deaths typically continue to occur over a period of seven days from the onset of clinical signs. The clinical course may vary depending on host age, immune status, and co-infection with *A. phagocytophilum* [8,10].



2-3. Brainstem, sheep: Virchow-Robin's spaces are expanded by 1-3 layers of lymphocytes and plasma cells. There is diffuse mild gliosis of the surrounding neuroparenchyma. (HE, 127X)



2-4. Brainstem, sheep: Higher magnification of the inflamed neuroparenchyma. Neuronal swelling and occasional satellitosis is seen. (HE, 3074X).

A 2011 serological study conducted at the Sligo Regional Veterinary Laboratory in Ireland reported a flock-level antibody prevalence of 9.8% to LIV, with higher seropositivity in adult sheep, suggesting repeated exposure and the potential for subclinical infection or age-related immunity in enzootic areas[1]. This study was geographically restricted and is not likely to be representative of the national sheep population, but did highlight the potential disease exposure in certain regions. Serology can support flock-level surveillance or confirm prior exposure, but interpretation is complicated by cross-reactivity with related flaviviruses, including tick-borne encephalitis virus (TBEV) and West Nile virus[3]. Although TBEV is not endemic in Ireland, its recent detection in southern England and potential for serological interference highlight the importance of test specificity and ongoing regional surveillance[7]. Whole genome sequencing of viral RNA is increasingly being used to distinguish closely related flaviviruses, which is especially important considering the differences in host ranges and zoonotic implications of the flavivirus group.

Environmental and land use factors are increasingly recognised as key determinants of LIV transmission dynamics. The distribution and seasonal activity of *I. ricinus*, the principal vector, are influenced by climate, vegetation, and host availability[11]. Warmer, wetter conditions associated with climate change are predicted to expand the tick's geographic range and extend its active season, thereby increasing the potential for LIV transmission[6,9] . It is notable that this outbreak occurred in December at a time of year when ticks would not be considered to be active. Organic farming systems may also contribute to increased tick exposure due to restrictions on acaricide use and greater contact with wildlife reservoirs such as

deer and red grouse[6]. Control of louping ill remains challenging. A previously available inactivated vaccine was withdrawn in 2017, and although a new formulation has been developed, at time of writing, this product is not widely available due to its limited commercial viability. While disease outbreaks can be devastating at flock level in endemic areas, the overall demand for vaccines against LIV remains low in global terms, making future availability uncertain. In the absence of a vaccine, prevention relies on integrated strategies including tick control, strategic grazing management, and research into the risks associated with wildlife reservoirs.

There are no gross lesions, and definitive diagnosis of louping ill requires laboratory confirmation, typically through detection of viral RNA in CNS tissue by reverse transcriptase polymerase chain reaction (RT-PCR) or demonstration of viral antigen by immunohistochemistry. These techniques are most sensitive when applied to CNS regions with the highest lesion burden and viral tropism, such as the cerebellum, brainstem, or cervical cord[3,8]. The most severe microscopic lesions are typically found in the cerebellum including Purkinje cell necrosis and gliosis in the molecular layer ('glial shrubbery')[3]; however, the current case illustrates that lesions are also found in more rostral regions such as the cerebrum and basal ganglia. Lesion distribution can vary with chronicity; typically polioencephalitis in acute cases progressing to meningoencephalomyelitis. Particular histopathological findings are highly suggestive of louping ill - neuronal necrosis, neuronophagia, satellitosis, and gliosis. However, these changes are not pathognomonic, and other common differential diagnoses for infectious nervous disease in sheep, such as listeriosis, sarcocystosis, and other viral encephalitides, must be considered especially if limited sections of brain tissue are available for examination making interpretation of the distribution of lesions more difficult. Diagnostic confidence increases when typical histopathological findings occur in animals from appropriate seasonal or geographic contexts.

This case highlights the continued relevance of LIV as a differential diagnosis in ovine neurological disease, particularly in hill and rough grazing systems where tick exposure is high and routine animal monitoring is limited. Although geographically restricted, louping ill can cause severe outbreaks with high mortality in naïve flocks, and the potential for clinical misdiagnosis underscores the importance of laboratory confirmation. Effective control depends on ongoing surveillance, recognition of co-infection dynamics, and understanding of risk in tick habitats where disease is endemic. Historically, louping ill has served as a model for understanding tick-borne viral encephalitis more broadly, contributing to early research into flavivirus infections of human patients[2]. In the context of changing environmental conditions, expanding tick ranges, and cross-reactivity with other flaviviruses, the recent emergence of TBEV in southern England highlights the zoonotic potential of this virus group. This reinforces the need for integrated, One Health approaches to managing tick-borne disease threats at the livestock–wildlife–human interface.

Contributing Institution:
Veterinary Sciences Centre, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
http://www.ucd.ie/vetmed/

JPC Diagnoses:
Cerebrum, thalamus: Meningoencephalitis, lymphoplasmacytic, subacute, multifocal, moderate with gliosis and rare neuronal necrosis.

JPC Comment:
The contributor has done a marvelous job of discussing the louping ill virus in its first-ever entry in the Wednesday slide conference. As the contributor mentions, the louping ill virus is a well-known member of the tick-borne encephalitis viruses. Other related tick-borne flaviviruses that cause encephalitis in small ruminants include the Turkish sheep encephalitis virus, Spanish sheep encephalitis virus, and the Greek goat encephalitis virus. Several tick-borne flaviviruses also affect humans, including Russian spring-summer encephalitis virus, Central European encephalitis virus, Omsk hemorrhagic fever virus, and the very old North American entry, Powassan virus. Mosquitoes may also transmit flaviviruses; examples of mosquito-borne flaviviruses in humans include West Nile Virus, Japanese encephalitis virus (WSC 2013, Conf 9, Case 3), St. Louis encephalitis virus, and Murray Valley encephalitis virus (WSC 2011, Conf 20 Case 1).

West Nile Virus, a mosquito-borne flavivirus, infects humans, horses, and numerous avian species. Birds are the natural reservoir and amplifying host for this virus, and humans and horses (less commonly, other mammals) are dead-end hosts. In horses, the virus causes polioencephalitis (or poliomeningoencephalitis), primarily in the brainstem or spinal cord (WSC 2002, Conf 2, Case 4). The WSC includes two cases of spinal cord polioencephalomyelitis in other dead-end hosts, including a harbor seal (WSC 2020, Conf 20, Case 2) and a cat (WSC 2007, Conf 14, Case 2).

Another mosquito-borne flavivirus affecting small ruminants and humans is Wesselsbron virus (WSLV). First identified in Wesselsbron, South Africa, in 1955, WSLV results in hepatitis, abortions, congenital abnormalities, and neonatal mortality in sheep, but has less commonly been identified in other ruminants, wildlife, and humans. Clinical signs overlap with the bunyavirus that causes Rift Valley fever in small ruminants and humans, and lead to diagnostic confusion. It may also be spread to humans via milk and exposure to placenta, and may result in hepatitis and, less commonly, neurologic disease as well.[12]

References:

1. Barrett D, Collins DM, McGrath G, Ó Muireagain C: Seroprevalence of louping ill virus (LIV) antibodies in sheep submitted for post mortem examination in the north west of Ireland in 2011. Ir Vet J. 2012;65:20. doi:10.1186/2046-0481-65-20

2. Buxton D, Reid HW: 120 years of louping-ill research: an historical perspective from the archive of the Journal of Comparative Pathology. J Comp Pathol. 2017;157:270–275.

3. Cantile C, Youssef S. Nervous system. In: Maxie MG, ed. Jubb, Kennedy, Palmer's Pathology of Domestic Animals. Vol. 1. 6th ed. Philadelphia, PA: Elsevier; 2016:373.

4. Fingerhood S, Mansfield KL, Folly AJ, et al.: Meningoencephalomyelitis and brachial plexitis in a dog infected with louping ill virus. Vet Pathol. 2025;62:87–91. doi:10.1177/03009858241265035

5. Flattery A, Lawlor A, O'Grady L, McKiernan F, Browne J, Gray J, et al.: The prevalence and distribution of Anaplasma phagocytophilum genotypes in Ixodes ricinus nymphs collected from farm- and woodland sites in Ireland. Ticks Tick Borne Dis. 2022;13:101901. doi:10.1016/j.ttbdis.2022.101901

6. Gray J, Kahl O, Zintl A: Pathogens transmitted by Ixodes ricinus. Ticks Tick Borne Dis. 2024;15:102402. doi:10.1016/j.ttbdis.2024.102402

7. Holding M, Dowall SD, Medlock JM, Carter DP, Pullan ST, Lewis J, et al.: Tick-borne encephalitis virus, United Kingdom. Emerg Infect Dis. 2020;26:90–96. doi:10.3201/eid2601.191085

8. Jeffries CL, Mansfield KL, Phipps LP, Gurney SMR, Troyer JL, King B, et al.: Louping ill virus: an endemic tick-borne disease of Great Britain. J Gen Virol. 2014;95:1005–1014. doi:10.1099/vir.0.063016-0

9. Ogden NH, Beard CB, Ginsberg HS, Tsao JI: Possible effects of climate change on Ixodid ticks and the pathogens they transmit: predictions and observations. J Med Entomol. 2021;58:1536–1545. doi:10.1093/jme/tjaa220

10. Reid HW, Buxton D, Pow I, Brodie TA, Holmes PH, Urquhart GM: Response of sheep to experimental concurrent infection with tick-borne fever (Cytoecetes phagocytophila) and louping-ill virus. Res Vet Sci. 1986;41:56–62.

11. Zintl A, Moutailler S, Stuart P, Paredis L, Dutraive J, Gonzalez E, et al.: Ticks and tick-borne diseases in Ireland. Ir Vet J. 2017;70:4. doi:10.1186/s13620-017-0084-y

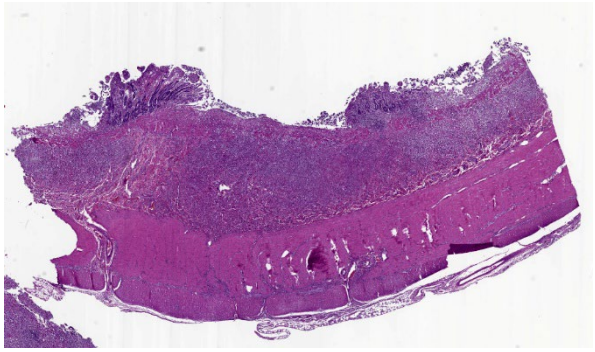
12. Mugisha KG, García-Nicol O, Benarafa C, Fevre E. Wesselsbron virus at the livestock–human interface: A practical guide to disease recognition and diagnosis. One Health 2026 23:101583

CASE III:

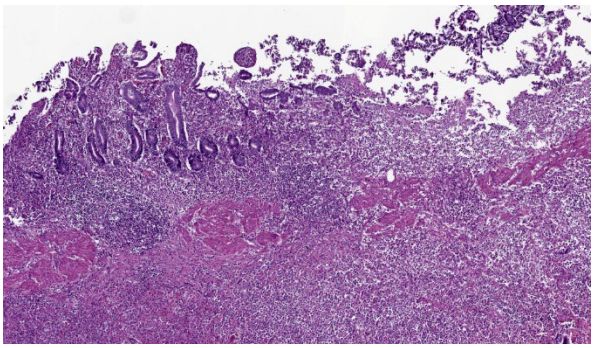
Signalment:
One-year-old, intact cryptorchid, male, Boxer, *Canis familiaris,* canine.

History:
The animal had a 9-month history of chronic diarrhea and inability to gain weight. There was limited clinical improvement with probiotics, antibiotics (tylosin and metronidazole), diet trials, and prednisone. Previously, the dog had a resolved giardiasis that was treated with panacur.

Abdominal radiographs revealed a diffusely thickened colon (approximately 1.3 cm), loss of mucosal layering, and a circumferentially ulcerative mass with gas infiltration into the intestinal wall from the transverse transition to the descending colon. There was also diffuse intra-abdominal lymphadenopathy on abdominal ultrasound. Bloodwork indicated an anemia and leukocytosis (neutrophilia, monocytosis, and basophilia). The animal was euthanized due to quality of life concerns and submitted for necropsy.



3-1. Colon, dog. A section of colon is submitted for examination. There is ulceration of the overlying mucosa. The section shows transmural infiltration by a densely cellular inflammatory infiltrate. It primarily infiltrates the muscularis within perivascular spaces. (HE, 20X)



3-2. Colon, dog. The inflammatory infiltrate expands and effaces the mucosa and extends downward through the muscularis mucosae. (HE, 93X)

Gross Pathology:
The entire colon and cecum were diffusely thickened up to 7mm with marked expansion of the mucosa and submucosa. The mucosal surface was pale tan, irregularly nodular with flattened mucosal folds, and multifocally ulcerated through the entire segment. On sectioning, multiple areas were firm, gritty, and fibrotic. The colonic lumen contained a moderate amount of mucoid to watery light brown fluid and the ileal lumen orally contained dark brown liquid digesta. The remainder of the small intestine was unremarkable. Many mesenteric lymph nodes were variably enlarged and measure up to 3.5 cm x 3 cm x 1 cm.

Laboratory Results:
Fecal culture was negative for significant bacteria including *Salmonella*, *Aeromonas*, *Escherichia coli* 0157:H7, and *Campylobacter*. The colonic sample smear revealed few Gram positive and gram negative rods, and the colon was missing normal anaerobic flora.

Microscopic Description:
Colon: Extensively expanding the colonic wall up to 1 cm thick, predominantly in the submucosa and deep mucosal layers, are dense sheets of a granulomatous inflammatory infiltrate composed of numerous epithelioid macrophages, as well as foamy histiocytes mixed with fewer scattered degenerate and viable neutrophils, lymphocytes, and plasma cells within mild edema and hemorrhage. Histiocytes have an abundant eosinophilic cytoplasm, occasionally contain pale eosinophilic variably sized vacuoles with cellular debris, have vesicular to hyperchromatic chromatin, with 1-3 nucleoli and low mitotic activity. The previously described infiltrate often obscures the variably ulcerated and eroded mucosa and lamina propria, replaces and separates the remaining colonic crypts, and extends into the muscularis externa with decreasing intensity. Minimal histiocytic aggregates are within the serosal adipose tissue. Colonic enterocytes lining the crypts have increased mitotic activity, and goblet cells in the mucosa are markedly reduced. Many endothelial cells lining blood vessel throughout all colonic layers are hypertrophic (reactive endothelium).

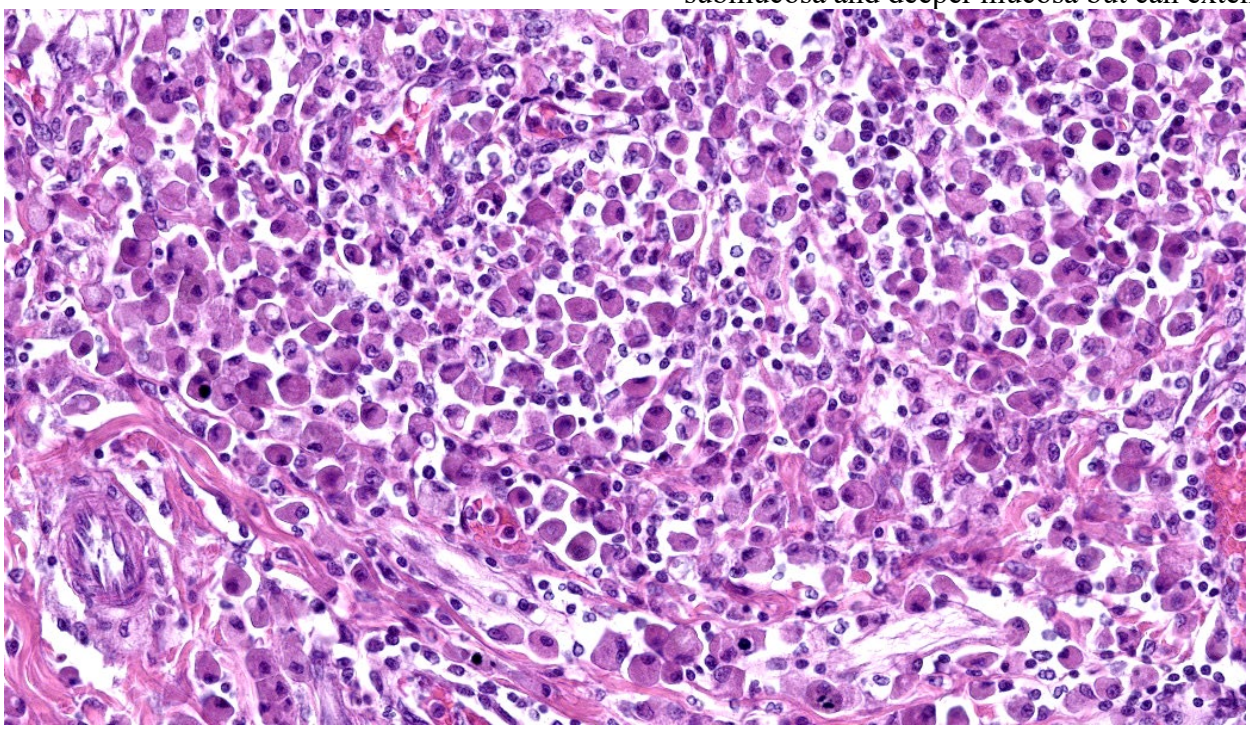
Additional stains not included: Numerous histiocytes in the colon have strong cytoplasmic reaction to PAS. Along the superficial mucosal surface, there are few Gram-positive coccobacilli. No organisms are highlighted with Fite's acid-fast staining.

Contributor's Morphologic Diagnoses:
Colon: Colitis, histiocytic, ulcerative, chronic, transmural, severe.

Condition: Histiocytic ulcerative colitis.

Contributor's Comment:
The syndrome histiocytic ulcerative colitis (HUC) is additionally referred to as granulomatous colitis and Boxer colitis accordingly as there is thickening of the colon with a band of numerous histiocytes in the submucosal and mucosal layers and certain breeds, notably Boxers and French Bulldogs, are more commonly represented[1]. However, this diagnosis

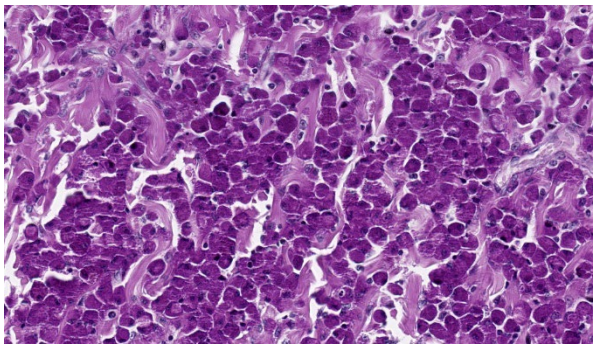
submucosa and deeper mucosa but can extend



3-3. Colon, dog. The inflammatory infiltrate within the markedly expanded submucosa is composed primarily of macrophages with abundant eosinophilic cytoplasm, admixed with numerous lymphocytes and fewer neutrophils and plasma cells. (HE, 564X)

is not restricted to Boxers, and other reported affected breeds include Mastiffs, Beagles[3], Alaskan malamutes, Doberman pinschers, and American Staffordshire Terriers[2]. A typical presentation involves a young dog with a chronic history of large bowel diarrhea, weight loss, and gross thickening of the colonic wall[1]. Gross thickening of the colon with ulceration is non-specific and differentials to consider are infectious colitis (particularly fungal or oomycotic), immune-mediated (idiopathic mucosal colitis), traumatic (foreign body), barium-associated, neoplasia, or, as in this case, histiocytic ulcerative colitis. The signalment, gross thickening and ulceration of the colon, and chronic history of mucoid to hemorrhagic diarrhea for this animal favored HUC as a top differential for the clinicians.

The characteristic histological findings of HUC include dense sheets of granulomatous inflammation centered on and expanding the transmurally, as observed in this case. Consideration should be taken by clinicians obtaining mucosal biopsies from the colon for evaluation as cases can have localization in the submucosa and selective sampling can miss the lesion entirely. Histiocytes with HUC have variable cytoplasmic reaction to periodic acid-Schiff reaction (PAS) staining[8]. Similar PAS positive histiocytes in this case were observed expanding the mesenteric lymph node sinuses method involves taking rectal scraping samples for cytological evaluation. Samples taken from a French Bulldog, in this proposed study likely from drainage. A novel proposed testing showed macrophages with a pink granular cytoplasm that stained bright pink with PAS for presumptive diagnostic of HUC later confirmed with histopathology and fluorescence *in situ* hybridization (FISH) analysis[8]. Supplementary testing with immunohistochemistry (IHC) analysis can also be used on colon tis-



3-4. Colon, dog. Macrophages have strongly PAS-positive cytoplasm. (Periodic acid-Schiff, 606X)

sue samples. Prior studies with IHC show increased numbers of immune cells: plasma cells (IgG, IgG3, IgG4), T cells (CD3), MHC class II cells, and cells staining with L1 suggesting an immune-mediated cause[9].

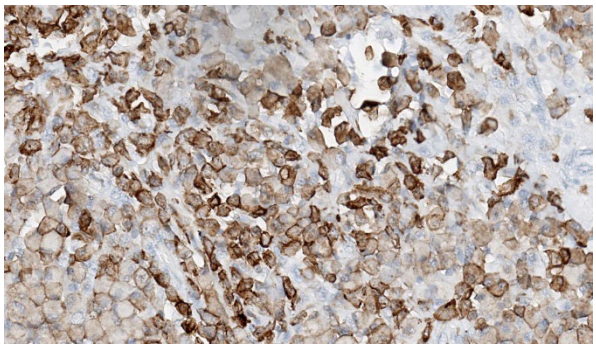
More recently HUC has been associated with infection with adherent and invasive *Escherichia coli* strains[5] through FISH analysis and 16S ribosomal DNA sequencing. The adherent-invasive *E.coli* (AEIC) phenotype, virulence gene profiles, and larger genome size observed from these strains resembled those cultured from patients with Crohn's disease but varied in serotype and genotype[4,5]. This connection with *E.coli* has further been supported by remission of cases with antibiotic therapy particularly with enrofloxacin treatment[4,6]. HUC has rarely been reported in felids, and these cases similarly have responded to enrofloxacin therapy[7]. This diverges from the previous theory that HUC was an idiopathic immune-mediated disease that failed to respond to a number of various empirical treatments. Treatment with enrofloxacin has greatly improved the overall prognosis of animals diagnosed with this condition[6].

Contributing Institution:
Department of Pathobiology - Auburn University College of Veterinary Medicine

JPC Diagnoses:
Colon: Colitis, ulcerative and histiocytic, chronic, transmural, marked, with crypt loss.

JPC Comment:
The contributor provides a concise yet thorough review of histiocytic ulcerative colitis in the dog, and the condition was first described by van Kruiningen *et al.* in 1965 in a colony of Boxer dogs with chronic hemorrhagic diarrhea. The frequency of this condition in French Bulldogs bears repeating due to the popularity of this breed and its frequency in surgical pathology caseloads today (and provides additional incentive to retire the term "Boxer colitis"). The particular susceptibility of Boxers (and likely French bulldogs as well) to HUC is thought to be due to a heritable anomaly that confers susceptibility to invasion and persistence by an adherent and invasive group of E. coli.[2,12] The rarity of disease in cats may suggest variation in hereditary and host susceptibility to HUC between dogs and cats.[11, 12]

Histopathological demonstration of macrophages with intracytoplasmic PAS-positive material has been accepted as the pathognomonic feature of HUC[8,11] (an especially important feature before the identification of AEIC *E.coli* as the causative agent and the development of specific testing protocols such as FISH to identify its presence in tissue samples). PAS-positive material is considered glycoprotein from bacterial cell walls.[7] This



3-5. Colon, dog. Macrophages demonstrate strong positivity for IBA-1. (anti-IBA-1, 696X)

technique uses fluorescent probes to specifically target and hybridize with E. coli 16S ribosomal rDNA, thus enabling identification and localization of the bacteria within HUC lesions.[4] In addition to allowing direct observation of *E. coli* within macrophages in tissue samples, FISH has several other advantages: it can be conducted on formalin-fixed paraffin-embedded sections, it is not confounded by normal flora present in the colon, and it can detect multiple bacterial species at once.[13]

Finally, fecal transplantation has been advocated as a potential treatment for this condition, replacing prolonged antibiotic administration. Commonly used antibiotics such as enrofloxacin may become less effective over time, suggestive of pathogen resistance. Other antibiotics that remain efficacious, such as amikacin, are nephrotoxic and are used as a last resort. In a single case report of fecal transplantation in a French bulldog with HUC, stool consistency stabilized within 48 hours, and the animal remained asymptomatic for three months after the second fecal transplant.[14]

References:

1. Uzal FA, Plattner BL, Hostetter JM. Alimentary System. In: Maxie MG, ed. *Jubb, Kennedy and Palmers Pathology of Domestic Animals*. 6th ed., vol. 2. Philadelphia, PA: Elsevier Ltd; 2016:97-98.
2. Argenta FF, de Souza SO, Meirelles LS, Snel GGM, De Lorenzo C, Ienes-Lima J, Horn F, Driemeier D, Pavarini SP. Histiocytic Ulcerative Colitis in an American Staffordshire Terrier. J Comp Pathol. 2018 Nov;165:40-44.
3. Carvallo FR, Kerlin R, Fredette C, Pisharath H, DebRoy C, Kariyawasam S, Pardo ID. Histiocytic typhlocolitis in two colony Beagle dogs. Exp Toxicol Pathol. 2015 Feb;67(2):219-21.
4. 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.
5. 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.
6. Davies DR, OHara, AJ, Irwin, PJ, Guilford, WG. Successful management of histiocytic ulcerative colitis with enrofloxacin in two Boxer dogs. *Australian Vet J*. 2004;82:58-61.
7. Matsumoto, I., Nakashima, K., Morita, H., Kasahara, K., Kataoka, O., & Uchida, K. (2019). *Escherichia coli*-induced granulomatous colitis in a cat. *JFMS open reports*, *5*(1), 2055116919836537.
8. Conrado, F. O., Jones, E. A., Graham, E. A., Simpson, K. W., Craft, W. F., & Beatty, S. (2022). Cytologic, histopathologic, and clinical features of granulomatous colitis in a French Bulldog. *Veterinary clinical pathology*, *50 Suppl 1*, 76–82.
9. German, A. J., Hall, E. J., Kelly, D. F., Watson, A. D., & Day, M. J. (2000). An immunohistochemical study of histiocytic ulcerative colitis in boxer dogs. *Journal of comparative pathology*, *122*(2-3), 163–175.
10. Van Kruiningen, JH, Montali RJ, Strandberg JD, Kirk RW. A granulomatous colitis of Boxer dogs with histologic resemblance to Whipple's disease. *Path Vet*, 1965; 2:521-544.

11. Nolte A, Junginger J, Baum B, et al. Heterogeneity of macrophages in canine histiocytic ulcerative colitis. Innate Immun 2017;23:228-239.

12. Wednesday Slide Conference, 2022-2023, Conference 5, Case 1.

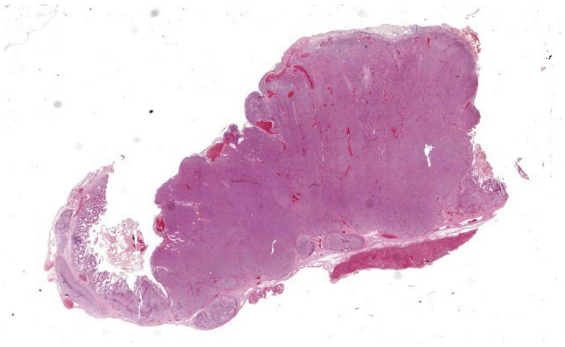
13. Sims CS, Nagle J, Tolbert MK, Anderson K, Linder K, Neel J. Correlation of cytology to histology in a case of granulomatous colitis in a Boxer dog. Vet Clin Pathol. 2022:50(Suppl.1):83-87.

14. Romano FS, Lallo MA, Romano RS, Isidoro LPS, Cardoso MR, Sodré LC, Melchert A, Guimarães-Okamoto PTC, Pappalardo MCF, Amaral AR, Vendramini THA. Fecal Microbiota Transplantation as a Treatment for Granulomatous Colitis in a French Bulldog: A Case Report. Microorganisms. 2025 Feb 8;13(2):366.

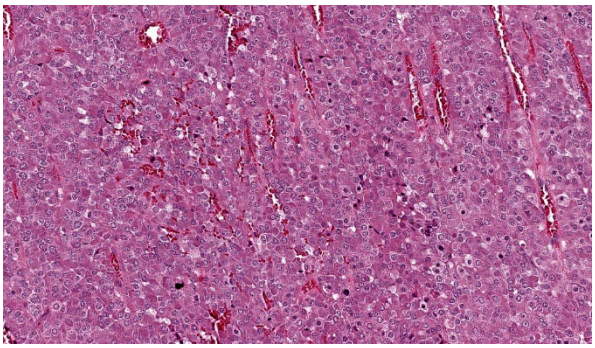
CASE IV:

Signalment:
8-year-old castrated male Boston Terrier, *Canis familiaris, dog*

History:
The dog had a long-standing history of adrenal insufficiency (Addison's disease) and was maintained on long-term low-dose steroid therapy. Recently, the owner observed decreased appetite, abdominal pain, occasional hematochezia, and intermittent episodes of syncope. Clinical examination revealed a severe regenerative anemia with a hematocrit of 20%. A blood transfusion was initiated. Abdominal ultrasonography revealed a mass within the wall of the gallbladder. Due to a suspicion of neoplasia, the gallbladder was surgically excised and submitted for histopathological evaluation.



4-1. Gallbladder, dog: An infiltrative neoplasm expands the wall of the gallbladder and focally effaces the overlying gallbladder mucosa. (HE, 10X)



4-2. Gallbladder, dog: Neoplastic cells are arranged in sheets, trabeculae, nests, and packets on a fine fibrovascular stroma. (HE, 381X)

Gross Pathology:
Gallbladder, measuring 4 × 3 × 2 cm, with a 3 × 1 × 1 cm intraluminal mass protruding from the wall.

Laboratory Results:
Cytokeratin (pan): negative

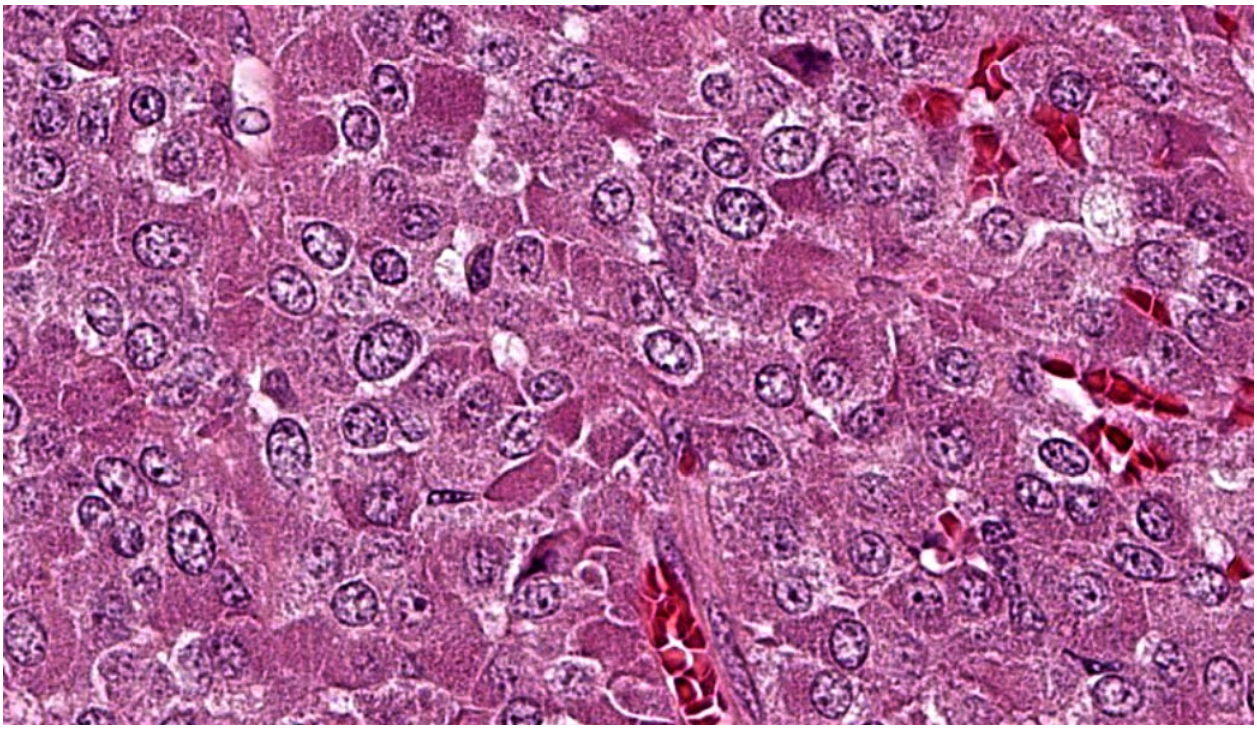
Synaptophysin: positive

Gastrin: negative

S100: negative

GATA-3: up to 100 % of nuclei positive

Ki-67: 1–3 positive nuclei per high-power field (0.237 mm^2)



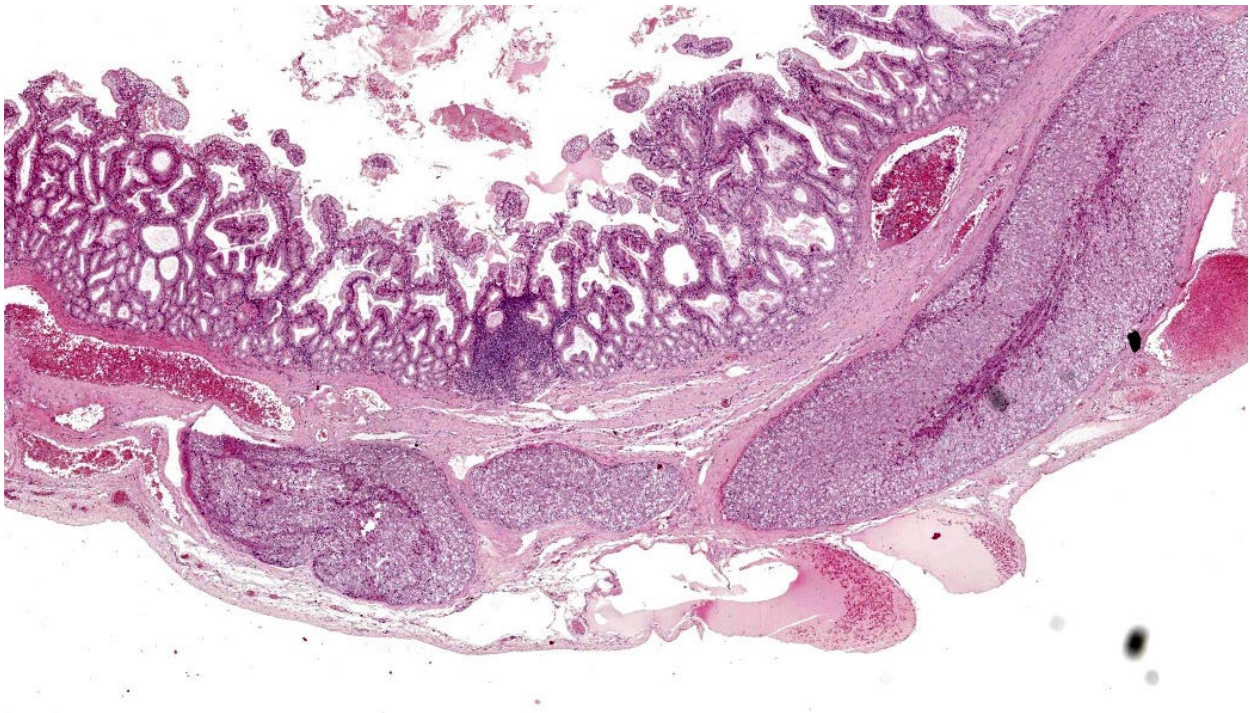
4-3. Gallbladder, dog: High magnification of densely granulated neoplastic cells. (HE, 1409X)

Microscopic Description:
Gallbladder: Infiltrating the mucosa and protruding into the lumen is a highly cellular, moderately demarcated, unencapsulated mass composed of round to polygonal cells arranged in nests, packets, and more solid areas supported by a fine fibrovascular stroma. Neoplastic cells have indistinct cell borders, abundant eosinophilic, finely granular cytoplasm, and a round to oval nucleus with clumped chromatin and a variably distinct nucleolus. There is mild anisocytosis and anisokaryosis and mitoses average one per ten high-power fields (2.37 mm^2). Many neoplastic cells appear shrunken and hypereosinophilic with condensed nuclei (degeneration). There is severe vascular invasion with frequent tumor cell emboli within blood vessels. The adjacent liver shows diffuse cytoplasmic vacuolization (fatty degeneration) with subserosal fibrosis, and hyperemia.

Contributor's Morphologic Diagnoses:
Gall bladder: Neuroendocrine tumor (carcinoid), malignant

Contributor's Comment:
Neuroendocrine tumors (NETs), historically termed carcinoids, originate from cells of the diffuse neuroendocrine system and may arise throughout the alimentary tract, including the stomach, intestine, pancreas, and biliary system[2, 3, 4]. In veterinary medicine, NETs have been documented in various species including dogs, cats, cattle, and more recently in reptiles such as bearded dragons[5]. Canine tumors often form small masses within the liver or the biliary tract.

Carcinoids often have a fine fibrovascular stroma with cells forming solid nests, cords or ribbons, or ideally rosettes mimicking an acinar pattern[4]. Special stains such as Grimelius (argyrophilic) can highlight the cytoplasmic



4-4. Gallbladder, dog: The neoplasm extends along the wall of the gallbladder along perineurial and perivascular spaces. There is marked cystic-glandular hyperplasia of the overlying remnant gallbladder mucosa and mild chronic lymphoplasmacytic cholecystitis. (HE, 44X)

granules, though this technique is not yet established in our laboratory[7]. Hemobilia and hemocholecyst have been described as complications of gallbladder carcinoids due to their invasive nature[6]. In the presented case the mass infiltrated the mucosa of the gallbladder leading to bleeding, hemobilia and severe anemia.

A recent retrospective study revealed a predisposition in Boston Terriers and reported frequent vascular invasion and metastasis to the liver and lungs[8]. The presented case also involved a Boston Terrier. A reported feline case was highly malignant, too[9].

Immunohistochemically, the neoplastic cells of the presented case were strongly positive for synaptophysin and GATA-3 and showed a relatively high proliferative activity (Ki-67: 1–3/HPF). The cells were negative for pan-cytokeratin, S100, and gastrin. The latter was tested due to clinical considerations of Zollinger-Ellison syndrome. The patient responded well post-surgically, and no metastatic lesions have been detected three months after diagnosis.

A similar case of an extrahepatic bile duct NET was presented at WSC 22/23 (conference #9, case 3), highlighting the importance of including NETs in the differential diagnosis of biliary tract masses in dogs.

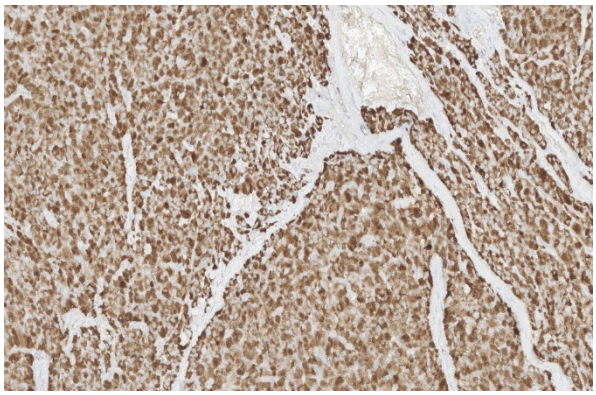
Contributing Institution:

Institut fuer Veterinaer-Pathologie, Justus-Liebig-Universitaet Giessen
Frankfurter Str. 96, 35392 Giessen, Germany
http://www.uni-giessen.de/cms/fbz/fb10/institute_klinikum/institute/pathologie

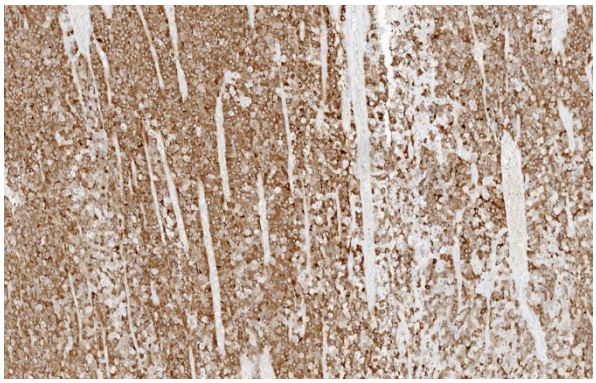
JPC Diagnoses:
Gallbladder: Neuroendocrine tumor (carcinoid).

JPC Comment:
The contributor has provided a concise but insightful overview of carcinoids in domestic species. The term carcinoid was first coined by German pathologist Siegfried Orberndorfer in 1907, to describe a series of six cases with a total of 12 small intestinal lesions[10] – but this term has been replaced by the World Health Organization with neuroendocrine tumor (NET) to more accurately reflect its cellular origin, with neuroendocrine tumor referring to a slow-growing indolent tumor, and with neuroendocrine carcinoma being used to classify more aggressive tumors, often with cellular atypia, areas of necrosis, and a high proliferation rate (as measured by Ki-67 of over 20%)[11]

Neuroendocrine cells (and consequently NETs) are found in most organ systems, with the musculoskeletal system as an exception. Neuroendocrine cells either secrete low-molecular-weight polypeptide hormones like secretin, somatostatin, and cholecystokinin or are a part of a larger amine precursor uptake decarboxylation (APUD) group which produces serotonin among other compounds.[12]



4-5. Gallbladder, dog: Neoplastic cells demonstrate strong diffuse cytoplasmic reactivity for chromogranin A. (anti-chromogranin A, 350X)



4-6. Gallbladder, dog: Neoplastic cells demonstrate strong diffuse cytoplasmic reactivity for synaptophysin. (anti- synaptophysin, 375X)

Grossly, neuroendocrine tumors are lobulated, firm, dark red to tan, and rarely larger than a few centimeters in diameter. They arise deep within the mucosa, commensurate with the location of neuroendocrine cells, causing submucosal nodules with ulceration and erosion of the overlying mucosa and often infiltrating transmurally into the mesentery. In the liver, there is frequent intrahepatic spread with metastasis to local lymph nodes, peritoneum, and lung, necessitating a diagnosis of neuroendocrine carcinoma.[12]

In humans, several paraneoplastic syndromes have been identified, such as cutaneous flushing, diarrhea, bronchospasm, and systemic fibrosis. Fibrosis is concerning as it can lead to dysfunction of the pulmonary and tricuspid valves followed by regurgitation and right heart failure. This syndrome, termed "carcinoid syndrome", is most common in patients who develop carcinoids in their respiratory tract (and this is one location in which the term carcinoid is still used). The right side of the heart is affected because monoamine oxidases in the lungs fail to inactivate vasoactive substances, which lead to the aforementioned cu-

taneous flushing and bronchospasm, key clinical indicators.[12,13] The mechanism of fibrosis in humans is unclear but is postulated to result from interactions between tumor cells and fibroblasts or hormones and peptides secreted by the carcinoid.[12,14]

The JPC morphologic diagnosis differs slightly from the contributor's diagnosis, as definitive evidence of malignancy is not seen in this section. There is no description of metastatic disease in the clinical history, no evidence of necrosis, a low mitotic rate, and we interpret the Ki67 positivity of 1-3 cells per 0.237mm^2 field as beneath the accepted rate of 20% required for diagnosis of neuroendocrine carcinoma (a mitotic rate of >20 per 2.37mm^2 has also been listed as criteria of malignancy in human gallbladder NETs).[15] (A recent publication of a GB-NEC in a dog had a significantly lower mitotic rate of 6/2.37mm^2 field, but also had hepatic and vascular invasion, which made a diagnosis of GB-NEC much more clear-cut.)

For these reasons (and also because solitary gallbladder NETs tend to pursue an indolent course), we prefer a diagnosis of "neuroendocrine tumor" in this case.

References:

1. Champion CP, Miller AD, Parry S, Demeter EA. Immunohistochemical and histomorphologic characterization of canine neoplasms of the disseminated neuroendocrine system. Vet Pathol. 2025:3009858251324652.
2. Cullen JM, Stalker MJ. Liver and biliary system. In: Maxie MG, ed. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. 6th ed. Philadelphia, PA: Elsevier; 2016; vol. 2:349.
3. Cullen JM: Tumors of the liver and the gallbladder. In: Meuten DJ, ed. Tumors in domestic animals. 5th ed. Ames, IA: Wiley Blackwell; 2017:624-626.
4. Kiupel M, Capen C, Miller M, Smedley R. Histological classification of tumors of the endocrine system of domestic animals. WHO, Washington, DC: Armed Forces Institute of Pathology; 2008,second series, vol. 12.
5. LaDouceur EB, Argue A, Garner MM. Alimentary Tract Neoplasia in Captive Bearded Dragons (Pogona spp). J Comp Pathol. 2022;**194**:28-33.
6. Lippo NJ, Williams JE, Brawer RS, Sobel KE. Acute hemobilia and hemocholecyst in 2 dogs with gallbladder carcinoid. J Vet Intern Med. 2008;**22**:1249-1252.
7. Morrell CN, Volk MV, Mankowski JL. A carcinoid tumor in the gallbladder of a dog. Vet Pathol. 2002;**39**:756-758.
8. O'Brien KM, Bankoff BJ, Rosenstein PK, Clendaniel DC, Sánchez MD, Durham AC. Clinical, histopathologic, and immunohistochemical features of 13 cases of canine gallbladder neuroendocrine carcinoma. J Vet Diagn Invest. 2021;**33**:294-299.
9. Shaw T. Gallbladder carcinoid in a cat. Can Vet J. 2024;**65**:241-244.
10. Soga J. The term "carcinoid" is a misnomer: the evidence is based on local invasion. J Exp Clin Cancer Res. 2009 Feb 10;28(1):15. doi: 10.1186/1756-9966-28-15.
11. Pathology Outlines; https://www.pathologyoutlines.com/topic/anussmallcell.html?mobile=off, accessed 10.21.2026.
12. Wednesday Slide Conference 2018-2019, Conference 20, Case 1.
13. Shinn BJ, Tafe LJ, Vanichakarn P. A case of carcinoid syndrome due to malignant metastatic carcinoid tumor with carcinoid heart disease involving four

cardiac valves. Am J Case Rep. 2018; 19:284-288.

14. Laskaratos FM, Rombouts K, Caplin M, Toumpanakis C, Thirlwell C, Mandair D. Neuroendocrine tumors and fibrosis: A mystery?. 2017; 123(24):4770-4790.

15. Wu YT, Kelly N, Langohr IM, Sokol S, Gerdin J, Liu CC, Butsch TJ, Johnston AN. Proliferation-Based WHO Grading and Heterogeneous Gastrin Expression in Canine Gallbladder Neuroendocrine Tumors. Vet Sci. 2025 Oct 14;12(10):989.

16. Wicker S, Johnson A, Olin S, Bevelock T, Fazio C, Mochizuki H. Cytologic, Clinicopathologic, and Histologic Findings in a Dog with a Gallbladder Neuroendocrine Carcinoma and Multiple Endocrinopathies. Vet Clin Pathol. 2026 Jul 27. doi: 10.1111/vcp.70123.