CASE I – CASE 1 771-2 NIEHS (AFIP 2799444).

Signalment: 2-year-old female Harlan Sprague-Dawley rat.

History: High-dose animal from a 2-year carcinogenicity/toxicity study.

Gross Pathology: Livers from the treated rats were enlarged and had granular surfaces and multicentric nodules and masses randomly scattered within the parenchyma.

Histopathologic Description: Cholangiocarcinomas were variably sized, irregular, non-circumscribed proliferations of dense fibrous connective tissue stroma containing dysplastic epithelium which formed numerous irregular ducts and duct-like structures that were lined by pleomorphic neoplastic epithelial cells. Small islands of dysplastic neoplastic cells were also interspersed among the ducts. Within the ducts, the neoplastic cells were flattened in some areas and cuboidal to columnar in others. In some ducts, there was segmental loss of epithelium. Bizarre epithelial cells were frequently present. Component neoplastic cells had scant to moderate amounts of basophilic cytoplasm and prominent markedly pleomorphic vesicular to hyperchromatic nuclei with one or more prominent nucleoli. Mitoses were sometimes numerous. Ducts often contained mucinous material, degenerate epithelial and inflammatory cells, and cell debris. Low numbers of mast cells, lymphocytes and neutrophils were scattered throughout the stroma. Additional alterations include nodular regenerative hepatocellular hyperplasia, hepatocellular hypertrophy and bile duct hyperplasia.

Contributor’s Morphologic Diagnosis: Liver: Cholangiocarcinoma, multiple.
Contributor’s Comment: The terminology for benign and malignant lesions of the biliary epithelium is often confusing reflecting the uncertainty surrounding the biology of the so-called more benign appearing or preneoplastic lesions. The latter have been called cholangiofibrosis, adenofibrosis, cholangiofibroma, cystic cholangioma, cholangiohepatitis, toxic hepatitis and toxic cholangitis. Cholangiofibrosis, cholangiofibroma, cystic cholangioma and cholangiocarcinoma are closely related lesions that are distinguished on the basis of the degree of proliferation and anaplasia of the biliary epithelium, evidence of invasion, and the quantity of fibrous connective tissue stroma. In this study, transplantation of the cells from the proliferative lesions grew and metastasized confirming the malignancy of these lesions.

AFIP Diagnosis: Liver: Cholangiocarcinoma, Harlan Sprague-Dawley rat, rodent.

Conference Comment: As pointed out by the contributor, the terminology for benign and malignant lesions of the biliary epithelium is often confusing. Furthermore, the distinction between cholangiofibrosis, cholangiofibroma, and cholangiocarcinoma has not been clearly defined. Morphologic features such as intrahepatic infiltration and microinvasion (disruption of basement membranes of glandular formations), absence of histological evidence of regression, piling up of epithelial cells lining glandular lumens, formation of branched or anastomosing glands, nuclear hyperchromasia and cellular dysplasia, increased mitotic figures and compression of the surrounding hepatic parenchyma support the potential malignancy of proliferative cholangial lesions.¹

There was much discussion about whether the lesion in this case was more consistent with cholangiofibrosis or cholangiocarcinoma. The gross appearance of cholangiofibrosis varies from multifocal microscopic foci to grossly visible firm, pearly white areas up to 5 cm in diameter. Lesions on the surface are depressed. Characteristic light microscopic findings of cholangiofibrosis include atypical glandular structures line by hyperbasophilic, sometimes dysplastic epithelium that ranges from flattened to large cuboidal cells with goblet cells and occasional Paneth cells. The glands often appear crescent-shaped due to tall columnar epithelium on one side of the gland and attenuated epithelium on the other side. Mitotic figures and necrotic cells are often present within the epithelium. The lumen is usually filled with mucin/necrotic debris. Mucin production is usually pronounced. The glandular structures are embedded within dense connective tissue with sclerosis in the more central areas of the lesion. Multifocal areas of cholangiofibrosis often coalesce.²
Cholangiocarcinomas are usually firm, white to grey masses with irregular borders and may protrude from the surface of the liver. They may have a spongy texture and exude clear to yellow fluid from cut surfaces in cystic areas. Microscopically, these tumors may have glandular, solid, or papillary patterns. They are comprised primarily of cuboidal to columnar cells with basophilic cytoplasm and prominent hyperchromatic nuclei. Cellular atypia and a high mitotic index are common. The epithelium lining dilated glands is occasionally piled up. Mucin production is highly variable. Abundant scirrhous stroma is often present. Cholangiocarcinomas usually exhibit microinvasion and can invade surrounding tissues, blood vessels, and may metastasize.

After much discussion, a diagnosis of cholangiocarcinoma was made due to the presence of glandular structures lined by one to multiple cell layers; prominent hyperchromatic nuclei; a high mitotic index; cellular atypia; and reported metastasis.

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**References:**

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**CASE II – 6-2156-A (AFIP 3030596).**

**Signalment:** 13-week-old female pig, Landrace.

**History:** Gilts found acutely dead, had appeared healthy, introduced to barn 2 weeks previously, recent increase in mortality within the barn, suspect circovirus.

**Gross Pathology:** Bilaterally involving the ventral half of all lung lobes, cranial mediastinum and entire pericardium, the serosal surfaces are diffusely overlaid by abundant amounts of fibrin. The lungs are heavy, dark red and wet and the regional lymph nodes are moderately enlarged and glistening. Throughout the abdominal cavity overlying the serosal surface of multiple loops of bowel and the entire splenic capsule, there is moderate fibrin deposition.
Laboratory Results: *Hemophilus parasuis* type 4 recovered from lung and mediastinal lymph node, PCR negative for swine flu and positive for porcine circovirus 2. Egg inoculation was negative for influenza virus.

Histopathologic Description: Lung: Diffusely overlying and multifocally extending below the visceral pleura, there are multifocal to coalescing accumulations of predominantly neutrophils with fewer macrophages and lymphocytes interspersed within variable amounts of fibrinous exudate and acute hemorrhage. The aforementioned inflammatory infiltrate multifocally expands and occludes alveolar and bronchiolar spaces and interlobular septa.

Contributor’s Morphologic Diagnosis: Lung: Pleuropneumonia, marked, diffuse, fibrinosuppurative, necrotizing, subacute.

Contributor’s Comment: The polyserositis noted in this case would have been sufficiently severe to have contributed significantly to antemortem morbidity and the loss of this animal. Special culture recovered *Hemophilus parasuis* type 4 from the lung and mediastinal lymph node and this pathogen was considered significant. In a previous case series, swine infected with *H. parasuis* featured dual infections and the most prevalent combination was with Circovirus 2. The contribution of Circovirus 2 in predisposing or exacerbating this infection is unknown.

Infection with *H. parasuis* typically presents with fibrinous polyserositis, polyarthritis, and meningitis. These bacteria are a commensal of the upper respiratory system (nasal cavity and trachea) with invasion into the lungs and development of clinical disease often associated with some stressor. The history of recent introduction to a new herd as in this case is a common factor in the development of clinical disease. Additional contributory factors include concurrent infections with swine influenza virus, pseudorabies, PRRSV, suboptimal environmental conditions or inclement weather.

AFIP Diagnosis: Lung: Pleuropneumonia, fibrinohemorrhagic and suppurative, diffuse, severe, Landrace pig, porcine.

Conference Comment: As pointed out by the contributor, *Haemophilus parasuis*, the cause of Glasser’s disease, results in severe serofibrinous to fibrinopurulent meningitis, polyserositis, and/or polyarthritis in young (5-12 weeks) pigs following a stressful episode. Polyarthritis is usually most severe in the carpal and tarsal joints and the atlanto-occipital joint. The primary differential diagnoses for fibrinous serositis in pigs are *Mycoplasma hyorhinus*, *Streptococcus suis* type II (zoonotic), and septicemic salmonellosis, and septicemic *E. coli*. Like *H. parasuis*, *M.
hyorhinus also causes polyarthritis; however, meningitis is not usually a feature of mycoplasmal infection. If meningitis is present, it is mild with lymphocytic inflammation. In addition to purulent meningitis and polyarthritis, S. suis type II can also cause endocarditis.3-6


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CASE III – PG06-2481 (AFIP 3026809).

Signalment: 8-month-old female Yucatan mini-pig.

History: The pig was naïve without prior medical treatment. It was euthanized after a sudden onset of cyanosis of extremities, lethargy and inappetence.

Gross Pathology: The pulmonary artery was markedly dilated, approximately 7 cm in diameter. The lumen of the pulmonary artery immediately proximal to the pulmonic valve was filled with yellow-white chalky vegetations. Smaller vegetations of similar consistency were also on the aortic valve leaflets. The rest of the heart appeared normal. There were small petechial hemorrhages in the
eyelids bilaterally. The brain was not examined. The lungs, kidney and spleen are grossly normal.

**Histopathologic Description:** Pulmonic valves and pulmonary arteries had fibrinosuppurative inflammatory infiltrates, mineral deposits, and large colonies of bacteria extending into the lumen from the intimal surface. The intimal aspect of the lesion had proliferating capillaries with plump endothelial cells and fibroblasts. Aortic valves had similar but smaller lesions. Pulmonary thrombi were present in large and small pulmonary arteries as well as alveolar capillaries. Evidence of early organization was present with fibroblasts and endothelialization of thrombi surfaces. The heart had foci of myocardial necrosis and suppurative inflammation. Acute microthrombi were also present in glomerular capillaries. The liver had centrilobular necrosis and congestion.

**Contributor’s Morphologic Diagnosis:** Pulmonary valve/artery: Vegetative endocarditis, subacute, severe with mineralization and large colonies of intrallesional bacteria; etiology *Streptococcus suis*.

**Contributor’s Comment:** Gram stain of affected tissue shows that bacterial colonies contain Gram-positive cocci. Foci of necrosuppurative myocarditis are present on some submission slides.

Endocarditis is usually bacterial but parasitic and fungal etiologies have been reported. Valves are most commonly affected beginning in the area of apposition of valve leaflets. The AV-valves are most commonly affected. Experimentally, the lesion has been reproduced with a single injection of bacteria intravenously. In swine, the most common cause is *Streptococcus suis*. *Erysipelothrix rhusiopathiae* is also a common etiologic agent in pigs.¹

*Streptococcus suis* has over 30 serotypes. *S. suis* type 1 generally causes disease in suckling piglets with septicemia, synovitis and meningitis. Type 2 causes disease in weaner and feed pigs causing bacteremia, synovitis, arthritis and meningitis; acute and chronic disease can occur. In addition, endocarditis, myocarditis and pericarditis can occur.⁴ *S. suis* is carried by healthy pigs in the tonsils, nasal cavity, genital and alimentary tract.²,³,⁴ Spread is via aerosols and close contact.² It also has been isolated from wild boars, horses, dogs, cats and birds.² *S. suis* is a zoonotic agent causing meningitis, septicemia and other inflammatory diseases in recent outbreaks in China.²,⁴
AFIP Diagnosis: Heart, pulmonary artery (per contributor): Endocarditis, valvulitis, and arteritis, fibrinosuppurative and granulomatous, chronic, diffuse, severe, with mineralization and colonies of Gram-positive cocci, Yucatan mini-pig, porcine.

Conference Comment: Streptococcus suis type II is carried in the palatine tonsils of pigs and infection is probably by the respiratory route. In addition to endocarditis, S. suis type II can also cause purulent meningitis, polyserositis, arthritis, and possibly pneumonia in pigs.3,4

As pointed out by the contributor, S. suis is a zoonotic agent causing meningitis, septicemia, septic shock, and residual deafness in man. Recently, scientists confirmed the first human case of S. suis meningitis in North America. Lack of reports of this disease in humans in the U.S. is most likely due to misidentification of the organism. S. suis infections in humans is most frequently observed in intensive pig farming areas or where people live or work in close contact with pigs (e.g., butchers, veterinarians, pig farmers).5,6,7

The pathogenesis of endocarditis involves the components of Virchow’s triad of thrombogenesis – turbulence of blood flow, endothelial injury and hypercoagulability. Turbulent intracardiac blood flow associated with congenital anomalies or intracardiac devices contribute to initiation of the lesion. Endothelial disruption of the valves allows bacteria to adhere, proliferate, and initiate an inflammatory reaction with subsequent fibrin deposition. In addition, preexisting extracardiac infections are often present in affected animals, such as gingivitis or dermatitis, which result in bacteremia.8

In cattle, valvular endocarditis is most frequently caused by Actinomyces pyogenes, and less commonly, streptococci of enteric origin. Horses rarely develop bacterial endocarditis, but it has been observed in association with Streptococcus equi, Actinobacillus equi, Escherichia coli, Pseudomonas aeruginosa, and Candida parapsilosis. Endocarditis is also rarely observed in dogs and cats, but can be associated with a variety of organisms including Streptococcus sp., Erysipelothrix rhusiopathiae, and E. coli.1,8

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References:

06WSC16 - 7 -
CASE IV – S1552/05 (AFIP 3032267).

Signalment: 3-year-old, female, German Improved Fawn breed, goat (Capra hircus).

History: The goat belonged to a flock of three goats and five sheep. Over a period of one week the animal was febrile and showed central nervous signs including temporary blindness and ataxia. Therapy with vitamin B1 and antibiotics was not successful. The goat was euthanized due to the poor prognosis.

Gross Pathology: The goat had a body weight of 26 kg, and was in good nutritional condition. The superficial cervical lymph nodes were markedly enlarged. The lung exhibited multifocal pale firm areas, and a moderate alveolar edema and emphysema. The spleen was slightly enlarged. The liver and the kidneys were mottled and showed multifocal pale foci with a diameter up to 5 mm (Fig. 1).

Laboratory Results: Antigen of rabies virus could not be detected by immunofluorescence microscopy.

In liver samples a severe deficiency of copper (0.74 mg/kg wet weight; reference value: 10 - 120 mg/kg wet weight), and vitamin E (2.34 mg/kg wet weight;
reference: >10 mg/kg wet weight), and a mild deficiency of selenium (0.213 mg/kg wet weight; reference value: 0.25-1.5 mg/kg wet weight) were detected.

Samples from the brain and the kidney were tested by PCR with primers designated for the glycoprotein B region of OvHV-2 and CpHV-2. The reaction product was cloned and sequenced. Comparing the nucleotide sequences to other herpesviruses similarities of 99.49% for OvHV-2 and 86.22% for CpHV-2 were observed.

**Histopathologic Description:** The renal cortex and medulla displays a multifocal interstitial nephritis characterized by infiltration of lymphocytes, macrophages and plasma cells. Occasionally, necrosis of single cells is observed. The inflammatory lesions are mainly associated with blood vessels. Numerous arteries and arterioles show lymphohistiocytic vasculitis with fibrinoid necrosis of the vascular wall. Some vessels exhibit destruction of endothelial cells and thrombi are present. The epithelium of the renal pelvis is moderately hyperplastic and focally there is a moderate lymphohistiocytic subepithelial infiltration.

In the superficial cervical lymph nodes, lung, spleen, liver, brain and spinal cord a severe vasculitis and perivasculitis of similar quality are found. Additionally, the lung shows a moderate granulomatous pneumonia with nematodes.

**Contributor’s Morphologic Diagnoses:**
1. Kidney: Interstitial nephritis, vasculitis and perivasculitis, lymphoplasmacytic and histiocytic, multifocal, subacute, severe, with fibrinoid necrosis of vessel walls and thrombi, German Improved Fawn breed, goat.
2. Renal pelvis: Pyelitis, lymphohistiocytic, focal, subacute, mild to moderate, with hyperplasia of epithelium, German Improved Fawn breed, goat.

**Contributor’s Comment:** Malignant catarrhal fever (MCF) is a fatal lymphoproliferative disease of ruminant species including domestic cattle and wild living ruminants. The disease is caused by a group of closely related gammaherpesviruses collectively referred to as malignant catarrhal fever (MCF) viruses. Epidemiologically, two primary forms of MCF in cattle have been described. Wildebeest-derived (WD)-MCF, caused by alcelaphine herpesvirus type 1 (AlHV-1), primarily seen in Africa, and the sheep-associated form (SA-MCF) due to ovine herpesvirus type 2 (OvHV-2) infection, which is found in North America, Europe, and Australia. In goats, a different gammaherpesvirus, termed caprine herpesvirus-2 (CpHV-2), has been identified. This virus caused MCF in white-tailed deer and in sika deer in the USA. However, infection associated lesions or disease have not been observed in goats. Interestingly, various ruminant species may be infected by MCF-viruses, but only few develop clinical disease. In goats, which can be infected by OvHV-2 and CpHV-2, lesions have not been described so far.
Domestic sheep represent the primary reservoir for OvHV-2. Clinical signs of MCF are not observed in domestic sheep or goats under natural conditions.\textsuperscript{1} Experimentally, MCF-like disease was induced in sheep after aerosol inoculation with OvHV-2.\textsuperscript{7} MCF was also described in Barbary sheep and pigs and OvHV-2 was identified by PCR as the causative agent.\textsuperscript{6,7}

Goetze described four clinical forms of MCF in cattle, termed “peracute”, “head-and-eye”, “intestinal”, and “mild”.\textsuperscript{8} The most frequent signs in cattle include high fever, corneal opacity, and lymph node enlargement. Neurological signs may be observed but are typically not that pronounced until the terminal stages of the disease. The animals are typically lethargic and appear obtunded.\textsuperscript{1} Head and eyes were not infected in the goat presented here.

Macroscopically, the present case showed enlargement of lymph nodes and visceral organs with multifocally distributed tiny white spots in liver and kidney (Fig. 1). In spontaneous cases of bovine MCF, the kidneys show similar lesions. Many bovine cases present generalized lymph node enlargement, which is particularly marked in the head and neck region, and visceral nodes.\textsuperscript{8}

Histopathologically, in cattle a lymphohistiocytic vasculitis with fibrinoid necrosis is characteristic and almost pathognomonic for MCF.\textsuperscript{1} The vasculitis in bovine is segmental and irregular in distribution, most readily seen in medium-sized arteries invariably accompanied by perivascular and intramural infiltration of mononuclear cells. There is frequently a striking degeneration, often fibrinoid in character, of infiltrated connective tissues and smooth muscle elements of vessel walls. Vascular lesions appear to have predilection sites including kidney, brain and meninges, hepatic triads, lung, capsule of lymph nodes and adrenal gland and the rete mirabile.\textsuperscript{8} Until now, a disease pattern similar to that in bovine has not been described in goats.

Though the pathology of MCF is well documented, the pathogenesis is still poorly understood. It is assumed that disease is caused by a lymphoproliferative disorder caused by a dysregulation of T-lymphocytes.\textsuperscript{1} In addition, there is evidence that MCF is an immunopathological condition. The essential defect in MCF pathogenesis appears to be an immune dysregulation attributable to a dysfunction of Natural Killer cells and uncontrolled proliferation of lymphoblastoid elements in many tissues. Whether MCF-lesions are due to a type III or Arthus-like hypersensitivity reaction is still discussed controversially.\textsuperscript{8}

Most episodes of spontaneous SA-MCF appear to be a sequel of close contact between cattle and infected sheep actively shedding the agent.\textsuperscript{9} The goat presented was kept together with sheep. Confirmation of the diagnosis was based on histological demonstration of generalized lymphoid vasculitis in multiple organs,
including the brain. Infection with viruses inducing MCF can be most easily demonstrated by PCR in post mortem tissues.\textsuperscript{1} Infection of goats with OvHV-2 or CpHV-2 has been demonstrated previously.\textsuperscript{9} Both viruses have the ability to infect other ruminants and to cause MCF\textsuperscript{2,4,6,7}. In this goat, an infection with OvHV-2 could be confirmed by PCR and discriminated by phylogenetic analysis.

AFIP Diagnosis: Kidney: Vasculitis and perivasculitis, lymphohistiocytic, diffuse, marked, with intimal and medial fibrinoid necrosis, hemorrhage, and few fibrin thrombi, German Improved Fawn breed goat (\textit{Capra hircus}), caprine.

Conference Comment: The contributor provides a thorough overview of malignant catarrhal fever. Other gammaherpesviruses include the following:\textsuperscript{10-12}
1. Herpesvirus saimiri (Saimiriine herpesvirus 2) – carried by squirrel monkeys, causes lymphoma in owl monkeys, tamarins and marmosets
2. Herpesvirus ateleus (Atleine herpesvirus 2) – carried by spider monkeys, causes lymphoma in owl monkeys, tamarins and marmosets
3. Marmoset lymphosarcoma virus – outbreak of spontaneous fatal lymphoproliferative disease in captive marmosets due to a novel lymphocryptovirus
4. Herpesvirus sylvilagus (Leporid herpesvirus 1) – lymphoma and infectious mononucleosis-like syndrome in cotton-tail rabbits
5. Epstein-Barr virus (human herpesvirus 4) – Infectious mononucleosis in humans; associated with Bukitt’s lymphoma, nasopharyngeal carcinoma, and Hodgkin’s disease

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References:

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