CASE 1: 060377 (JPC 4065817).

Signalment: 20-year-old quarter horse (*Equus ferus caballus*) gelding.

History: This 20-year-old gelding horse had been part of a small herd that was used for antibody production against various antigens. It suddenly presented with muddy-red fluid discharge from the prepuce and physical examination revealed a large, proliferative mass within the sheath that was bleeding and friable. The animal was anesthetized for a more thorough exploration of the mass. The mass was determined to be deep within the prepuce, palpable up to, and involving, the body wall. Due to the location and infiltrative nature of the mass, resection was not possible and the animal was euthanized. A full necropsy was not performed; only the penis and prepuce was removed for post mortem examination.

This animal was part of a research project conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.

Gross Pathology: Extending from the base of the glans penis, involving the surrounding prepuce, and extending deep to the body wall is a 13x13x12 cm friable, proliferative, ulcerated mass.

*The preputial mucosa is replaced by an exophytic multilobular infiltrative mass composed of islands of keratinizing squamous epithelium. (HE,6X)*
**Laboratory Results:** N/A

**Histopathologic Description:** Prepuce: Multifocally, effacing and replacing normal tissue architecture, affecting approximately 70% of the tissue section, extending to cut borders, is an unencapsulated, infiltrative, well-demarcated, moderately cellular neoplasm composed of polygonal cells arranged in nests and packets, often surrounding variably sized areas composed of keratin, admixed with degenerate neutrophils, necrotic cellular debris, sloughed, often necrotic, neoplastic cells, and hemorrhage, on a moderately dense desmoplastic stroma. Neoplastic cells have distinct cell borders, moderate to abundant amounts of brightly eosinophilic cytoplasm, a round to oval nucleus with finely stippled chromatin, and up to 4 variably distinct nucleoli. The mitotic rate is brisk, with up to 5 mitotic figures in some high-powered fields. Anisocytosis and anisokaryosis are marked. Neoplastic cells at the periphery of nests and packets occasionally surround concentric lamellations of brightly eosinophilic material (keratin pearls), and often exhibit dyskeratosis and single cell necrosis.

Multifocally, neoplastic cells are found within expanded lumens of lymphatics. There are multifocal aggregates of lymphocytes and plasma cells that infiltrate the neoplasm and surrounding fibrovascular connective tissue. The surrounding connective tissue is edematous with dilated lymphatics.

**Contributor’s Morphologic Diagnosis:** Prepuce: Squamous cell carcinoma.

**Contributor’s Comment:** Squamous cell carcinoma (SCC) is the most common genital malignant tumor in horses and is considered a “classic” neoplastic lesion in the study of veterinary pathology. SCCs are epithelial neoplasms that arise from keratinocytes, and often develop at mucocutaneous junctions, such as the eyelids, perineum, anus, and external genitalia in stallions and geldings. Gross lesions are often proliferative and may exhibit ulceration, hemorrhage, necrosis, and infection. Histologically, neoplastic cells are usually well-differentiated, keratinization is almost always present, and the neoplasm is frequently infiltrated by neutrophils and/or...
eosinophils. Historically, potential promoters such as ultraviolet light, chronic inflammation, and smegma accumulation have been associated with development of the neoplasm, although the role of these elements remains unclear.

There are numerous recent reports that reveal increasing evidence to support a potential causal relationship between equine papillomavirus type 2 (EcPV2) and equine penile squamous cell carcinoma (SCC). Studies have suggested that equine penile papillomas, in situ carcinomas, and invasive carcinomas belong to a continuum of papilloma-induced disease, which is supported by detection of EcPV2 DNA in horses with characteristic SCC lesions, and in various lesions of the penis in a proportional number of control cases. When comparing the levels of EcPV2 viral load in equine SCC lesions, penile non-SCC, or precursor disease lesions, and tissues without observable lesions from SCC-prone sites on clinically normal horses, EcPV2 DNA was present significantly more often, and in higher copy numbers, in the equine penile SCC lesions than the others. The presence of EcPV2 DNA has also been demonstrated in anal lesions, a lymph node, and contact metastases. Additionally, in one report, viral mRNA was detected in all examined EcPV2 DNA positive lesions, while only 2.6% of specimens from healthy horses had detectable mRNA. This finding provides evidence of intralesional viral transcriptional activity which further supports an active role of the virus in equine SCC disease.

Note: Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

JPC Diagnosis: Prepuce: Squamous cell carcinoma.

Conference Comment: Conference discussion included a review of neoplastic features of malignancy observed within this neoplasm, including marked anisocytosis and anisokaryosis, invasion of lymphatics, elevated mitotic rate, infiltrative pattern, and desmoplasia. As noted by the contributor, additional features included dyskeratosis, areas of necrosis, infiltration of a mixed population of inflammatory cells as well as few areas of mineralization. Although the specific anatomic location could not be determined histologically in this case, participants briefly discussed common locations for SCC in horses, including the urogenital area, ocular/periocular regions, and the stratified squamous portion of the stomach. Locations for squamous cell carcinoma in other species include the nares, pinnae and lips of white cats, the vulva of sheep and goats and the nonglandular stomach of rodents and pigs. In addition to horses, squamous cell carcinomas of the eyelid epithelium occur in other species, most commonly cattle, cats, and dogs. Squamous cell carcinomas develop through a stepwise process, often consisting of precancerous stages and the formation of a papilloma. Tumor development begins with initiation, which is an irreversible genetic change typically caused by solar radiation. The next step is promotion, which includes the growth of genetically altered or initiated cells within a favorable environment. Promotion is followed by progression, which results in increasing malignancy of the developing tumor and involves both genetic and
epigenetic changes to tumor cells.\textsuperscript{7} Ultraviolet radiation induced squamous cell carcinoma of the eyelid occurs most commonly in Hereford cattle which have nonpigmented eyelids.\textsuperscript{7} The ultraviolet radiation induces DNA damage and subsequent mutation; of the three wavelengths of ultraviolet light, UVB is thought to be most associated with development of cutaneous neoplasms. The energy in UV light absorbed is by DNA and results in covalent crosslinking of pyrimidine bases and the formation of pyrimidine dimers, preventing proper base pairing. It is postulated that nucleotide excision repair mechanisms are overwhelmed with continued UV exposure, which may result in the propagation of cells with genomic mutations.\textsuperscript{6}

As mentioned by the contributor, recent evidence supports an association between equine penile squamous cell carcinoma and equine papillomavirus 2 (EcPV2). The expression of two proteins, E6 and E7, has been demonstrated in the human papillomavirus associated with cervical cancer, which interact with proteins associated with cell cycle regulation.\textsuperscript{10} Zhu et al. demonstrated that a subset of equine penile squamous cell carcinomas contained the E6/E7 nucleic acid of EcPV2, and a majority of neoplastic cells contained virus, providing additional evidence for the role of EcPV2 in penile and preputial SCCs in horses. Additionally, in that study the E6/E7 onecogenes of EcPV2 were present in metastatic SCCs. There was also evidence of solar damage in cases of penile and preputial SCC not associated with EcPV2.\textsuperscript{10}

\textbf{Contributing Institution:}
\textbf{USAMRIID Pathology Division}
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http://www.usamriid.army.mil/

\textbf{References:}


**CASE II: 14-42715 (JPC 4066859).**

**Signalment:** 10 year old, male neutered, domestic shorthair cat, feline (*Felis catus*).

**History:** The cat was diagnosed with suspected idiopathic pulmonary fibrosis approximately eleven months prior to date of death. On the morning of the cat’s death, the owner found their German shepherd dog standing over the cat’s body, and there were numerous puncture wounds on the cat’s dorsum and limbs.

**Gross Pathology:** The animal was in fair nutritional condition evidenced by scant visceral and subcutaneous adipose tissue stores. There was a cutaneous puncture wound on the right dorsal trunk, and another ventral to the right scapula. There were multiple sites of subcutaneous hemorrhage over the trunk and appendages.

All lung lobes were firm and displayed a prominent nodular pattern, with nodules ranging from 0.2 cm to 0.5 cm in diameter. The pancreas consisted of discrete, multifocal, 3 mm diameter and smaller, white to tan nodules. The splenic capsule contained few, multifocal, 2 mm diameter white to tan, firm, slightly raised patches. The right thyroid gland was moderately enlarged relative to the left.

**Laboratory Results:** No virus was detected via virus isolation of lung tissue. Rare to very rare coliforms, *Streptococcus* (Group D), and *Trueperella pyogenes* bacteria were isolated from lung samples.

**Histopathologic Description:** Lung. Effacing 70% of the lung parenchyma, mostly located subpleurally and around large airways, are multifocal to coalescing areas of fibrosis. The subpleural foci of fibrosis are retracted and efface the normal lung parenchyma architecture. These foci of fibrosis are composed of aggregates of mature fibroblasts admixed fewer lymphocytes and plasma cells, and with a marked accumulation of collagen. In these areas the pleural mesothelial cells are multifocally hyperplastic. At the periphery of foci of fibrosis are some areas with less mature fibroblasts pilling and arranged in parallel (foci of fibroplasia), producing small amounts of collagen. In between these areas of fibrosis are
variably distended and confluent alveoli with ruptured septa (emphysema, honey combing aspect) giving the lung a nodular architecture.

 Approximately 80% of the section is replaced by fibrous connective tissue primarily focused on airways. (HE 4X)

Terminal bronchiolar smooth muscles are frequently markedly hyperplastic and remaining alveoli often filled with foamy macrophages and fewer lymphocytes, plasma cells and rare neutrophils. Alveoli are also often lined by plump cuboidal pneumocytes (type II pneumocyte hyperplasia).

Within bronchiolar and bronchial lumens, is abundant basophilic, slightly fibrillar material (mucus) admixed basophilic nuclei and nuclear debris (necrotic neutrophils). Bronchial glands are mildly hyperplastic. The tunica media of vessels is markedly thickened (hyperplastic).

**Contributor’s Morphologic Diagnosis:** Lung, chronic, severe, multifocal to coalescing interstitial fibrosis, with alveolar honey combing, mild chronic histiocytic, lymphocytic, and plasmacytic interstitial pneumonia, and chronic moderate catarrhal and suppurative bronchitis.

**Contributor’s Comment:** Idiopathic pulmonary fibrosis (IPF), also known as cryptogenic fibrosing alveolitis, is a form of interstitial lung disease (ILD), and is the most prevalent idiopathic interstitial pneumonia in humans. Human IPF is defined as a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause, occurring primarily in older adults, and limited to the lungs. It is further characterized by progressive worsening of dyspnea, and carries a poor prognosis. A consensus published in 2000 by a joint collaboration between the American Thoracic Society (ATS) and the American College of Chest Physicians (ACCP) classified human IPF as a distinct clinical entity with the histologic appearance of usual interstitial pneumonia (UIP), which includes a patchwork distribution of fibrosis leading to honeycombing (predominantly in the subpleural/paraseptal regions), smooth muscle metaplasia or hyperplasia, and the presence of fibroblastic foci.

Diagnosis of human IPF relies on the exclusion of other known causes of interstitial lung disease (ILD), the presence of a UIP pattern on high resolution CT scans (HRCT), or specific combinations of HRCT and lung biopsy patterns in patients where lung biopsy samples are obtained.

Human IPF carries a variable and unpredictable natural history, and remains a poorly understood. While an exact etiology for human IPF remains unknown, several risk factors have been identified, including cigarette smoking, environmental exposures (metal and wood dusts, farming, raising birds, hairdressing, stone polishing), microbial agents (Epstein-Barr virus, ...
hepatitis C virus), and gastroesophageal reflux. Genetic factors have also been identified in the context of human IPF. For example, strong associations with familial IPF have been identified with mutations in the surfactant protein C gene.

Animal models used to study IPF often fail to mimic morphological changes seen in human IPF, nor do they follow a similar progression of disease. Beginning in 1996, IPF has been recognized in cats, characterized as a novel spontaneous chronic, progressive respiratory disease with morphologic features of UIP. One study found that the average age of onset is 8.7 years, and average duration between onset of disease and death/euthanasia is 5.5 months. The disease in cats shares critical features with human IPF in the context of gross pathology, histopathology, cell differentiation markers, and ultrastructural details. No other spontaneous disease of induced animal model is able to reproduce the criteria for UIP as in feline IPF.

Large areas of fibrosis focus on and efface airways throughout the section. (HE, 20X) (Image courtesy of University of Illinois College of Veterinary Medicine Department of Pathobiology and Veterinary Diagnostic Laboratory. http://vetmed.illinois.edu/path/)

Grossly, lungs are mottled tan to red, and display a distinct cobblestone appearance on pleural surfaces that typically involves large regions of the lungs. Additionally, the extensive areas of fibrosis typically form plaque-like regions that are discernable from normal lung tissue and extend deep into the parenchyma. Grossly discernable honeycombing is an uncommon finding.

The histopathologic hallmark (and most important diagnostic criterion) is that of a heterogeneous appearance at low magnification, characterized by fibrosis with scarring and honeycomb change that alternate with regions of less affected or even normal parenchyma. Other changes include foci of fibroblasts/myofibroblasts, and interstitial smooth muscle hyperplasia. The amount of inflammation is variable, but not a prominent feature, however, large foci of alveolar macrophages is a common finding. In some (69%) cats, mucous cell metaplasia is noted. In cats that do not demonstrate mucous cell metaplasia, the lining cells are either type II pneumocytes (well differentiated) or columnar cells of an unknown phenotype.

In typical cases, lesions are often patchy or multinodular, rather than diffuse. The histopathologic changes often preferentially affect the subpleural and paraseptal parenchyma. This case is atypical in that lesions efface the entire pulmonary parenchyma, with virtually no normal tissue remaining. Similar cases have been documented by other authors, with the conclusion that the disease was too advanced to appreciate any temporal heterogeneity. Similarly, this case likely represents advanced disease, as this animal survived approximately one year after detection of clinical signs (average duration is 5.5 months). This animal died after a traumatic encounter with a dog. Thus, the cause of death was concluded to be respiratory compromise in the face of increased respiratory demand due to the traumatic incident.

**JPC Diagnosis:**
Lung: Fibrosis, interstitial, multifocal to coalescing, marked with smooth muscle hypertrophy and hyperplasia, type II pneumocyte hyperplasia, and moderate alveolar histiocytosis.
Lung: Bronchitis and bronchiolitis, chronic-active, multifocal, moderate with squamous metaplasia.

**Conference Comment:** The conference description was aligned very closely with the contributor’s description. The nodularity of the fibrosis, especially in subpleural areas correlates well with the contributor’s high quality gross image. The overall cellularity of the interstitial fibrous component, albeit variable in severity in some areas, was also a major discussion point. Conference participants speculated on the presence of myofibroblasts in some areas, which are thought to be an important cellular component of feline idiopathic pulmonary fibrosis. Important ancillary changes described included alveolar macrophages variably filling alveolar lumina, prominent alveolar epithelial lining cells (in this case, interpreted as type II pneumocyte hyperplasia), luminal narrowing of arteries, pleural mesothelial hypertrophy, and an inflammatory component within bronchi and bronchioles that varied between slides.

Most agreed that the mostly neutrophilic inflammatory infiltrate within the larger airways along with mucous exudation was a secondary process and may have been related to the positive bacterial culture in the lungs.

The precise pathogenesis of feline idiopathic pulmonary fibrosis is unclear, but current scientific literature suggests a defect in type II pneumocytes and alveolar repair are precursory findings. The loss of these cells may have an important role in the development of pulmonary fibrosis. Chronic interstitial inflammatory stimulation resulting in fibrosis has also been implicated in cases of interstitial fibrosis. However, in feline idiopathic pulmonary fibrosis, inflammation may not have an essential role and the primary defect and stimulus may involve the type II pneumocytes. In this case, conference participants noted that, overall, interstitial inflammation was mild at best and not a significant feature. Additionally, ultrastructural changes suggest surfactant protein C may play a role in the disease. This case was submitted to the Department of Pulmonary and Mediastinal Pathology at the Joint Pathology Center (JPC) for comparative pathology consultation regarding idiopathic pulmonary fibrosis in humans. Their consultation revealed similar histologic features as those discussed in the conference, but proposed an alternative interpretation to these changes. Their interpretation is that of an “organizing pneumonia characterized by airway luminal, airspace, and interstitial loose fibroblastic connective tissue”. Though difficult to discern due to overwhelming and diffuse injury, they favored an airway-centered process and described organizing pneumonia, in general terms, as a subacute process that is most commonly the sequelae of infection and, in many cases, difficult/impossible to document. The larger cartilaginous airways were described as showing “submucosal and adventitial chronic inflammation with varying degrees of destruction including complete obliteration of the small airways.” In their opinion, this case resembles progression of subacute organizing pneumonia to non-specific interstitial pneumonia (with fibrosis) with the histomorphologic features less consistent with unusual interstitial pneumonia (UIP) as it occurs in people. However, also noted in their detailed consultation, interstitial pulmonary fibrosis in cats (i.e. a novel spontaneous

Many alveoli are expanded and filled with numerous foamy macrophages. (HE, 320X)
chronic, progressive respiratory disease with morphologic features of UIP in humans) may have variations in histologic appearance as compared to humans suggesting possible species differences.

**Contributing Institution:**
University of Illinois College of Veterinary Medicine Department of Pathobiology and Veterinary Diagnostic Laboratory.  
http://vetmed.illinois.edu/path/

**References:**


**CASE III:** 12-269-84 (JPC 4032912).

**Signalment:** Black rats (*Rattus rattus*) (unknown age and gender).

**History:** In November 2011, a trapping campaign was organized in a French zoological park to assess the prevalence of capillariasis in wild rodents. The campaign was initiated after the diagnosis of capillariasis in a primate from the zoo. Eighty rats were trapped and their livers were sampled and fixed in 10% buffered formalin.

**Gross Pathology:** Most livers were grossly unremarkable. In a few cases, livers had a slightly irregular surface and, in some areas, contained tightly packed pinpoint yellow foci and small irregular tracts.

**Laboratory Results:** None

**Histopathologic Description:** There is some variability between slides regarding the severity and the type of lesions. Livers show various degrees of bridging fibrosis characterized by fine fibrous septae connecting portal tracts to portal tracts or to centrilobular spaces (the so-called *septal fibrosis*). As a consequence, in the most affected areas, the architecture is reminiscent of the porcine liver. This pattern is highlighted with Masson’s trichome stain. In addition, there are various degrees of lymphoplasmacytic and, to a lesser extent, eosinophilic infiltration in septae and around portal tracts and centrilobular veins. Sections usually have one or two aggregates of parasitic eggs surrounded by macrophages and multinucleated giant cells, and a peripheral rim of fibrosis with lymphocytes, plasma cells and eosinophils (granulomas). In older granulomas, eggs are mainly surrounded by fibrosis. Eggs are typically barrel-shaped, about 80 µm in length, bi-operculate and have a thick shell, the inner layer of which is refractile and the outer layer
striated (Fig. 3). The content is eosinophilic and granular (morphology consistent with unembryonated eggs of *Capillaria hepatica*). Some granulomas also contain areas of necrosis and/or dystrophic mineralization. Some slides show sections of adult worms: they measure 100-150 µm in diameter, have a pseudocoelom and a digestive tract (nematodes), a polymyarian-coelomyarian musculature and two bacillary bands (hypodermal bands with nuclei) (aphasmid nematodes) (Fig. 4). This morphology is consistent with adults of *Capillaria hepatica*. Some worms are surrounded by numerous eosinophils and macrophages.

**Contributor’s Morphologic Diagnosis:** Liver: Hepatitis, granulomatous and eosinophilic, chronic, multifocal, moderate, with intralesional eggs and adult nematodes consistent with *Capillaria hepatica* (adults are not present on all slides). Liver: Porto-portal and porto-central bridging fibrosis, multifocal, moderate to severe (septal fibrosis).

**Contributor’s Comment:** *Capillaria hepatica* (also known as *Calodium hepaticum*), the cause of hepatic capillariosis, is an aphasisid nematode that mainly infects rodents and lagomorphs, and occasionally other vertebrates such as dogs or primates (including humans). Rodents of the genus *Rattus* are considered the main reservoir. As such, they tend to be highly infected and are

There is mild bridging portal fibrosis present diffusely throughout the section which separates and surrounds individual hepatic lobules. (HE, 76X)

A Masson’s trichrome stains highlights the degree of bridging fibrosis. (Masson’s trichrome, 100X) (Image courtesy of: Unité d’Histologie, Embryologie et Anatomie pathologique, Département des Sciences Biologiques et Pharmaceutiques Ecole Nationale Vétérinaire d’Alfort, France www.vet-alfort.fr)
often the only source of parasites in urban environments. Infection of other mammals and humans results from incidental ingestion of water or food contaminated by embryonated eggs.

*Capillaria hepatica* is the only known nematode with a direct life cycle requiring death of the host to be completed. Following their ingestion, embryonated eggs hatch in the intestine and release first stage larvae that cross the cecal barrier and reach the liver through mesenteric and portal veins. The migration of larvae within the hepatic parenchyma produces areas of hepatic necrosis. After three weeks, mature females start to lay eggs around portal tracts until they die (up to seventy days later). After their death, adults progressively disintegrate. Eggs elicit a mixed inflammatory response composed of macrophages, multinucleated giant cells, lymphocytes, plasma cells and eosinophils that leads to the formation of granulomas.

The peculiarity of hepatic capillariasis is that eggs are kept within the hepatic parenchyma instead of being released through the biliary tract as for other hepatic parasites. Eggs can only be released in the environment after death and decomposition of the host or after its ingestion by a predator. This predator represents a paratenic host that, although not necessary for completing the cycle, greatly promotes the maturation and the dissemination of the eggs.

A peculiar finding in rats infected with *Capillaria hepatica* is the development of septal fibrosis, a type of bridging fibrosis in which portal tracts are connected to portal tracts (or rarely to centrilobular spaces) by thin strands of connective tissue containing collagen, fibroblasts and lymphocytes. This pattern gives the hepatic parenchyma a porcine liver-like architecture. In humans, septal fibrosis is an important, frequent and non-specific finding in chronic liver diseases. For this reason, rats infected with *Capillaria hepatica* represent a good model for hepatic fibrosis. It has been shown that in this model, septal fibrosis is not preceded by hepatic necrosis or overt chronic inflammation. The first step of septal fibrosis appears to be star-shaped expansions of portal spaces containing fibroblasts and blood vessels that sprout from perportal spaces. The presence of proliferating blood vessels is a characteristic feature.

In the present case, other rodent species (ex: Greater white-toothed shrew (*Crocidura russula*)) have been trapped and revealed to be infected by *Capillaria hepatica* but only rats developed septal fibrosis. For veterinary pathologists, this example illustrates how different can be the patterns of tissue reaction among species, even with the same initiating cause. Another well-known example for that is tuberculosis.
<table>
<thead>
<tr>
<th>PARASITE</th>
<th>DEFINITIVE (DH) OR INTERMEDIATE (IH) HOSTS</th>
<th>LOCATION / COMMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>WANDERING LARVAE (NUMEROUS SPECIES)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cysticercus tenuicollis (Taenia hydatigena)</td>
<td>IH: sheep, goat, cattle, squirrel, swine</td>
<td>Cysts in peritoneal cavity / Fatal hepatic hemorrhages in lambs severely infected. Associated with Black disease</td>
</tr>
<tr>
<td>Cysticercus pisiformis (Taenia pisiformis)</td>
<td>IH: rabbit, squirrel, small rodents</td>
<td>Cysts in liver capsule</td>
</tr>
<tr>
<td>Cysticercus fasciolaris (Taenia taniaeformis)</td>
<td>IH: rodents, rabbit</td>
<td>Cysts in liver / Associated with the development of fibrosarcoma</td>
</tr>
<tr>
<td>Larvae of Ascaris suum</td>
<td>DH: swine</td>
<td>Causes milk-spotted liver. Adults in intestine</td>
</tr>
<tr>
<td>Larvae of Stephanurus dentatus</td>
<td>DH: swine</td>
<td>Adults in urinary system</td>
</tr>
<tr>
<td>Larval strongyles (Strongylus edentatus, S. equi, S. vulgaris)</td>
<td>DH: horse</td>
<td>Causes villous perihepatitis</td>
</tr>
<tr>
<td>CESTODES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stilesia hepatica</td>
<td>DH: ruminants (Africa)</td>
<td>Only cestodes that inhabit the bile ducts</td>
</tr>
<tr>
<td>Thysanosoma actinioides</td>
<td>DH: ruminants (America)</td>
<td></td>
</tr>
<tr>
<td>Echinococcus granulosus</td>
<td>IH: humans, cattle, swine, sheep, deer, horse, small rodents, moose etc.</td>
<td>Liver</td>
</tr>
<tr>
<td>Echinococcus multilocularis</td>
<td>IH: humans, cattle, swine, sheep, deer, horse, small rodents, moose etc.</td>
<td>Liver</td>
</tr>
<tr>
<td>Mesocestoides corti / M. lineatus</td>
<td>IH: dog, cat, other mammals, reptiles, rodents</td>
<td>Liver, peritoneal and pleural cavities, lungs, other organs</td>
</tr>
<tr>
<td>NEMATODES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillaria hepatica</td>
<td>DH: small rodents, rabbit, humans etc.</td>
<td>Liver</td>
</tr>
<tr>
<td>TREMATODES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasciola hepatica</td>
<td>DH: ruminants, dog, cat, horse</td>
<td>Bile ducts / Associated with Black disease and bacillary hemoglobinuria.</td>
</tr>
<tr>
<td>Fasciola gigantica</td>
<td>DH: cattle, sheep</td>
<td>Bile ducts</td>
</tr>
<tr>
<td>Fascioloides magna</td>
<td>DH: cattle, sheep, horse, pig</td>
<td>Liver</td>
</tr>
<tr>
<td>Dicrocoelid flukes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eurytrema pancreaticum</td>
<td>DH: sheep, goat, cattle</td>
<td>Pancreatic and bile ducts and duodenum</td>
</tr>
<tr>
<td>Concinnum procyonis</td>
<td>DH: raccoon, fox, cat</td>
<td>Pancreatic and bile ducts</td>
</tr>
<tr>
<td>Dicrocoelium dendriticum</td>
<td>DH: sheep, goat, dog, pig, deer</td>
<td>Bile ducts / Associated with Black disease.</td>
</tr>
<tr>
<td>Dicrocoelium hospes</td>
<td>DH: cattle (Countries south to the Sahara)</td>
<td>Bile ducts</td>
</tr>
<tr>
<td>Platynosomum fastosum</td>
<td>DH: cat (North America and Amazonia)</td>
<td>Liver, bile ducts</td>
</tr>
<tr>
<td>Athesmia foxi</td>
<td>DH: New World monkeys</td>
<td>Bile ducts</td>
</tr>
<tr>
<td>Opisthorchid flukes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metorchis albidus</td>
<td>DH: dog, cat, fox</td>
<td>Bile ducts</td>
</tr>
<tr>
<td>Metorchis conjunctus</td>
<td>DH: cat, dog, fox, mink</td>
<td>Bile ducts</td>
</tr>
</tbody>
</table>
### Table: Helminth Infections

<table>
<thead>
<tr>
<th>Species</th>
<th>DH</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Metorchis bilis</em></td>
<td>DH: red fox</td>
<td>Bile ducts</td>
</tr>
<tr>
<td><em>Opisthorchis felineus</em></td>
<td>DH: cat, dog, fox (Europe, Russia)</td>
<td>Bile ducts</td>
</tr>
<tr>
<td><em>Opisthorchis sinensis</em></td>
<td>DH: dog, cat, pig, humans, fox</td>
<td>Bile duct and duodenum. Associated with cholangiocarcinoma in humans.</td>
</tr>
<tr>
<td><em>Opisthorchis tenuicollis</em></td>
<td>DH: dog, cat, fox, pig</td>
<td>Pancreatic and bile ducts</td>
</tr>
<tr>
<td><em>Pseudamphistomum truncatum</em></td>
<td>DH: dog, cat, fox</td>
<td>Bile ducts</td>
</tr>
<tr>
<td><em>Paramphistomatidae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gigantocotyle explanatum</td>
<td>DH: cattle, buffalao</td>
<td>Bile ducts</td>
</tr>
<tr>
<td><em>Schistosomatidae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Schistosoma japonicum, S. mansoni, S. bovis, S. spindale etc.</em></td>
<td>DH: various species (humans, monkey, cat, dog, ruminants etc.)</td>
<td>Adults reside in veins (portal v., mesenteric v. etc.)</td>
</tr>
</tbody>
</table>

### JPC Diagnosis:
Liver: Hepatitis, granulomatous and eosinophilic, chronic, multifocal, moderate with bridging fibrosis and adult nematodes and eggs.

### Conference Comment:
Conference participants also noted slide variation as mentioned above, with absence of adult nematodes in some sections and variation in the degree and type of inflammation around nematodes. Eosinophilic inflammation is present in some sections and others are dominated by granulomatous inflammation. The stichosome, which is a row of basophilic esophageal gland cells surrounding the esophagus and is one of the most distinctive characteristics of aphasim nematodes, is seen only in a few sections and the eggs are described as being unembryonated. Other ancillary changes described include the pitted and undulating surface of the liver as well as the hepatocellular degeneration and necrosis directly surrounding adult nematodes in some sections.

Aphasim nematodes derive their name from the lack of a tiny set of sensory papillae (phasmids) on their caudal end, which are not identifiable histologically, but differentiate them from the phasmid nematodes (i.e. strongyles, rhabditoids, ascarids, oxyurids, rhabditoids and spirurids). Histologically visible characteristics that help distinguish them from the phasmids include absence of lateral cords, the presence of hypodermal/bacillary bands, and as mentioned above, the presence of a stichosome, which would only be seen in sections with esophagus. The eggs of some species are bipolar plugged / bioperculate as seen in this case, but may be embryonated or unembryonated depending on the specific parasite species.

As mentioned above by the contributor, portal bridging fibrosis is a prominent feature of this entity. However, unlike many other types of portal fibrosis, hepatic stellate cells do not seem to participate in the pathogenesis of this lesion. Experimentally, septa are visible around the 23rd and 27th day after rat inoculation with embryonated eggs, and prominent angiogenesis precedes deposition of collagen, which is a key component in the development of hepatic fibrosis. The earliest changes are seen in portal areas, which may be located some distance away from the location of the actual nematodes, and may emanate from multiple portal areas simultaneously. In addition to angiogenesis, studies have found that proliferating cells...
responsible for the formation of prominent septa include fibroblast like cells as well as pericytes and myofibroblasts. Other cells, such as eosinophils, may also be present, but evidence for involvement of hepatic stellate cells is absent. Interestingly, angiogenesis also seems to play a role in regression of fibrosis as it occurs in rats infected with *C. hepatica*.

**Contributing Institution:**

**Reference**

**CASE IV:** N14-367 (JPC 4066359).

**Signalment:** 4 weeks of age, male, Duroc porcine (*Sus scrofa domesticus*)

**History:** The submitted animal was one of approximately 20 pigs displaying respiratory distress. This pig died enroute to the veterinary clinic. This animal came from a 600-head porcine facility in Puerto Rico.

**Gross Pathology:** A male red porcine in adequate body condition (BCS 3.5/5) presented in a mild state of autolysis. A 6 cm wide area of alopecia was present on the dorsal aspect of the body extending from the neck to the base of the tail. Alopecia was also noted around the snout and the left dorsal carpal region had a focal circular hairless area of 3x3 cm. The lungs were markedly thickened with a meaty consistency and did not collapse or float in formalin solution (interstitial pneumonia). They were diffusely

1There is diffuse consolidation of the lung as a result of severe interstitial pneumonia. (HE, 4X)
mottled pink to red and contained multifocal to coalescing dark red circular areas ranging from 2.5x 2 cm to 2x1 cm. The right caudal lung lobe had a 4x1 cm dark red area at its border. Blood oozed from cut surfaces of the lung (congestion). The pericardial sac contained 5-6 ml of transparent amber colored fluid. The myocardium was diffusely pale. The nasal turbinates were diffusely hyperemic. The spleen surface had multiple circular plum colored areas measuring 1.5 cm in diameter, which extended into the parenchyma. The small and large intestine were diffusely gas-distended.

**Laboratory Results:** Bacteriology was assessed in-house and samples for virology and nutritional analyses were sent to the Diagnostic Center for Population and Animal Health at Michigan State University.

**Virology:** Lung tissue was positive for PRSSV by PCR. Samples were negative for Swine influenza virus and pseudorabies virus.

**Culture and Sensitivity:** *Streptococcus pyogenes* was isolated from the lung. The isolate was susceptible to carbenicillin, and not susceptible to tobramycin, norfloxacin, vancomycin, gentamycin, sulfamethoxazole and erythromycin. Nutritional analysis for vitamin E levels in the liver were normal. Trace nutrient analysis of the liver revealed adequate levels of selenium, zinc and copper. Iron levels were slightly elevated at 674 micrograms/gram (normal reference interval is 300-600). Cobalt and molybdenum were also analyzed, but reference values for pigs were unavailable.

**Histopathologic Description:** Lung: Diffusely, the interstitium is moderately to markedly expanded by predominantly macrophages, lesser lymphocytes and plasma cells, and few neutrophils. Alveolar lumina are markedly distended with fibrin and proteinaceous karyorrhectic cellular debris, numerous viable and degenerate neutrophils, macrophages, and lesser lymphocytes and plasma cells. Greater than 80% of alveolar walls are necrotic characterized by loss or replacement with fibrinoid necrosis. There is also scattered type II pneumocyte hyperplasia. In severely affected areas, bronchiolar lumina contain moderate amounts of fibrillar to homogenous eosinophilic material (fibrin and protein) with karyorrhectic
necrotic cellular debris and neutrophils, macrophages, lymphocytes and plasma cells (Fig. 3). Occasional vessels contain fibrin thrombi. There is BALT hyperplasia and interstitial congestion in less inflamed areas.

Heart: The subendocardium contains a locally extensive area of myocardial hemorrhage with a mild infiltrate of lymphocytes (Fig. 4). Within this area and the adjacent myocardium there is myofiber separation due to expansion of the interstitial space (edema). There is moderate perivascular edema around multiple epicardial vessels.

Contributor's Morphologic Diagnosis:
Lung: Severe diffuse lymphohistiocytic and necrotizing interstitial pneumonia, etiology PRRSV.
Lung: Mild to moderate multifocal suppurative bronchointerstitial pneumonia.
Heart: Mild focally extensive lymphocytic and hemorrhagic myocarditis.
Lymph node and Peyer's patches: Severe diffuse lymphoid hyperplasia.
Haired skin: Moderate to severe diffuse orthokeratotic hyperkeratosis with intracorneal yeasts and bacteria and mild suppurative crusting.
Haired skin: Mild superficial perivascular lymphplasmacytic dermatitis.

Contributor’s Comment: The cause of death in this pig was a severe interstitial pneumonia associated with porcine reproductive and respiratory syndrome (PRRS) virus, as identified in lung tissue by PCR. Porcine reproductive and respiratory syndrome is caused by an arterivirus in the family Arteriviridae.

There are 2 clinical manifestations of PRRS: A reproductive phase and a respiratory phase. The reproductive form of the disease typically lasts 1-4 months, and is characterized by increased numbers of stillborn and mummified fetuses, late term abortion, and weak-born piglets. Anorexia and agalactia are also often present further contributing to preweaning mortality. Suckling piglets characteristically develop a “thumping” respiratory pattern. The respiratory form of the disease is characterized by a chronic interstitial pneumonia clinically associated with dyspnea with no associated cough. Post-weaning pigs are primarily affected and have a significant reduction in daily weight gain and increased mortality up to 10-25%.1 Secondary bacterial infections are common. Histologically, the virus results in a severe necrotizing interstitial pneumonia with sparing of the bronchiolar epithelium. Alveolar septa are thickened by infiltrates of lymphocytes and macrophages,
which also infiltrate the alveolar lumina, along with small numbers of neutrophils. Scattered alveoli will contain karyorrhectic cellular debris. There may be variable type II pneumocyte hyperplasia. Lymphoid hyperplasia is a characteristic feature of PRRSV infection, along with scattered apoptosis of follicular lymphocytes. Perivascular infiltrates of lymphocytes, plasma cells and macrophages may be present in several organs including the nasal mucosa, heart, kidney, and brain.

This virus is very contagious among pigs, and is transmitted via several routes of exposure including parenteral, intranasal, intramuscular, oral, vaginal and intrauterine. The virus replicates in monocyte-derived cells and is shed in blood, semen and oral fluids. Subclinical carriers are common, attributing to the endemic nature of the disease and difficulty in eradication once it has been introduced into a herd.5

In this case, the suppurative bronchointerstitial pneumonia is secondary to opportunistic bacterial invasion. Streptococcus pyogenes was isolated from the lung in this case. This is a human pathogen, warranting consideration of this being a potential zooanthroponosis or a possible contaminant. The lesions in the heart are likely a sequela to the PRRS virus. Another differential to rule-out for this lesion is encephalomyocarditis virus. This animal had a rough hair coat and areas of alopecia, which were histologically associated with hyperkeratosis and intracorneal coccioid bacteria and yeasts, suggestive of Candida sp. Similar mycotic agents and numerous rod bacteria were also present in the lumen of the intestine. The overgrowth of yeasts and bacteria is likely due to an immunosuppressive state of the animal secondary to viral infection. Prolonged antibiotic use can also cause a similar overgrowth of yeast.

JPC Morphologic Diagnosis:
1. Lung: Pneumonia, interstitial, necrotizing, subacute, diffuse, marked with type II pneumocyte hyperplasia.
2. Lung: Bronchopneumonia, neutrophilic, acute, multifocal, moderate.

Conference Comment:
The slide description and conference discussion of the histologic features in the submitted section of lung closely aligned with those by the contributor. Most conference participants agreed the process likely began in the alveolar interstitium with septal degeneration and necrosis, and subsequently progressed to loss of alveolar septa and the influx of inflammatory cells. Hyaline membranes are prominent in some areas secondary to exudation of fibrin; rare syncytial cells are also observed. Although BALT hyperplasia is not a prominent feature in most slides, there is slide variation and some participants felt BALT hyperplasia was an important microscopic finding when present. Additionally, a secondary process affecting medium and large airways is present. Bronchioles contain variable numbers of viable and degenerate neutrophils, admixed with fewer sloughed epithelial cells. Participants attributed the inflammatory lesions of the conducting airways to secondary bacterial infection, most likely due to Streptococcus pyogenes based on the contributor’s laboratory information and comments above. The differential diagnosis considered in this case included Mycoplasma hyopneumoniae and swine influenza virus.

There are multifocal areas in the myocardium of hemorrhage, edema, and infiltration by small numbers of lymphocytes. (HE, 200X). (Image courtesy of: Tuskegee University College of Veterinary Medicine, Nursing and Allied Health, 1200 Old Montgomery Rd., Tuskegee, AL 36088 http://www.tuskegee.edu/academics/colleges/cvmnah/school_of_veterinary_medicine.aspx
The PRRS virus results in significant economic losses in the swine industry worldwide, with annual cost estimates around $600 million in the United States alone. It is a species specific virus that has a high mutation rate, which may result in outbreaks in previously vaccinated herds. As mentioned above, routes of transmission and shedding are diverse and duration of infection and shedding may vary depending on virus strain. Additionally, prolonged infection is a key feature of the virus, as with other arteriviruses.3

Clinically, PRRS disease typically occurs in clinical stages, the first beginning with viral infection and replication in alveolar macrophages followed by spread to alveolar pneumocytes, most likely via leukocyte trafficking. The virus appears to have a predilection for infection of macrophages. The presence of infected alveolar macrophages results in alveolar damage and acute interstitial pneumonia. Degeneration and necrosis of pneumocytes occur as a result of the acute inflammatory response. Being an enveloped virus, the agent is able to infect the cell without causing cell death. However, both infected and non-infected macrophages release proinflammatory cytokines, recruiting additional inflammatory cells, and causing significant tissue damage. Histologically, the results of the cytokine cascade are seen as alveoli filled with inflammatory cells and debris, as observed in this pig case. Infected macrophages drain to regional lymph nodes where they infect lymphocytes and other macrophages, resulting in clinically large, firm, grossly edematous nodes. In the second clinical stage of disease, the virus spreads to other organ systems and becomes a persistent infection. This typically results in more generalized infection of lymph nodes and the reproductive organs, among other tissues such as tonsil and spleen.

At the molecular level, the PRRS virus is able to prevent infected macrophages/monocytes from functioning properly during phagocytosis, antigen presentation and cytokine release, thus hampering the immune response.4 Specifically, infection results in impaired expression of cytokines such as TNFα, IL-12 and IFNγ.2 Additionally, the virus increases the release of IL-10 from infected cells, which is immunosuppressive, affecting both the innate and adaptive immune response.4

References:
3. Perez AM, Davies PR, Goodell CK. Lessons learned and knowledge gaps about the epidemiology and control of porcine reproductive and respiratory syndrome virus in North America. JAVMA. 2015;246(12):1304-1317.