CASE I: ITPA Berne 2011/2 (JPC 4002463).

Signalment: 15-year-old female alpaca, (Lama pacos).

History: The patient presented with a history of 2 weeks loss of appetite, apathy and weight loss. An intra-abdominal neoplasia was suspected at ultrasound examination.

Gross Pathology: The alpaca was in a poor body condition. At necropsy, in the second compartment of the stomach (C2), there was a 30x20x15 cm, non-encapsulated, infiltrative, and relatively well demarcated mass which on cut surface contained abundant fibrous connective tissue. The mucosa was focally, extensively ulcerated and covered by a thick layer of necrotic material and pus. In the mesentery and intestinal serosa, there were multiple white nodules
of firm consistency, approximately 1x1x1 cm size. White nodules were also present within the liver capsule and parenchyma, approximately 1.5x1x1 cm size. In the colon and third compartment of the stomach (C3), there were multiple chronic mucosal ulcerations. The abdomen and thorax contained approximately 500 ml of red, transparent (serosanguinous) fluid.

**Histopathologic Description:** Stomach, C2: The wall of C2 is severely disrupted by an ulcerated, infiltrative growing, non-encapsulated, poorly demarcated, densely cellular neoplasm, projecting from the mucosa and extending transmurally to the serosa, consisting of islands, nests and anastomosing cords of neoplastic epithelial cells separated by a moderate to large amount of fibrovascular stroma. The cells are polygonal, up to 30 µm in diameter with abundant eosinophilic to amphophilic fibrillar cytoplasm with a large vesicular, central oval nucleus and prominent, single to multiple nucleoli. Some nests of neoplastic cells exhibit gradual individual cell keratinization, prominent concentrically lamellated keratin “pearls”, or contain cellular debris admixed with inflammatory cells, mainly lymphocytes and degenerate neutrophils. Intercellular bridges between keratinized cells are prominent. There are approximately 5 mitoses per 400x field with frequent bizarre mitotic figures. There is severe anisocytosis and anisokaryosis. Multifocally within the neoplasm there are large areas of necrosis, hemorrhage and a mixed inflammatory infiltrate of lymphocytes, plasma cells, neutrophils and some macrophages. Many submucosal and subserosal vessels contain clusters of neoplastic cells (tumor emboli) as well as fibrin thrombi. Overlying the ulcerated mucosa, there is abundant fibrillar eosinophilic material (fibrin exudation) and hemorrhage, admixed with cellular and karyorrhectic debris (necrosis) and bacterial colonies. Throughout the mass, but especially along the serosa, there are multiple nodules or bands of abundant fibrous connective tissue (scirrhous response).

**Contributor’s Morphologic Diagnosis:** C2: Gastric squamous cell carcinoma.

**Contributor’s Comment:** This was an interesting case of a rare presentation of gastric squamous cell carcinoma (SCC) with carcinomatosis and metastases to the liver and mesenteric lymph nodes in an alpaca. There are numerous reports of neoplasia in South American camelids, including lymphoma, urogenital tumors, cutaneous and mucocutaneous neoplasia, oral, intraocular, gastrointestinal, pulmonary, neuroendocrine and brain neoplasia (Table 1).5

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Total no. tumors</th>
<th>Alpacas (no.)</th>
<th>Llamas (no.)</th>
<th>Mean age alpacas (yrs)*</th>
<th>Mean age llamas (yrs)</th>
<th>Location(s)</th>
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</thead>
<tbody>
<tr>
<td>Fibroma/fibropapilloma</td>
<td>12</td>
<td>8</td>
<td>2</td>
<td>5.6</td>
<td>11.5</td>
<td>Face; nose; lip; distal limb; gingiva; hard palate</td>
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<tr>
<td>Carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cutaneous</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td></td>
<td>13.5</td>
<td>3rd eyelid</td>
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<tr>
<td>Ocular</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>U</td>
<td>14</td>
<td>Metastatic to local lymph node; disseminated</td>
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<tr>
<td>Mammary</td>
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<td>2</td>
<td></td>
<td>17</td>
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<tr>
<td>Un-differentiated</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td>12</td>
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<tr>
<td>Adenocarcinoma</td>
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<tr>
<td>Biliary</td>
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<td></td>
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<tr>
<td>Pancreas</td>
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<td>1</td>
<td>0</td>
<td>10</td>
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<tr>
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<td>0</td>
<td>1</td>
<td></td>
<td>8</td>
<td>Disseminated</td>
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<tr>
<td>Lymphoma</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>1.5</td>
<td>15</td>
<td>Disseminated</td>
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<tr>
<td>Fibrosarcoma</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>13</td>
<td>Lip; gingiva; maxilla; comea</td>
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<tr>
<td>Lipoma</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td></td>
<td>12</td>
<td>Mesentery; subcutis</td>
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<tr>
<td>Melanocytoma</td>
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<td>0</td>
<td>1</td>
<td></td>
<td>11</td>
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<tr>
<td>Leiomyoosarcoma</td>
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<td>0</td>
<td>1</td>
<td></td>
<td>12</td>
<td>Uterus</td>
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<tr>
<td>Intersitial cell tumor</td>
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<td>1</td>
<td>0</td>
<td>9</td>
<td></td>
<td>Ovary</td>
</tr>
<tr>
<td>Primitive stromal tumor</td>
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<td>1</td>
<td>0</td>
<td>U</td>
<td></td>
<td>Testis</td>
</tr>
</tbody>
</table>

* U = unknown.
Gastric squamous cell carcinoma in ruminants is usually quite rare and only few have been reported in llamas and alpacas.\textsuperscript{3,5} However, in a 5 year (2001-2006) study at Oregon State University it was reported that cutaneous and mucocutaneous squamous cell carcinoma (SCC) was the most frequent malignant neoplasm identified, although in previous studies lymphoma was most commonly reported.\textsuperscript{5}

Histologically, C1 and portions of C2 are composed of non-keratinized stratified squamous epithelium, whereas the rest of C2 and all of C3 have a mucinous glandular epithelium.

In previous cases, neoplastic cells most commonly originated from squamous mucosal epithelium of compartments 1 and 2, but it could arise also from the glandular mucosa of compartment 3.\textsuperscript{3} In this case, the neoplasm was most closely related to the stratified squamous epithelium of C2. SCC of glandular mucosa could derive from metaplasia of glandular epithelium, from growth of heterotopic squamous cell rests, or from multipotent cells of the crypt gland base, as suggested in previous reports in dogs.\textsuperscript{3}

Commonly described sites of metastases are the liver, diaphragm, mesentry, and myocardium. The mode of metastatic spread is by implantation and vascular dissemination, as seen in horses with gastric SCC.\textsuperscript{3}

Several factors have been associated with the development of gastric papillomas and squamous cell carcinomas in humans and other species including host, dietary, genetic, environmental conditions and infectious agents. In cattle, papillomas of the esophagus and reticulorumen are caused by bovine papillomavirus 4 (BPV-4), whereas fibropapillomas of the esophagus, esophageal groove and rumen are caused by bovine papillomavirus 2. It is very seldom for viral particles to be seen in the tumor. Malignant neoplasia of bovine esophagus and forestomach is extremely rare.\textsuperscript{2,3} On the contrary, SCC involving male and female genitalia, ocular and periocular tissue and stomach is most commonly reported in the horse. Equine gastric SCC metastasized most frequently to retropharyngeal lymph nodes.\textsuperscript{4}

The frequency of llamas and alpacas as patients in the veterinary hospital is increasing; therefore it is important to improve the knowledge about possible lesions, prevalence and predisposing factors causing diseases in these animals.

Table 1. Summary of 40 tumors reported in 20 llamas and 18 alpacas between 2001-2006 at Oregon State University.\textsuperscript{5}

**JPC Diagnosis:** C2: Squamous cell carcinoma.

**Conference Comment:** The contributor provided a very good characterization of squamous cell carcinomas (SCC) in alpacas, as well as comparisons to other species such as the horse, in which SCC is the most common gastric tumor. Conference participants readily agreed upon the diagnosis; however, during the discussion a question was raised regarding histological determination of the location of the specimen (C2).

Following is a brief summary of new world camelid gastric anatomy and histology: As the contributor described, new world camelid stomachs are composed of three compartments :C1, C2, and C3, which are sometimes referred to as the proximal compartment (PC), intermediate compartment (IC) and the distal compartment (DC), respectively. C1 is often compared with the rumen, and constitutes the largest chamber (comprising approximately 83% of the gastric volume). C2 is somewhat kidney-shaped and is the smallest of the three compartments. C3 is tubular and elongated and accounts for approximately 11% of the gastric volume. Histologically the majority of C1 is nonglandular, covered by stratified squamous epithelium which is supported by a dense collagenous lamina propria. This tissue is arranged in folds (rather than papillae as in ruminants) which are more prominent when the stomach is contracted. Smaller, more ventral portions of C1 (known as cranial and ventral glandular sacculles) are lined by columnar epithelium and deep tubular glands which are supported by a smaller amount of looser connective tissue. C1 communicates with C2 through the ventricular furrow. C2 has thick walls, and histologically is divided into two regions: a dorsal nonglandular and a ventral glandular portion, each of which are similar to those described for C1. Lymphoid tissue may be found in the lamina propria of the glandular portions of C2. The muscularis mucosae is absent in the nonglandular regions of both C1 and C2, and is present in glandular areas, but is incomplete. C2 communicates with C3 via the channel of isthmus, a small tubular continuation of the ventricular furrow that is lined by stratified epithelium. C3 is otherwise covered by a glandular mucosa, with 3 regions differing histologically: the proximal region has abundant lymphoid tissue in the mucosa and submucosa; the central region has simple mucous-secreting tubular glands; and the caudal region has fundic glands in the ventral portion and simple tubular glands in the dorsal (pyloric) region. C3 has a well-developed muscularis mucosae and a thin submucosa. Surrounding all three gastric compartments is a muscularis comprised of inner circular and outer longitudinal layers.\textsuperscript{1}


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**References:**
CASE II: GC-12 (JPC 4018081).

**Signalment:** Nine-month-old Red Angus steer, *Bos taurus*.

**History:** This steer belonged to a herd of 250 animals that were weaned one month prior to presentation. This steer had a brief history of inability to stand up and was treated with 6.5 cc Draxxin® (tulathromycin). Ten other animals in this herd were treated for respiratory disease and twenty were seen limping in the previous week. Another steer in this herd had an enlarged stifle that was tapped and cytology revealed inflammatory arthropathy.

**Gross Pathology:** Brain: There were multifocal random foci of malacia and hemorrhage in the gray and white matter of the cerebrum, cerebellum, and brainstem. The meninges in the ventral aspect of the brain contained a moderate accumulation of fibrinosuppurative material. The thoracic cavity contained approximately 50 mL of serous fluid with fibrin strands. The lungs failed to fully collapse upon opening the thoracic cavity. The right cranial and middle lobes and the left cranial lobe were consolidated and attached to the thoracic wall by a thick mat of fibrin (approximately 50% of the total lung). On cut sections of these lobes, there was exudation of small amounts of whitish suppurative material. Fibrin adhesions were also seen in the caudal portion of the right caudal lobe. The pulmonary parenchyma at this level was grossly normal. Stifle joints in both sides contained increased volume of yellowish and watery synovial fluid with fibrin clots. No gross lesions were found in the heart.

**Laboratory Results:** Bacterial culture from cerebral swabs and fresh samples of brain yielded growth of *Histophilus somni*. Mycoplasma bovis culture from joint swab was positive. Polymerase chain reaction (PCR) for detection of *Mycoplasma bovis*, *Chlamydia* spp., bovine viral diarrhea virus, bovine respiratory syncytial virus, bovine coronavirus, bovine herpesvirus type 1, and parainfluenza type 3 from lung tissue were all negative. Bacterial cultures from the lung yielded no growth of significant pathogens.

**Histopathologic Description:** Brain: Sections of cerebrum, cerebellum, or brainstem contain multiple, variably-sized, and random foci infiltrated by moderate to large numbers of neutrophils and lesser numbers of macrophages associated with hemorrhage, edema, degeneration, necrosis and loss of gray and white matter. The meninges over these areas are expanded by sheets of neutrophils and hemorrhage. The tunica media of numerous small to medium sized blood vessels in the meninges and cerebral parenchyma is necrotic and is often effaced by moderate amounts of fibrin (fibrinoid necrosis), and their lumen contains fibrin thrombi. Colonies of Gram negative bacilli are frequently observed within the blood vessels and in surrounding neuropil.

**Contributor's Morphologic Diagnosis:** Meningoencephalitis, necrosuppurative and hemorrhagic, multifocal, acute, severe, with necrotizing vasculitis, thrombosis, with intralesional colonies of Gram negative bacilli, etiology consistent with *Histophilus somni*.

**Contributor’s Comment:** *Histophilus somni* (previously known as *Haemophilus somnus*) is a Gram negative coccobacillus that causes septicemia and a number of clinical signs in cattle, termed altogether as “*Histophilus somni disease complex*” or “histophilosis” 6,7,8 The clinical signs in cattle include...
pneumonia; myocarditis; polyarthritis; mastitis; genital infection; abortion; reproductive failure; and thrombotic meningoencephalitis (TME), the neurological form of the disease.\textsuperscript{1,3,6,7} Septicemia caused by \textit{H. somni} develops in cattle in many age groups, but it is more prevalent in young growing cattle in feedlots during the winter time.\textsuperscript{3,6,8} Other species that are affected include sheep, bison, and bighorn sheep.\textsuperscript{1}

The lesions observed in the central nervous system in this steer are compatible with the neurological form of \textit{H. somni} disease complex, or TME, which was confirmed by bacterial culture from cerebral swabs and
fresh samples of the brain. Historically, TME was the most common disease presentation of the bacterial infection; however, in recent years, the most common presentations are pleuropneumonia and myocarditis.\textsuperscript{6,8,9} The gross lesions of TME are random, but most often localized at the cortical gray matter-white matter junction of the cerebral cortex and in the thalamus, where it is presumed that the bacteria can lodge and replicate in the blood vessels due to changes in vessel diameter and flow patterns.\textsuperscript{6,7} The lesions consist of multiple variable sized dark foci of necrosis and hemorrhage.\textsuperscript{6,7} The meninges over the hemorrhagic areas are usually affected.\textsuperscript{6,7} Similar lesions are also observed in the brainstem and spinal cord.\textsuperscript{6,7} Microscopic lesions are characterized by severe vasculitis and thrombosis with infarction, and infiltrates of neutrophils and macrophages in the site.\textsuperscript{6,7}

\textit{H. somni} is a commensal of the respiratory and reproductive tracts.\textsuperscript{2} The pathogenesis of the septicemia leading to TME in cattle is unknown, but it is believed that some virulent bacterial strains first colonize the surface of the mucous membranes in the upper respiratory tract and invade the circulatory system.\textsuperscript{3,7} The bacteria adhere to the endothelial cells, causing vasculitis, thrombosis, and infarction and continue replicating in the thrombus, triggering an inflammatory response.\textsuperscript{7} Several studies have shown that virulent strains of \textit{H. somni} produce a multitude of virulence factors aimed at evasion of host defenses for colonization of tissues. Virulence factors in \textit{H. somni} include, but are not limited to, lipooligosaccharides (LOS), attachment and induction of apoptosis in bovine endothelial cells, intraphagocytic survival, immunoglobulin Fc binding proteins (IgBPs), biofilm formation, histamine production, and integration of phosphorylcholine (ChoP) into its LOS.\textsuperscript{2,9} Disease in TME likely occurs as a result of apoptosis of endothelial cells and host inflammation due in part to the presence of endotoxin and the activation of the coagulation cascade, and the recruitment of polymorphonuclear leukocytes and macrophages to sites of infection.\textsuperscript{3,9}

**JPC Diagnosis:** Cerebrum: Meningoencephalitis, fibrinopsuppurative, multifocal, severe, with vasculitis, fibrinoid necrosis, rarefaction, and intravascular bacterial colonies.

**Conference Comment:** The hallmark lesion of TME is vasculitis and thrombus formation due to endothelial cell damage and subsequent exposure of the underlying extracellular matrix. Although the mechanism of vascular injury by \textit{H. somni} is not fully understood, it is thought to be due to apoptosis caused by direct effects of the bacteria and its lipopolysaccharide (LOS) on endothelial cells.\textsuperscript{3,5} However, because there is often endothelial damage in areas devoid of bacterial antigen, studies in more recent years have attempted to elucidate additional mechanisms that may contribute to this vasculitis. These studies have suggested that activated platelets likely play an important role in the vascular damage. Although the primary role of platelets lies in maintaining hemostasis, platelets also contribute to vascular inflammation and injury by expressing chemotactic factors (platelet factor 4, lipooxygenase products, RANTES), cytokines (IL-1β), platelet activating factor, and surface molecules (CD40L, FasL, P-selectin)\textsuperscript{3} that can interact with leukocytes and endothelial cells.\textsuperscript{4,5} Additionally, it has been found that platelets activated by \textit{H. somni} induce endothelial apoptosis via caspases 8 and 9, and promote endothelial cell production of reactive oxygen species (ROS), which further enhances apoptosis. Conversely, inhibitors of either caspase 8 or 9, or the disruption of ROS activity, were found to decrease endothelial apoptosis.\textsuperscript{5} Interestingly, bovine (and human) endothelial cells appear to be resistant to Fas-mediated apoptosis, and it has been suggested that activated platelets may induce apoptosis of endothelial cells via a novel pathway that utilizes caspases 8 and 9 as well as ROS.\textsuperscript{5} These findings contribute to our developing understanding of mechanisms of endothelial cell damage not only in \textit{H. somni} infections, but in bacterial sepsis in general as well.

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**References:**


CASE III: V11-07180 (JPC 4003694).

Signalment: 5-month-old male Weimaraner, Canis lupus familiaris, dog.

History: The dog was presented for anorexia. The dog developed melena. Additional abnormalities included severe thrombocytopenia, anemia, and worsening hepatopathy. The owner opted to euthanize due to lack of response to therapy and the poor prognosis.

Gross Pathology: The dog was in good body condition with minimal postmortem decomposition. The carcass was icteric. The liver was enlarged and friable. The liver was mottled yellow, tan and dark red in random coalescing foci and in a reticular pattern with small numbers of petechiae. Multiple lymph nodes, including the submandibular, mediastinal, pancreatic, and mesenteric lymph nodes, were dark red with hemorrhage. The lungs were congested and edematous. There were small numbers of petechiae on the endocardium of the heart. There were multiple petechiae in both right and left kidneys and the brain. The mucosal epithelium of the stomach contained rare petechiae with small numbers of erosions and ulcers in the pylorus. The small intestine contained long segments with extensive hemorrhage with smaller segments of the small intestine that contained petechiae. The stomach, small intestine, and large intestine contained digested blood.

Laboratory Results: Fluorescent antibody testing was performed at the Colorado State University Veterinary Diagnostic Laboratory. The liver was positive for canine adenovirus using a commercial antibody against canine adenovirus (CAV). Research monoclonal antibodies against canine adenovirus type 1 (CAV-1) and canine adenovirus type 2 (CAV-2) were used to further define the type of CAV present in the liver. Using the research monoclonal antibodies, the liver was positive for CAV-1. CAV-2 was not detected in the liver.

Histopathologic Description: The liver contains numerous foci of centrilobular to midzonal necrosis with occasional extension of necrosis from one centrilobular area to another (bridging necrosis). The sinusoids in the foci of necrosis are dilated and filled with blood. There is a sharp distinction between the foci of necrosis in the centrilobular areas and the intact periportal areas that do contain rare apoptotic hepatocytes and swollen vacuolated hepatocytes. The necrotic foci and the adjacent sinusoids contain small numbers of macrophages with lesser numbers of lymphocytes and rare neutrophils. The portal areas contain small numbers of macrophages, lymphocytes, and rare neutrophils. Moderate numbers of Kupffer cells and macrophages and lesser numbers of intact hepatocytes contain magenta to basophilic intranuclear inclusion bodies with occasional karyomegaly. There is apoptosis of small numbers of macrophages and Kupffer cells.

Contributor’s Morphologic Diagnosis: Severe, acute, centrilobular to midzonal bridging hepatic necrosis with mild lymphohistiocytic hepatitis and magenta to basophilic intranuclear inclusion bodies; etiology consistent with canine adenovirus type 1; infectious canine hepatitis.

Contributor’s Comment: Canine adenovirus (CAV) is divided into two types: canine adenovirus type 1 (CAV-1) and canine adenovirus type 2 (CAV-2). CAV-1 and CAV-2 are within the genus Mastadenovirus in the family Adenoviridae, and are antigenically and genetically closely related. CAV-1 is the causative agent of infectious canine hepatitis (ICH) in Canidae and Ursidae. CAV-2 causes respiratory disease (infectious tracheobronchitis) mainly in kennel dogs.

Infection with CAV-1 in canids can range from subclinical infection to severe clinical infection leading to death. In domestic dogs, infectious canine hepatitis is an uncommon clinical disease due to the routine vaccination of domestic dogs. However, there are sporadic individual cases and outbreaks of infectious canine hepatitis in unvaccinated domestic dogs. In the wild canid population, subclinical infection with CAV-1 is most likely widespread due to the high incidence of neutralizing antibodies against CAV-1 in this population.
When a susceptible dog is oronasally exposed to CAV-1, the virus localizes in the tonsils and travels to the regional lymph nodes. The virus then traverses the lymphatic system to the thoracic duct where the virus enters the blood. After the dog becomes viremic, CAV-1 is disseminated to the tissues at which point the virus is shed in body secretions. The viremia usually lasts 4 to 8 days postinfection. During viremia, the hepatocytes and the vascular endothelial cells of many tissues are the prime targets for CAV-1 replication. Severe widespread hepatic and vascular damage, which may result in disseminated intravascular coagulation (DIC), often result in death in dogs that do not mount an adequate neutralizing antibody response. Dogs that respond with a high neutralizing antibody response by day 7 postinfection often recover from the disease. Dogs that demonstrate a partial neutralizing antibody response can develop chronic hepatitis. Dogs with a high neutralizing antibody titer before infection can develop mild or inapparent disease. In dogs that do recover from clinical illness with infectious canine hepatitis, the virus localizes in the kidney 10 to 14 days postinfection leading to secretion of the virus in the urine that can last 6 to 9 months.

The gross lesions of ICH are consistent with what one would expect considering the cellular tropism of CAV-1. The liver is slightly swollen with sharp edges, turgid, and friable with fine yellow mottling throughout the liver. The gallbladder is often edematous with occasional intramural hemorrhages. There are serosal hemorrhages in the small intestine and stomach. The lymph nodes are enlarged and congested with hemorrhages. The lungs may contain hemorrhages with occasional hemorrhagic pneumonia in the caudal lung lobes. There may be hemorrhage infarcts in the cortices of the kidneys. The brain and the medullary cavity of the long bones may also contain hemorrhages.
The microscopic lesions of ICH also coincide with the cells CAV-1 infects. In fatal cases of ICH, the liver is the organ with the predominate lesions. The liver contains centrilobular to midzonal necrosis with dilated sinusoids filled with blood in the areas of necrosis. There can be extension of the necrotic foci from one centrilobular area to another isolating the portal area. There is often a sharp distinction between the foci of necrosis and the normal liver in the periportal areas. The periportal hepatocytes are often normal, but can have increased apoptotic cells or be swollen and vacuolated. The leukocytic infiltrate is mild and surrounds the necrotic focus. The infiltrating leukocytes consist of neutrophils and mononuclear cells. Many of the Kupffer cells throughout the liver are necrotic. Intranuclear inclusion bodies can be seen in the intact hepatocytes, Kupffer cells, and macrophages. The microscopic lesions in the other affected organs consist of hemorrhage due to the widespread endothelial damage, which may lead to DIC. Intranuclear inclusion bodies can be seen in the endothelial cells, renal tubular epithelial cells, lymphoid follicles, red pulp of the spleen, and macrophages throughout the body.

**JPC Diagnosis:** Liver: Hepatitis, necrotizing, centrilobular to midzonal, diffuse, acute, with intranuclear viral inclusions.

**Conference Comment:** The contributor did an excellent job of characterizing canine adenoviral infections. Conference participants discussed the classic distribution of this disease (centrilobular to midzonal) in comparison to the often random distribution of other viruses associated with hepatitis. Additionally, there was some discussion regarding the morphologic diagnosis, with some participants favoring “degeneration and necrosis” because of the presence of so few inflammatory cells; however, the majority of the group felt that “hepatitis” was more appropriate.

Historically, CAV-1 is synonymous with “fox encephalitis”, described in 1933, and “Rubarth’s disease”, described in 1947. CAV-1 can affect dogs, foxes, wolves, coyotes, and bears. Common lesions include hepatitis, anterior uveitis with corneal edema, and interstitial nephritis. Chronic changes seen in animals that survive the initial infection include hepatic fibrosis, interstitial fibrosis, glaucoma, and/or phthisis bulbi.

**Contributing Institution:** New Mexico Department of Agriculture Veterinary Diagnostic Services


**References:**
CASE IV: 1 (JPC 4019128).

**Signalment:** Adult female Holstein Friesian, bovine (*Bos taurus*).

**History:** This cow came from an intensive livestock farming facility of approximately 450 cows (200 lactating cows + 250 heifers, calf and dry cows). The farm had an 8 month history of several cows (approximately 15%) developing severe uncontrollable diarrhoea, including animals of 24 months of age. Cows affected were progressively slaughtered (30 animals / 230 lactating cows) with no additional investigations. The increasing disease severity and number of animals involved induced the breeder to ask for a full necropsy on one of his cows. The veterinarian sent multiple tissues for microscopical evaluation and a gross report indicating the presence of a severe and diffuse thickening of the intestine.

**Gross Pathology:** Severe and diffuse thickening of the jejunum, ileum and large intestine and multiple intestinal hemorrhages were reported by the referring veterinarian.

**Laboratory Results:** All cows were tested for paratuberculosis and bovine viral diarrhoea (ELISA). All livestock aged 24 months and older were tested for Paratuberculosis by ELISA. All animals including 2 month calving heifers (26 months old) were tested for BVD (PCR and serology for ns 2-3 proteins). Approximately 30% of the cows of 24 months and older tested positive for paratuberculosis and only one heifer was BVD positive, persistently infected (viremic animal without antibodies). All the other tested animals were positive for BVD antibodies (according with a high viral environmental circulation). After these results, the BVD positive cow was slaughtered. Vaccination against BVD was performed in the farm. Ziehl-Nielsen stain demonstrated numerous acid-fast bacilli in the cytoplasm of macrophages.

**Histopathologic Description:** Ileum: approximately 90% of intestinal villi are blunted, shortened and variably fused and lamina propria is severely expanded and obliterated by epithelioid macrophages, large foamy reactive macrophages and occasional multinucleated giant cells with up to six often horseshoe arranged nuclei (Langhans type cells). Occasionally, 1-2 micron rod shaped negative stained organisms (Mycobacteria) can be seen in the cytoplasm of giant cells. In the lamina propria, histiocytes are associated with moderate numbers of small mature lymphocytes and lesser numbers of plasma cells and occasional eosinophils. Mucosal lining is detached and multifocally missing (ulceration). Crypts are variably, multifocally dilated and lumens often contain homogeneous eosinophilic material associated with basophilic, granular debris and by karyorrhectic neutrophils (crypt necrosis and crypt abscesses). Crypt epithelium is multifocally ulcerated. Scattered

4-1. Small intestine, ox: The mucosal epithelium is diffusely thickened by a marked cellular infiltrate which effaces both villi and crypts. (HE 70X)
aggregates of epithelioid cells extend into the inner circular muscle cell layer. In the subserosal lining occasional granulomas composed of macrophages and lymphocytes are also present.

**Contributor’s Morphologic Diagnosis:** Jejunum, severe chronic diffuse granulomatous enteritis with intraluminal mycobacteria. Bovine.

**Contributor’s Comment:** Microscopic findings were diagnostic for bovine paratuberculosis. In some sections occasional fungal hyphae with neither inflammation nor necrosis were also observed suggesting severe immune depression. Paratuberculosis or Johne's disease was first described in 1895 by Johne and Frothingham, who reported "a peculiar case of tuberculosis" in a cow with chronic enteritis characterized by diffuse thickening and corrugation of the intestinal mucosa and the presence of acid-fast bacilli in the lesions.\(^5\) Paratuberculosis is caused by *Mycobacterium avium* subsp. *paratuberculosis*. This organism was originally named *Mycobacterium enteritidis chronicae pseudotuberculosa bovis john* and the name changed into *Mycobacterium johnnei* followed by *Mycobacterium paratuberculosis* and, most recently, *Mycobacterium avium* subspecies *paratuberculosis* (MAPS).\(^4,5\)

The etiologic agent of Johne's disease has been reduced to the subspecies status within *M. avium* on the basis of the high (>90%) DNA homology among typical paratuberculosis strains and type strains of *M. a. avium*. The Johne's disease agent, which in culture is slow-growing and dependent on mycobactin as a source of iron, possesses some unique cultural and biochemical traits. Genetically, a distinct difference from *M. a. avium* is the presence of the insertion sequence, IS900, of which *M. a. paratuberculosis* has 15-20 copies per organism.

Paratuberculosis is a chronic wasting enteritis that affects mainly domestic and wild ruminants worldwide but occurs also in camels, lagomorphs, rodents, carnivores, birds and rarely in equids. Infection can also be experimentally reproduced in pigs, rabbits, mice, hamsters and primates.\(^2,4\)

*Mycobacteria* are shed in feces, and the main route of infection is fecal-oral, but microorganisms are shed in milk, semen and urine and are able to cross the placental barrier. Infection occurs most commonly during the first days of life, when MAPS is ingested via colostrum or by contaminated foodstuff. Calves are especially prone to the infection due to the lack of a functional hematointestinal barrier. After ingestion

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4-2 Small intestine, ox: The lamina propria is expanded by numerous epithelioid macrophages and rare multinucleated giant cell macrophages. (HE 200X)
MAPS binds to the luminal surface of M cells and reaches (through phagosomes) Peyer’s patches where it can be phagocyted by tissue macrophages. The virulence attributes of M. a. paratuberculosis are poorly understood, but presumably reside in resistance to killing in macrophages, through inhibition of the conversion of phagosomes to phagolysosomes. MAPS are highly adapted for survival within bovine mononuclear phagocytes. The organisms proliferate in cytoplasmic vacuoles, and transmit to adjacent macrophages, expanding the population of infected cells and recruiting elements of the humoral and cell-mediated immune system to the lesional tissue. Both IL-14 dependent and IL-14 independent mechanisms appear to be involved in attenuation of phagosome acidification and phagolysosome fusion. Mycobacteria acquire iron from ferritin stored in macrophages and, since the availability of iron is greatest in tissue macrophages of ileocecal junction, this is the site with initial and more severe lesions characterized by a diffuse granulomatous enteritis. Infected tissue macrophages spread via leukocyte trafficking in afferent lymphatic vessels to ileocecal lymph nodes leading also to a granulomatous lymphadenitis.

Chronic, continuous or intermittent, often intractable, diarrhea, and emaciation are the most common and are associated with severe milk drop and reduced fertility. Low total protein as a consequence of protein malabsorption and loss is the most common clinical chemistry abnormality associated with low albumin serum while globulin values are usually unaffected.

Specific gross lesions occur in the intestine and regional lymph nodes. The classical intestinal change is diffuse thickening of the mucosa, which is folded into transverse rugae, the crests of which may be congested. When well developed, the mucosal folds cannot be smoothed out by stretching. The intestinal serosa often has a slight granular and diffusely opaque appearance and foci of ulceration may be present. The mesenteric nodes, particularly the ileocecal, are always enlarged, pale, and edematous, especially in the medulla. Lymphangitis is the most frequent and common change and can be occasionally the only gross lesion granting a presumptive diagnosis of Johne's disease at necropsy. Advanced cases have signs of cachexia with loss of muscle mass, serous atrophy of fat deposits, intermandibular edema and effusions in body cavities. Plaques of intimal fibrosis and aortic mineralization have been reported. Focal granulomatous lesions can be occasionally found in liver, kidney and lungs.

When gross lesions are well developed, the characteristic microscopic change, transmural granulomatous enteritis, is obvious. Characteristic histopathological finding in bovines is a transmural granulomatous enteritis, lymphadenitis and lymphangitis. Masses of epithelioid macrophages may accumulate in the submucosa. Foci of necrosis may occur within these aggregates of macrophages but in cattle, caseation and mineralization are extremely rare. Giant cells may be present. The inflammatory infiltrate may abnormally separate and displace crypts, which are elongate, with hyperplastic epithelium. Crypts may be distended with mucus and exfoliated cells. These lesions are characteristic of the multicellular “lepromatous” phase of the disease as was in this case. In cattle in which gross changes are minimal or absent, the microscopic abnormalities are more subtle. In these the lamina propria is diffusely infiltrated with lymphocytes and plasma cells, and a large number of eosinophils. There may be very few macrophages, and the most characteristic change is an infiltrate of lymphocytes and plasma cells in the submucosa, and associated with the submucosal and mesenteric lymphatics. Lymphangitis is one of the most consistent changes. Initially the lymphatics are surrounded by lymphocytes and plasma cells and many contain plugs of epithelioid cells in the lumen. Granulomas may form in the wall and project into the lumen. These nodules may undergo central necrosis. Granulomatous lymphadenitis occurs in mesenteric lymph nodes in advanced cases. In the early stages, there is histiocytosis of the subcapsular sinus. Ultimately, nodular or diffuse infiltrates of epithelioid macrophages and giant cells may replace much of the cortex, and infiltrate the medullary sinusoids.

In sheep and goats diarrhea is uncommon and the disease is characterized by chronic wasting. Enteric gross lesions are often mild, with little obvious thickening, and no transverse ridges; they are easily missed at necropsy. Gross lesions are similar but mineralized tubercle-like lesions have been reported.

Mycobacterium avium spp. paratuberculosis is an acid-fast, weakly Gram positive bacillus identifiable in the cytoplasm of macrophages as a rod shaped Ziehl-Neelsen positive organism. The organism is usually readily evidenced in macrophages and giant cells in the lesions when appropriately stained by acid-fast techniques. However, in some clinical cases, especially the ovine paucibacillary form, an extensive search may be needed for individual macrophages or giant cells bearing a few acid-fast bacilli. Johne's disease in sheep and especially in goats may resemble tuberculosis, on account of caseation and mineralization in granulomatous foci, and for such cases specific identification of the etiologic agent is necessary.

The antemortem diagnosis of Paratuberculosis in cattle is challenging and mirrors the evolution of immunopathological mechanisms elicited by MAPS. Serological tests such as ELISA and PCR are available,
but bacteriological assay is still considered the gold standard diagnostic technique (1; OIE http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/). Vaccination has been reported as an effective strategy for Paratuberculosis control in several countries.1

Mycobacterium avium spp. Paratuberculosis has been identified in intestinal tissue from patients with Crohn's disease by culturing the organisms and by PCR.16 Additionally, some patients with Crohn's disease respond to antimycobacterial therapy.3 The detection of genome or the isolation of MAPS could be caused by ingestion of contaminated food, but the hypothesis that multiple exposure to MAPS via alimentary route could be involved in the pathogenesis of Crohn's patients has been taken into account.16

**JPC Diagnosis:** Small intestine: Enteritis, granulomatous, chronic, diffuse, severe, with crypt loss and abscessation, villar blunting and fusion, lymphangectasia and numerous intracytoplasmic acid-fast bacilli.

**Conference Comment:** The contributor provides an excellent description and characterization of *Mycobacterium avium* subsp *paratuberculosis* infection. Conference participants compared and contrasted the lepromatous (diffuse) and the tuberculoid forms of disease associated with *M. avium* subsp *paratuberculosis* in ruminants. Classically, clinical disease in cattle infected with *M. avium* subsp *paratuberculosis* is associated with a lepromatous reaction; however, in sheep and goats with subclinical to clinical paratuberculosis, a spectrum of lesions between lepromatous and tuberculoid inflammation can be observed.8

The type of granulomatous inflammation and clinical disease elicited by persistent, poorly degradable pathogens such as *Mycobacterium* species is dependent upon the host's immune response.16 Lepromatous inflammation is characterized by macrophages and epitheloid macrophages arranged in sheets within tissue. In tuberculoid lesions, the macrophages are arranged in distinct nodules (granulomas) that generally have a central necrotic core surrounded by a layer of macrophages, epitheloid macrophages and multinucleated giant macrophages, further surrounded by a layer of lymphocytes and plasma cells and bounded by fibroblasts and collagen.16 The lepromatous form is thought to be associated with a strong Th2 (humoral) immune response, whereas the tuberculoid form is associated with a strong Th1 (cellular) immune response.6 With a strong Th1 response, fewer *Mycobacterium* organisms are present; therefore this form is also referred to as the “paucibacillary” form. On the contrary, with a strong Th2 response, less effective intracellular bacterial killing results in more numerous intralosomal bacteria; hence the lepromatous form of disease is also referred to as the “multibacillary” form.6,16

Recent studies of cytokines in red deer infected with *M. avium* subsp *paratuberculosis* suggest that Th2 and Treg immune responses may not play a direct role in clinical disease; but rather, may control the immunopathology of the disease and that it is the loss of these responses that leads to the development of clinical disease.13

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