CASE I: EV-UFMG 08/1116 (JPC 3136273).

Signalment: 4-month-old male Holstein calf, Bos Taurus.

History: This was an experimental calf. Soon after birth it was placed in an arthropod-free containment facility. The calf was inoculated with Anaplasma marginale when it was two months old. At the end of the experimental protocol, prior to release into the field, the calf was subjected to premunition with Babesia bigemina and B. bovis, after which it developed apathy and quickly progressed to death.

Gross Pathologic Findings: The calf was emaciated with moderate anemia and mild icterus. There was mild hydrothorax and
hydroperitoneum. In the lung there were multifocal mildly consolidated areas ranging from 0.3 to 0.5 cm in diameter distributed in all pulmonary lobes. The liver was markedly enlarged yellowish with focally extensive dark-red areas. There were multiple miliary white nodules in the parenchyma. There was also small amount of purulent exudate draining on the cut surface, and small thrombi were grossly observed. The spleen was moderately enlarged. Gross findings did not support babesiosis as a cause of death.

**Laboratory Results:** Microbiology: *Salmonella* sp. was isolated from the liver.

**Histopathologic Description:** In the liver there was multifocal random degeneration and necrosis associated with an intense neutrophilic infiltrate with some lymphocytes and macrophages. There were several intralesional bacterial colonies. A lymphoplasmacytic and histiocytic infiltrate predominates in portal areas. Several blood vessels had fibrinoid necrosis in the vascular wall associated with thrombosis, particularly in centrilobular veins. Some veins are completely obliterated by thrombosis.

In the spleen (section not submitted) there were numerous hemosiderin-laden macrophages and increased erythrophagocytosis, associated with moderate lymphoid depletion.

In the lungs (sections not submitted), there was an interstitial pneumonia characterized by marked thickening of the alveolar wall with interstitial infiltration of macrophages, lymphocytes and a few neutrophils. There was multifocal intra-alveolar accumulation of fibrin and a few neutrophils.

**Contributor’s Morphologic Diagnosis:** Liver: Hepatitis, necrotizing, histiocytic and neutrophilic, multifocal, random, acute, associated with thrombosis and intralesional bacterial colonies.

**Contributor’s Comment:** *Salmonella enterica* comprises more than 2,000 serotypes, including serotypes Dublin and Typhimurium that are often isolated from cattle. The most common clinical manifestation of *Salmonella* infection in cattle is an enteric disease characterized by diarrhea that is usually associated with acute fibrino-necrotizing enteritis. However, systemic salmonellosis in cattle may occur in the absence of enteric disease, as observed in the present case.

*Salmonella* is highly invasive, crossing the intestinal epithelial barrier inducing ruffling of the apical surface of the epithelial layer, which results in internalization of the organism within a cytosolic membrane bound vacuole. This process is dependent on the expression of several effector bacterial proteins that are translocated into the host cells through a type III secretion system encoded by the *Salmonella* pathogenicity island 1 (SPI-1). Once the organism crosses the epithelial layer, it quickly localizes in the lamina propria, where it is found mostly within...
macrophages. Salmonella processes another type III secretion system encoded by SPI-2 that is activated as soon as the bacteria localizes in the phagosome. This SPI-2-encoded type III secretion system is essential for intracellular survival of the organism in macrophages and, therefore, systemic dissemination of the infection.

In our empirical experience, Salmonella is usually not observed in HE-stained section, both in enteric and systemic sites of infection. Interestingly, in the present case there were abundant intralobular bacterial colonies, which likely represent post-mortem overgrowth of the organism.

**JPC Diagnosis:** Liver: Hepatitis, necrotizing, multifocal, random, with vasculitis, thrombosis, and rare paratyphoid nodules.

**Conference Comment:** The accumulations of mixed mononuclear inflammatory cells multifocally scattered throughout the liver in this case are called “paratyphoid nodules” and are characteristic for the typhoidal form of salmonellosis. Paratyphoid nodules or granulomas may also be found in the kidney, spleen, lymph nodes and bone marrow in cases of septicemic salmonellosis. These contribute to the gross miliary pattern in affected organs, most often in the liver and spleen. It is unclear whether these nodules are the result of a cell-mediated immune response or accumulate due to bacterial replication within macrophages.

Typhoidal serotypes of Salmonella spp. are less common than the nontyphoidal forms in most species, including humans with the exception being in mice. Mice regularly develop septicemia with oral inoculation of Salmonella isolates, often without exhibiting gross and microscopic changes in the alimentary tract.

The nontyphoidal or enteric form of salmonellosis, characterized by self-limiting enterocolitis or diarrhea in the absence of systemic disease, is a major cause of morbidity and mortality in calves and often over one billion human cases per year. While both are incriminated in cattle, Salmonella enterica serovar Typhimurium infects all species and is the most common isolate in people, while S. enterica serovar Dublin is more specific to cattle. The similarity in enteric disease manifestation between cattle and humans and has led to their use as an experimental model for human infections. Characteristic histopathology of nontyphoidal salmonellosis is the massive influx of neutrophils, which is also suggested as the...
major triggering event of gastrointestinal necrosis and diarrhea. Vasculitis and thrombosis is also prominent, and in severe cases can lead to rectal strictures in pigs who have poor collateral circulation. Fibrinous cholecystitis is pathognomonic for acute enteric salmonellosis in calves. Chronic enteric salmonellosis is characterized by discrete foci of necrosis and ulceration called “button ulcers” and seen most often in pigs but also cattle and horses.

The colonies of coccobacilli and large numbers of rods found in some areas of the slide are both gram-positive, leading conference participants to conclude these are postmortem bacterial overgrowth not related to the Salmonella infection in this case.

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References
**CASE II: 08-4562 (JPC 3103756).**

**Signalment:** 3-4 year-old ewes.

**History:** Chronic weight loss.

**Gross Pathology:** None.

**Laboratory Results:** None.

**Histopathologic Description:** The mucosa and submucosa is diffusely expanded and crypts are widely separated by extensive infiltration of epithelioid macrophages, a few multinucleated giant cells, plasma cells and lymphocytes. The macrophages are distended with cytoplasm containing fine, granular basophilic material which was demonstrated as acid fast bacilli (mycobacteria) by Ziehl Nielson stain. There is villous blunting and fusion. There are multifocal areas of necrosis in the mucosa. The submucosa contains dilated lymphatics. There is mild edema of serosa with small numbers of lymphocytes and plasma cell infiltration. In the associated mesentery there are multifocal areas of adipocyte necrosis and mineralization. The mesenteric blood vessels are infiltrated and surrounded by moderate numbers of lymphocytes and plasma cells. There is marked perivascular fibrosis.

**Contributor’s Morphological Diagnoses:** Small intestine: Enteritis, granulomatous, diffuse, severe, chronic with villous atrophy, lymphangectasia and macrophages containing acid fast bacilli seen with Ziehl-Nielson stain.

Mesentery: Fat necrosis and mineralization, multifocal with lymphoplasmacytic perivasculitis.

**Contributor’s Comment:** Johne’s disease (paratuberculosis) is a chronic intestinal disease of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis*. This disease is responsible for extensive economic losses worldwide related to fatality and loss of productivity. In small ruminants, Johne’s disease is characterized by chronic wasting, decreased milk production and is rarely associated with diarrhea.\(^2\) Gross thickening of bowel is not as remarkable as in cattle. Caseating granulomas (often mineralized), generally not seen in cattle, are commonly present in the intestine, lymphatics and lymph nodes of small ruminants.\(^2\) Atrophy of fat due to malabsorption is a common finding in Johne’s disease. However, in this case there is fat necrosis and mineralization. Widespread or focal necrosis of abdominal fat is frequently found in sheep and the pathogenesis is unknown.\(^2\)

The route of disease transmission is fecal-oral route. After ingestion, the mycobacterium undergoes endocytosis by M cells of the dome epithelium over lymphoid follicles. It is proposed that integrin receptors on the apical surface of M cells bind fibronectin-opsonized bacteria, facilitating phagocytosis by these cells.\(^7\) From M cells, the mycobacterium is transported in vacuoles to macrophages in subepithelial and intraepithelial areas of Peyer’s patches and lamina propria.\(^1,2\) After uptake by macrophages, the bacteria resist the degradative and killing
mechanisms of the macrophage via sulphatide production, which prevents phagosome-lysosome fusion, escape from the phagosome into the cytoplasm, glycolipid-mediated inhibition of nitric oxide production, and inhibition of the respiratory burst and oxidative killing mechanisms by superoxide dismutase and glycolipid production.\(^1\)

Ovine Johne’s disease has been noted as a problem in Australia and Europe. The disease in sheep can have three different forms with majority of the animals being asymptomatic. Among the clinically affected sheep, 30% are affected by paucibacillary (tuberculoid) form characterized by large number of T-lymphocytes, fewer eosinophils, macrophages and fewer bacteria in the gut. The rest of 70% have multibacillary (lepromatous) form with high levels of bacteria along with epithelioid macrophages and B lymphocytes infiltration,\(^5-8\) as seen in this case. What determines the outcome of clinical disease is not entirely understood but various factors are implicated including the size of the infective dose, route of infection, mycobacterial strain virulence,\(^11\) local and systemic immune status and age of the host, host resistance genes affecting antigen presentation and intracellular killing, and environmental factors.
In paratuberculosis, cell mediated immunity (CMI) plays a critical role in development of lesions and onset of clinical disease. CMI develops relatively early in infection and, if effective, will lead to clearance of organisms and a resistant state. The paucibacillary form is mediated by Th-1 response where bacterial growth within infected macrophage is controlled by IFN- and TNF-α production. The multibacillary form is mediated by Th-2 response with high production of IL-10 and little IFN-secretion and hence less control of intracellular bacterial growth. It is interesting to note that the asymptomatic sheep are infected with mycobacterium but do not show any clinical or pathologic symptoms. The mechanism of disease resistance in asymptomatic animals is obscure. This differential activation of immune response to mycobacterium is speculated to be due to innate receptor engagement and signaling. Recently it is shown that different forms of sheep paratuberculosis have differential expression of pattern recognition receptors, including Toll-like receptors.

**JPC Diagnosis:**
1. Intestine: Enteritis, granulomatous, diffuse, marked, with villar blunting, lymphangitis, and crypt loss.
2. Intestine: Fat necrosis with saponification.

**Conference Comment:** Johne’s disease is problematic for livestock producers, as extended incubation times and average of 40% prevalence in infected herds coupled with intermittent bacterial shedding hinder eradication efforts. The problem is further exacerbated in small ruminant herds where diarrhea is not a typical clinical sign. The disease only develops in older animals (over 19 months) and this complex epizootiology amounts to Johne’s disease being one of the most important of the dairy industry. Fecal culture is still the standard diagnostic test, but its poor reliability has led to investigation of many methods from qPCR of fecal samples to liver biopsies with mixed results, often requiring late stage disease development to establish meaningful sensitivity and specificity.

The contributor discusses the pertinent differences of the cell-mediated immune response in relation to the two forms of disease, and we confirmed the lepromatous form in this case by repeating the acid-fast stains which revealed numerous intrahistiocytic bacteria. Clinical disease occurs due to the abundant granulomatous inflammation, and the lesions are often restricted to the ileum, though the transmural granulomatous infiltration may also be observed in the colon and the organism has been cultured from a variety of organs. Grossly the mesenteric lymph nodes are always enlarged and the classical intestinal change of diffuse mucosal thickening into transverse rugae is usually present in cattle. In sheep and goats, the disease is characterized by chronic wasting and submandibular edema due to hypoproteinemia; the feces is usually normal in consistency and the gross enteric lesions are minimal, often missed at necropsy.

**Contributing Institution:** Kansas State University

**References:**


CASE III: 07-27354 (JPC 3105594).

Signalment: Young adult female caribou, *Rangifer tarandus*.

History: This specimen is one of a number of hunter-killed caribou that were submitted to the Canadian Cooperative Wildlife Health Centre due to concerns about the quality of the meat. In this case, only the head was submitted with the concern being the presence of “pus in the nose”.

Gross Pathologic Findings: The head is from a young adult female caribou. Over the bridge of the nose about 2 cm behind the nasal plenum there is a 3 cm diameter area of thickened skin covered by a crusty surface exudate. The parotid lymph nodes are slightly enlarged. On section of the head, the anterior 4 cm of both nasal cavities contain a large amount of muco-purulent yellow-brown tenacious exudate. The underlying mucosa is roughened and red. The nasal turbinates and pharyngeal mucosa appear normal.

Laboratory Results: N/A.

Histopathologic Description: Haired skin from nose. Segmentally, the epidermis is thickened and composed of or replaced by a variably thick layer of parakeratotic hyperkeratosis, fibrin, necrotic debris, bacterial colonies, pustules, fragmented hair shafts and vacuolated epithelial cells. Full thickness necrosis of the epithelium is present in many areas. Multifocally, throughout the dermis, hypodermis and underlying muscularis there are myriad 200 to 400 µm diameter intracellular protozoal cysts. A thick pale eosinophilic to pale blue capsule surrounds the bradyzoites contained within a parasitophorous vacuole. There is minimal inflammation or tissue reaction around most cysts. Occasionally cysts, particularly within the more superficial layers of the skin, appear to be dead and are infiltrated with macrophages, and multinucleate giant cells with smaller numbers of lymphocytes and plasma cells.

Contributor’s Morphologic Diagnosis: Haired skin: Dermatitis, necrotizing, locally extensive, severe, chronic with myriad intracellular protozoal cysts consistent with *Besnoitia* sp.

Contributor’s Comment: *Besnoitia* are apicomplexan parasites that parasitize numerous species including caribou, cattle, horses, reindeer, lizards, rodents and rabbits. Currently, there are 9 different named species of *Besnoitia*. Whether these are all truly distinct species is unclear as currently the life cycle of only a small number of...
these species has been described and the taxonomy of the rest are based upon the species of the intermediate host.\textsuperscript{2}

\textit{B. tarandi} is the species of \textit{Besnoitia} known to affect caribou and the closely related reindeer. The life cycle of this species is not currently known. The domestic cat is the definitive host for the 3 species with a known life cycle, but the definitive host for the \textit{B. tarandi} is unknown although other \textit{Felid} species such as cougars and lynx may be a possibility.\textsuperscript{2}

An epizootic event of besnoitiosis occurred in a zoo in Manitoba starting in 1983.\textsuperscript{4} The initial cases were diagnosed in 2 caribou (\textit{Rangifer tarandus caribou}) that died of pneumonia. Over the course of 3 years besnoitiosis spread to mule deer (\textit{Odocoileus hemionus hemionus}), reindeer (\textit{Rangifer tarandus tarandus}) and a second isolated group of caribou. Twenty-eight caribou, 10 mule deer and 3 reindeer were euthanized or died as a result of this epizootic. The means of transmission in this case was thought to be biting flies.

In wild woodland caribou, besnoitiosis appears to be a relatively common (23\% infection rate in one study) chronic and mild disease.\textsuperscript{6} Lesions are most often confined to the skin of the extremities causing alopecia with mild skin thickening and crusting. In some cases however, the disease appears to be much more widespread and severe with ulceration of the skin, cysts in the sclera, conjunctiva, nasal mucosa, visceral serosa, tendons, tendon sheaths, hoofs and testes.\textsuperscript{1,4} In this case, cysts were found within the nasal mucosa, sclera, conjunctiva, lymph nodes and meninges as well as the skin.

Although \textit{B. tarandi} is not of zoonotic concern and meat from affected animals should be safe to eat, the relatively common prevalence, poorly understood natural history of the disease and the potential for transmission to other wildlife species makes besnoitiosis a disease of potential emerging concern for translocation of reindeer and caribou from arctic regions to more southern ones.

\textbf{JPC Diagnosis:} 1. Haired skin: Protozoal cysts, numerous. 2. Haired skin: Hyperplasia and hyperkeratosis, epidermal, diffuse, severe, with segmental epidermal necrosis and numerous mixed bacterial colonies.

\textbf{Conference Comment:} Most slides contain two sections of skin in this case which are variably affected with epidermal necrosis, parakeratotic hyperkeratosis and epidermal hyperplasia. While the protozoal cysts are diffuse throughout all areas, the most severe areas of hyperkeratosis also contain numerous aggregates of mixed bacteria
including many with long filaments of parallel rows of coccoid bodies resembling railroad tracks. These are consistent with *Dermatophilus congolensis* and conference participants deliberated about the level of contribution the two infectious organisms played in lesion development. The parakeratosis and epidermal hyperplasia is quite characteristic of *D. congolensis* infections; however, histopathology from *Besnoitia* spp. infections is variable and nonspecific.5

*Besnoitia* spp. have a two-host life cycle with disease occurring in the definitive host.3 The disease can be divided into acute, subacute, and chronic stages. The acute stage is characterized by tachyzoite proliferation within vascular endothelial cells. Bradyzoites proliferate during the subacute and chronic stages within mesenchymal cells resulting in the cyst formation such as observed in this case.5

Recently immunohistochemistry and other special stains were used to characterize the tissue cyst layers of *Besnoitia besnoiti* in cattle. Tissue cysts were found in multiple organs including the corium of the claw where they contributed to chronic laminitis. The cysts are composed of host cell wall with enlarged nuclei containing a parasitophorous vacuole with bradyzoites. Additionally, an inner and outer cyst wall were distinguished, and in chronic stages, extracystic zoites were observed.5

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**References:**
**CASE IV:** P678-12 (JPC 4032916).

**Signalment:** 2-year-old, female Scottish Highland heifer, *Bos Taurus.*

**History:** This heifer had a history of mild respiratory difficulty since birth. In the last month, multiple cutaneous nodules developed on the head, thorax and hind limbs, with pus draining from a few. This animal was the only one affected on the farm.

**Gross Pathology:** The animal was in poor body condition. There was diffuse facial swelling and deformity. On cut section, there were numerous encapsulated and fistulating abscesses, up to 5 cm in diameter, in the subcutaneous tissues and muscles of the maxillary and mandibular regions, and, minimally, the tongue; the pus was thick, yellowish, and no "sulfur granules" were detected. Similar abscesses were also present in: 1) the walls of the pharynx and proximal esophagus, 2) the cervical, preacapular, inguinal and tracheobronchial lymph nodes, 3) the subcutaneous tissues of the thorax and both hind limbs, and 4) the lungs. Multiple, often coalescing ulcers, 4 mm in diameter, were observed on the gingiva, tongue, soft palate and, to a lesser degree, esophagus.

**Laboratory Results:** The heifer tested negative for bovine viral diarrhea pestivirus by fluorescent antibody testing (FAT) on the oral and esophageal mucosa. Bacterial culture of abscesses in skin, lymph nodes and lung yielded a heavy growth of *Actinobacillus lignieresii*, in pure culture except in one lymph node in which *Trueperella pyogenes* was also identified.

**Histopathologic Description:** The submitted slide is a section of pharyngeal/cranial esophageal wall stained with hematoxylin-eosin-phloxin-saffron. The normal architecture is extensively obliterated by irregularly sized collections of degenerate neutrophils with variable numbers of surrounding and/or admixed macrophages (pyogranulomas), and dense fibrovascular (granulation) tissue infiltrated mainly by lymphocytes and plasma cells between pyogranulomas. Within these pyogranulomas, there are numerous structures composed of pale amorphophilic, finely granular material (bacteria) surrounded by radiating, deeply eosinophilic, club-shaped material (Splendore-Hoeppli phenomenon). This material is multifocally and variably mineralized, with occasional associated multinucleated giant cells. A Gram stain showed the bacteria to be Gram-negative coccobicilli.

**Contributor’s Morphologic Diagnosis:** Severe, multifocal chronic pyogranulomatous pharyngitis/esophagitis with Splendore-Hoppli material and intralesional Gram-negative coccobicilli.

**Contributor’s Comment:** The final diagnosis was actinobacillosis. Actinobacillosis is mainly a disease of soft tissues of cattle and sheep, but it has also been reported in other species including...
pigs, goats, horses and dogs. *Actinobacillus lignieresii* is an opportunist Gram-negative coccobacillary bacterium that is part of the normal flora of the oral cavity and rumen of cattle and sheep. Following trauma to the oral mucosa (e.g. plant material, teeth, etc.) or skin, *A. lignieresii* invades the underlying soft tissues and typically causes pyogranulomatous lesions, often with lymphogenous extension to regional lymph nodes (and thus lymphangitis and lymphadenitis).\(^1\)\(^2\)\(^3\) Iatrogenic cases have been described, e.g. surgical wound contamination during cesarean sections.\(^1\)\(^3\) *Actinobacillus lignieresii* is also a cause of bite wound infections in humans, especially horse bites.\(^3\)\(^4\) Grossly, the lesions appear as variably-sized abscesses with abundant fibrous tissue, in which small granules, known as "sulfur granules", are usually seen. Microscopically, these lesions correspond to pyogranulomas, with abundant inflamed fibrous/fibrovascular tissue, centered on masses of coccobacilli surrounded by radiating club-shaped eosinophilic material (Splendore-Hoeppli phenomenon), and grossly seen as the granules.\(^2\)\(^3\) Splendore-Hoeppli material can be seen in some bacterial, fungal and parasitic infections, but also in some non-infectious conditions; it is considered to be made up of immune complexes,\(^2\) among others. Other bacteria that can cause similar microscopic lesions in cattle include *Staphylococcus aureus and Pseudomonas aeruginosa* (botryomycosis), and also *Actinomyces bovis*; the latter causes mainly a mandibular osteomyelitis, known as "lumpy jaw", but can sometimes involve soft tissues.\(^1\)\(^2\)\(^7\)

In cattle, the most frequent presentation is a chronic glossitis known as "wooden tongue", due to the very firm consistency imparted by the abundant fibrous tissue.\(^1\)\(^2\) The disease is usually sporadic, but small outbreaks have been reported.\(^1\)\(^2\)\(^3\) Other reported locations include the skin of the head, neck and limbs, forestomachs, lungs, pharynx, esophagus and lungs.\(^1\)\(^2\)\(^3\) Clinical signs depend on lesion size and distribution; subclinical lesions, especially in the tongue and lymph nodes of the head and neck, may be found as incidental findings at slaughter.\(^3\) Intravenous sodium iodine is the treatment of choice, but successful surgical exeresis of localized lesions has been described.\(^1\)\(^7\) In the present case, the tongue was minimally involved and no sulfur granules were observed; an underlying cause was not found. Dystrophic mineralization was prominent, especially in "older" lesions. The multiple ulcerations in the oral and proximal esophageal mucosae were considered to be the result of *A. lignieresii* infection, as they were associated with underlying pyogranulomatous lesions.
JPC Diagnosis: Fibrovascular tissue: Cellulitis, pyogranulomatous, diffuse, severe, with Splendore-Hoeppli phenomenon, and numerous bacterial colonies.

Conference Comment: This is a classic case with challenging tissue identification as most sections lack discernible anatomic landmarks; however, the Splendore-Hoeppli phenomenon and club colonies leave few differentials for this lesion in the ox. Wooden tongue may be most aptly confused with lumpy jaw, the other classic large colony forming bacterial entity of the bovine oral cavity. Both Actinobacillus lignieresii and Actinomyces bovis form club colonies, but the colonies in actinomycosis are much larger with smaller and less discrete clubs. The two can be readily distinguished by the invasion of bone in lumpy jaw or by gram stain as only Actinomyces is gram positive.

As the contributor nicely explains, the bacteria is considered normal flora thus most cases of wooden tongue are sporadic with prevalence at slaughter of up to 3.6%. Interestingly, herd outbreaks have occurred with up to 73% morbidity and are likely associated with abrasive feedstuffs and crowded conditions.

Contributing Institution: http://www.medvet.umontreal.ca

References: