Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2022-2023

Conference #7 5 October 2022

CASE I:

Signalment:
A 17-day-old, male, Japanese Black, bovine

History:
A Japanese black male calf was born at full-term. The calf weighted 30 kg and received colostrum. The calf presented with fever (40 ℃), cough, nasal discharge, poor suckling, wobbler, and bilateral ocular cloudiness on day 4 of life, after which he developed astasia. On day 5 of life, the calf became recumbent and presented with dizziness and nystagmus. The calf was treated with medications such as antibiotics, non-steroid anti-inflammatory drugs, and vitamin B1, but he died on day 17 of life.

Gross Pathology:
Multiple white nodules with dark red edges and a diameter of 1 - 3 cm were found scattered in the peritoneum, including the spleen, stomach, and diaphragm. Many white nodules were also observed in the liver serosa and parenchyma. The surface of the forestomach mucosa was covered with a gray-yellowish caseous substance. Multiple erosions and ulcers were observed in the forestomach and the abomasum. The abomasum mucosa was extensively dark red. The calf had an increased volume of the cerebrospinal fluid, which was muddy; thymic hypoplasia; and persistent urachus.

Laboratory Results:
Bacteriological examination: Yeast-like fungi were isolated from the samples collected from the liver, kidney, lung, brain, and cerebrospinal fluid, which were identified as *Candida albicans* (99.12%) on sequence analysis of the ITS region.

Virological examination: Bovine adenovirus (BAdV) type 4 was detected on the PCR for BAdV and sequence analysis of DNA extracted from paraffin-embedded sections of the liver, reticulum, and ileocecal colon.

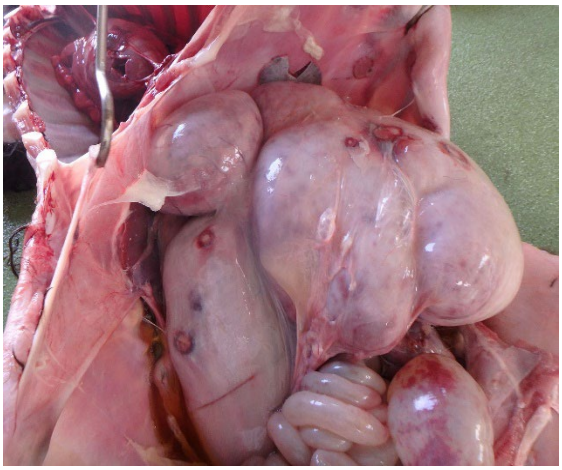

Figure 1-1. Abdominal viscera, ox. Numerous 1-3mm white nodules with a hemorrhagic border are scattered along the serosa of the gastrointestinal tract. (Photo courtesy of: National Institute of Animal Health, National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5Kannondai, Tsukuba, Ibaraki 3050856, Japan, (WSC ID95), http://www.naro.affrc.go.jp/english/niah/index.html)

Microscopic Description:
Multiple extensive necroses were observed from the mucosal epithelium to the submucosal tissue of the abomasum. In the necrotic area, typical structures were replaced by hemorrhage, acidophilic cell debris, and mostly necrotic infiltrated neutrophils. Numerous thrombi were observed in small-to-medium blood vessels from the lamina propria to the submucosa. Numerous fungal hyphae were found in the necrotic lesions. Presence of fungi was also found in thrombi and on the walls of blood vessels. The submucosa was highly edematous, with numerous neutrophil infiltrations, mild macrophage infiltrations, and presence of fibrin. Grocott's staining and PAS reaction revealed the presence of multiple species of fungi in the lesion. On the mucosal surface, a large number of yeasts and pseudohyphae were found mixed with inflammatory cells and cell debris. A large number of hyphae were found from the necrotic area to the submucosa. They were 5-8 μm wide, non-parallel, thin walled, and irregularly branched and had no septum. Such hyphae had also infiltrated the thrombus and walls of blood vessels. In some areas, these hyphae were mixed with pseudohyphae, which invaded the blood vessels. In the submucosa adjacent to these areas, a large number of hyphae were focally observed in the blood vessels, blood vessel walls, and surrounding tissues. They had slightly thicker walls; were 4-5 μm wide, parallel, and sharply branched; and contained septum. Immunohistochemically, these fungi reacted positively to anti-*Candida* (Biogenesis, UK), anti-*Rhizomucor* (Clone Mab-WSSA-RA-1, DAKO, USA), and anti-*Aspergillus* (Clone Mab-WF-AF-1, DAKO, USA) antibodies, respectively.

Furthermore, amphoteric-to-basophilic Cowdry A-type or full-type intranuclear inclusion bodies were observed in endothelial cells of most blood vessels. Electron microscopy of vascular endothelial cells revealed adenovirus-like particles with a diameter of approximately 80 nm, consistent with intranuclear inclusion bodies.

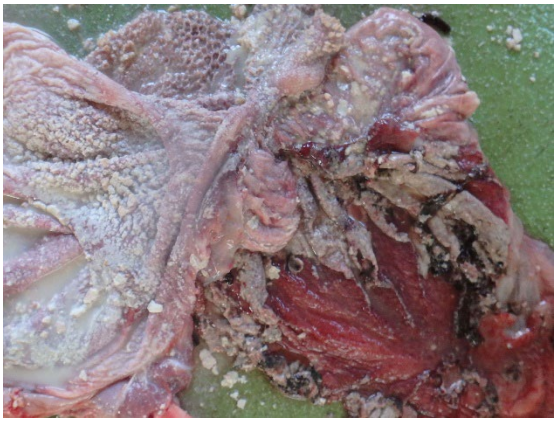

Figure 1-2. Abomasum, ox. The mucosa of the abomasum is partially covered by a fibrinonecrotic membrane. (Photo courtesy of: National Institute of Animal Health, National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5Kannondai, Tsukuba, Ibaraki 3050856, Japan, (WSC ID95), http://www.naro.affrc.go.jp/english/niah/index.html)

In the forestomach, severe hyperkeratosis or parakeratosis, mild-to-moderate erosion of the epithelium, and intoraepithelial accumulation of neutrophils (microabscesses) were observed. A large number of anti-*Candida* antibody-positive yeasts and pseudohyphae were observed in these lesions. In addition, numerous thrombi and extensive necrosis of the surrounding tissues were also observed in the rumen submucosa. In the necrotic tissue and blood vessels, anti-*Aspergillus* antibody-positive hyphae were observed. Anti-*Aspergillus* antibody-positive hyphae were also observed in the serosa. In other regions, a large number of anti-*Rhizomucor* antibody-positive hyphae in the vascular wall and necrotic foci of the lamina propria were observed. There were numerous viral intranuclear inclusion bodies in the vascular endothelium of the forestomach. In the ileum, swelling of vascular endothelial cells, presence of

thrombi with numerous intranuclear inclusions scattered from the lamina propria to the submucosa, and necrosis with hemorrhage were widely observed.

Many focal necroses or granulomas with mild hemorrhage were scattered in the brain and the spinal cord. A large number of anti-*Candida* antibody-positive pseudohyphae were observed in these lesions. Mild mononuclear cell infiltration was observed in the meninges, and one mycotic granulomatosis was observed in the choroid plexus. Intranuclear inclusion bodies were rarely observed in vascular endothelial cells.

Focal extensive necrosis with thrombosis was observed in the liver. Many hyphae that reacted against the anti-*Aspergillus* antibody were observed in thrombi and vascular wall. Furthermore, numerous focal necroses with anti-*Candida* antibody-positive pseudohyphae were randomly scattered. In addition, a number of intranuclear inclusion bodies were found in the endothelium or hepatocytes. A few anti-*Candida* antibody-positive pseudohyphae were observed around small vessels in the kidney, heart, and lung.

In the thymus, there was severe depletion of lymphocytes in both the cortex and medulla.

Contributor's Morphologic Diagnoses:
Abomasum: abomasitis, necrotizing, severe with vasculitis, vascular thrombosis, numerous multiple species of fungi, and viral intranuclear inclusion bodies

Etiology: *Candida albicans*, *Aspergillus* spp., Mucoraceae, and BAdV type 4

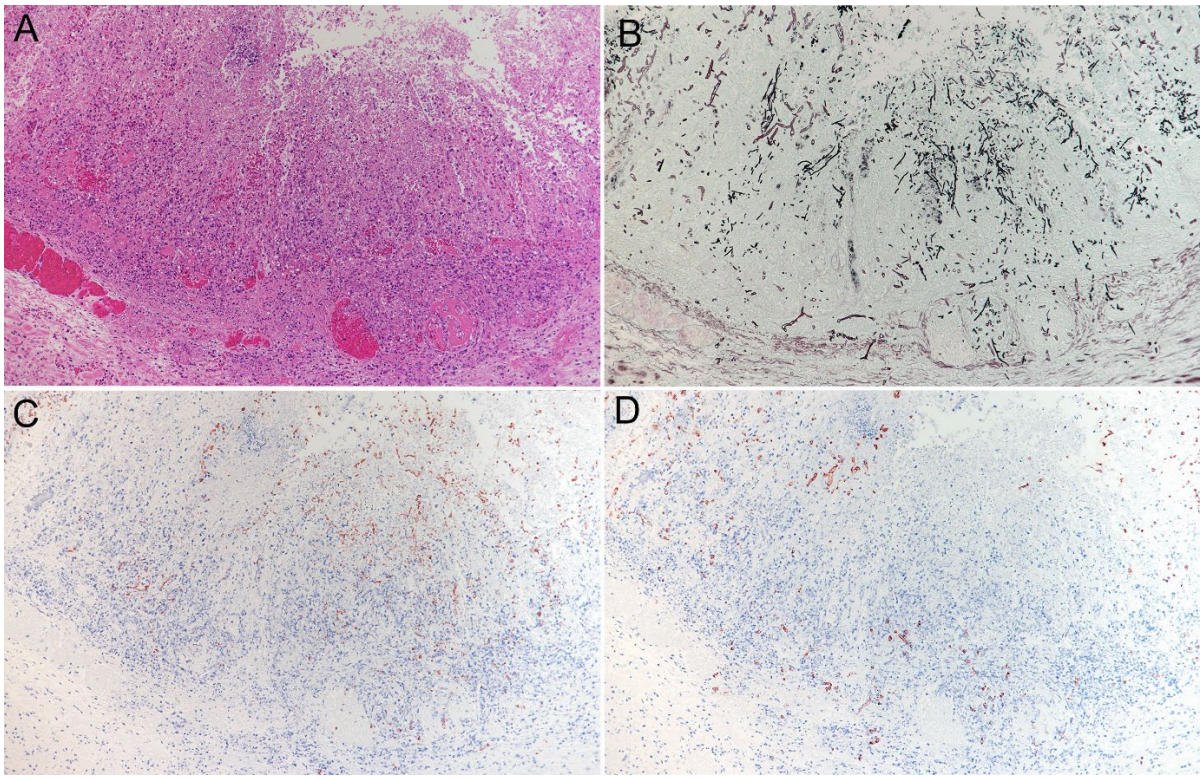

Figure 1-3. Abomasum, ox. A) There are multifocal areas of full-thickness necrosis of the abomasal mucosa. B) A Grocott methenamine silver stain highlights multiple fungal morphologies within the areas of necrosis. C) Some of the fungal hyphae are immunopositive for antibodies directed against Candida sp. D) Some of the fungal hyphae are immunopositive for antibodies directed at Rhizomucor. (Photo courtesy of: National Institute of Animal Health, National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5Kannondai, Tsukuba, Ibaraki 3050856, Japan, (WSC ID95), http://www.naro.affrc.go.jp/english/niah/index.html)

Contributor's Comment:
In this case, fungal lesions caused by *C. albicans* were systemically disseminated in many organs. Co-infection with *Aspergillus* spp. and Mucoraceae was observed in fungal lesions of the gastrointestinal tract and liver. Systemic BAdV type 4 infection was also observed.

Deep-seated mycosis is a fungal infection that forms lesions in the internal organs or subcutaneous tissue. In cattle, mastitis, endometritis, ulcerative gastroenteritis, pneumonia, and abortion have been reported.[16] Deep-seated mycosis occurs in animals with weakened immunity, and the main causative fungi are *Candida* spp., *Aspergillu*s spp., and Mucoraceae,[2,4,7,15,16] either alone[1,5,6,12] or in combination[2,3,14]. In cattle with deep-seated mycosis, co-infection with two types of fungi[3,13,14] and mixed infection with fungi and protozoa have been reported.[13] Many cases of systemically disseminated mycosis in cattle caused by Mucoraceae, resulting in infection of central nervous system tissues, have been reported.[1,4-6,12,13] In contrast, a few cases of central nervous system lesions caused by *Aspergillus* spp.,[3] without the presence of *Candida* spp. have been reported. In the present case, co-infection with three species of fungi was observed. *C. albicans* was identified as the causative fungi for lesions systemically disseminated to multiple organs, especially meningoencephalomyelitis.

A large number of *Aspergillus* spp. hyphae were observed in the liver, rumen, and abomasum, and a large number of Mucoraceae hyphae were observed in the rumen and abomasum. Both fungi were associated with thrombosis, vasculitis, and extensive necrotic lesions. Numerous yeasts and pseudohyphae of *C. albicans* were observed in the hyperkeratotic and parakeratotic mucosal epithelium and superficial lamina propria lesions of the forestomach, and they were not mixed with other fungi. However, in the abomasum, pseudohyphae of *C. albicans* were observed in the necrotic foci mixed with Mucoraceae hyphae along with invasion into the deep mucosa or blood vessels. These findings suggest that *C. albicans* was disseminated systemically through necrosis and vascular lesions caused by Mucoraceae in the abomasum.

On the other hand, viral intranuclear inclusion bodies were observed in vascular endothelial cells throughout the body, such as in the gastrointestinal tract, liver, and brain. Electron microscopy and sequence analysis of DNA extracted from FFPE samples showed that the inclusion bodies were BAdV type 4. BAdV disease is caused by BAdV belonging to the Mastadenovirus genus or the Atadenovirus genus of the Adenoviridae family.[18] It is mainly characterized by respiratory and digestive diseases. BAdVs are commonly isolated from the feces of healthy cattle,[11] which are rarely onset by BAdV alone. There are 10 serotypes of BAdV, and BAdV type 7 is highly pathogenic, [18] causing severe hemorrhagic diarrhea and calf frailty syndrome. Only a few cases of BAdV disease have been reported in Japan.[8,9] BAdV type 4 was identified only in one of these case via bovine testis cell culture.[8] Gastric erosion, ulcers, and hemorrhagic colitis have been reported in previous cases of BAdV infection.[17,18] In the present case, mucosal necrosis without fungal infection was observed in the ileum. It was suggested that BAdV type 4 injured blood vessels and induced gastrointestinal lesions. Furthermore, it was considered that *C. albicans* entered the bloodstream and invaded the parenchyma of the brain and liver through the vascular walls systemically damaged by the virus, causing inflammation or necrosis.

In addition, thymic hypoplasia, which was observed in the present case, may have contributed to these systemic infections.

Many cases of zygomycosis are angioinvasive and disseminated systemically.[1,5,6] However, no systemic dissemination of Mucoraceae was observed in the present case, probably because the calf developed fatal meningoencephalomyelitis due to *C. albicans* before the dissemination of Mucoracease.

Contributing Institution:
National Institute of Animal Health, National Institute of Animal Health, National Agriculture and Food Research Organization (NARO)
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http://www.naro.affrc.go.jp/english/niah/index.html

JPC Diagnosis:
1. Abomasum: Abomasitis, necrotizing, multifocal to coalescing, marked, with vasculitis, thrombosis, and numerous mucosal and submucosal fungal pseudohyphae, hyphae, and yeasts.
2. Abomasum, vessels: Vasculitis, necrotizing, multifocal, moderate with endothelial intranuclear viral inclusions.

JPC Comment:
The contributor has provided an excellent example of an endotheliotropic virus causing vascular damage, creating a permissive environment for the growth and invasion of multiple opportunistic fungal organisms, and setting the stage for systemic spread of *Candida albicans*. A remarkably versatile fungus, *C. albicans* has evolved numerous mechanisms of pathogenicity and adaptability that enable it to spread and cause disease in a wide range of host tissues.

C. albicans has three main morphologies: yeast, hypha, and pseudohypha. The yeast form exists at a lower pH and generally higher density than the hyphal form and is associated with fungal dissemination, whereas the hyphal form tends to invade tissue. A number of adhesins, including agglutinin-like sequence (Als) 3 and hyphal wall protein (Hwp) 1, facilitate adherence to tissue and formation of antibiotic and immunoresistant biofilms. *C. albicans* has two methods of invading cells. Surface integrins (including Als3) can interact with host ligands (such as E-cadherin) to stimulate endocytosis; or it can actively penetrate the cell through methods not fully elucidated.[10]

In order to survive in a variety of tissues with varied nutrient availability, *C. albicans* has developed metabolic adaptability. Glycolysis is its major source of energy in tissues rich in glucose (blood) or glycogen (liver), but in phagocytic cells such as macrophages, the fungus subsists on lipids and amino acids using gluconeogenesis and, later, the glyoxylate cycle. In other tissues, the fungus secretes proteases and subsists on liberated amino acids. [10]

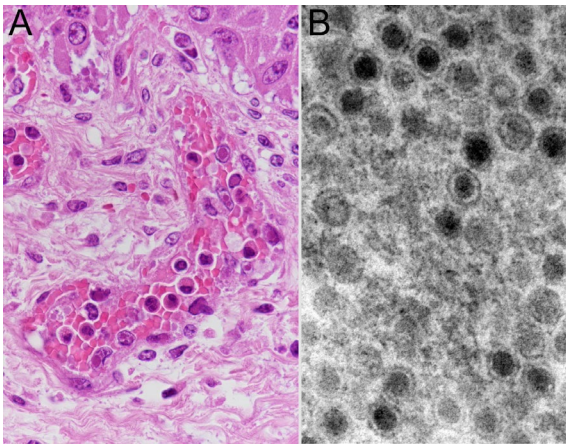

Figure 1-4. Abomasum, ox. A) Endothelial cells occasionally are swollen and contain a karyomegalic eosinophilic intranuclear viral inclusion with varying degrees of halo formation. B) Transmission electron microscopy of the affected nuclei demonstrates 80nm adenovirus-like particles. (Photo courtesy of: National Institute of Animal Health, National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5Kannondai, Tsukuba, Ibaraki 3050856, Japan, (WSC ID95), http://www.naro.affrc.go.jp/english/niah/index.html)

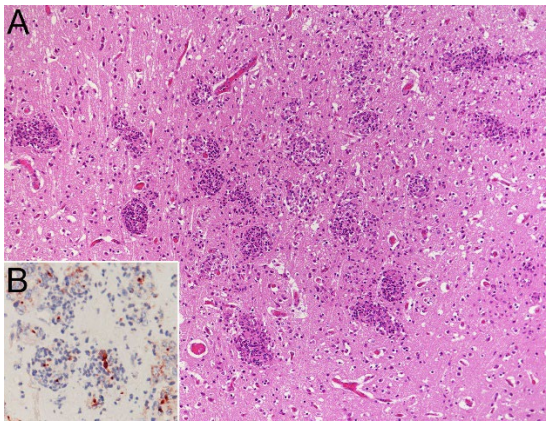

Figure 1-5. Brain, ox. A) The brain contained numerous foci of granulomatous inflammation. B) Within these foci, a large number of anti-Candida antibody-positive pseudohyphae are present. (Photo courtesy of: National Institute of Animal Health, National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5Kannondai, Tsukuba, Ibaraki 3050856, Japan, (WSC ID95), http://www.naro.affrc.go.jp/english/niah/index.html)

Interestingly, amino acid absorption indicates a less permissive environment, and, in response, the yeast will actively secret ammonia to stimulate its own pH receptors and induce hyphal formation. The pH is detected using surface receptors Dfg16 and Rim21 which act through Rim101 transcription factor to mediate various cellular responses. In addition to switching morphologies, the fungus can respond to change in pH by altering expression of cell wall beta glycosidases Phr1 and Phr2 to maintain virulence in neutral-alkaline and acidic pHs, respectively.[10]

C. albicans has evolved a few mechanisms to obtain iron, another key nutrient for survival. While it does not produce its own siderophores, *C. albicans* absorbs iron from the siderophores of other microbes using the transporter Sit1. Alternatively, it can use Als3 to acquire iron from host ferritin and transferrin, or it may acquire iron from hemoglobin and heme using a variety of proteins.[10]

The moderator, Dr. Francisco (Paco) Uzal, discussed differentials for this case and highlighted the fact that bovine adenovirus 1 can cause identical clinical signs and intranuclear inclusion bodies as BAdV-4. In general, however, gastrointestinal disease caused by adenoviruses in cattle is rare.

References:

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CASE II:

Signalment:
6-month old, male (intact), pygmy goat *Capra aegagrus hircus*

History:
The goat was euthanized following several days of diarrhea and a 24-hour period of "mental inappropriateness." No additional history was provided.

Gross Pathology:
The liver was enlarged, with 1 mm, soft, white, foci widely disseminated throughout capsular and cut surfaces. A focal, 1 cm in diameter ulcer was present in the abomasum near the pylorus, with adhered margins and subadjacent, firm, pale tan thickening of the wall (fibrosis).

Laboratory Results:
FA negative for rabies
FA positive, brain and liver, for *Listeria monocytogenes*
Aerobic culture, very light growth unidentified Gram-negative bacterium, enrichment broth culture negative for *L. monocytogenes*

Microscopic Description:
Hepatocytes are uniformly small, forming thin cords accentuated by moderate sinusoidal congestion. The parenchyma contains

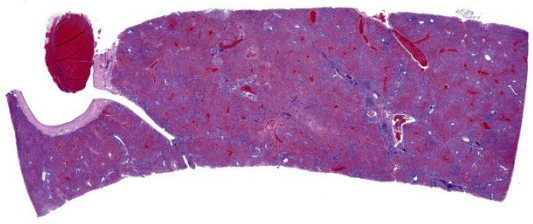

Figure 2-1. Liver, goat. One section of liver is submitted for examination. At subgross magnification, portal areas are outlined by biliary hyperplasia and a cellular infiltrate which occasionally fills bile ducts. There is diffuse edema of the connective tissue surrounding the gallbladder. (HE, 9X)

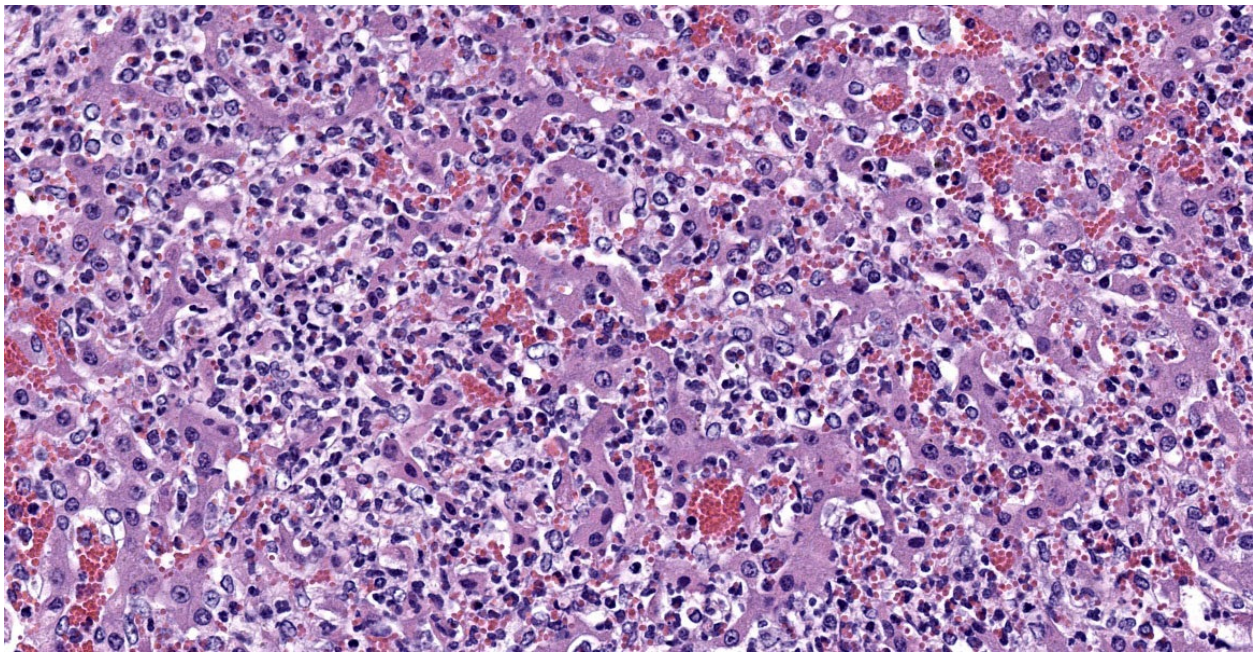

Figure 2-2. Liver, goat. Foci of lytic necrosis are scattered through the section. These areas contain large numbers of neutrophils which efface hepatocytes and fill adjacent sinusoids. (HE, 381X)

multifocal to coalescing, irregular, well-demarcated, variably sized, randomly distributed foci of hepatocellular degeneration and necrosis infiltrated by moderate to large numbers of predominantly neutrophils. There is widespread expansion of periportal areas by mixtures of edema, fibroplasia, proliferating bile ducts, and mixed inflammatory infiltrates predominated by lymphocytes and plasma cells, with smaller numbers of neutrophils. Biliary hyperplasia frequently links adjacent portal areas. Primarily larger bile ducts are ectatic, with lumens distended by large numbers of neutrophils, necrotic cellular debris and occasional unsporulated coccidial oocysts. The epithelium of affected bile ducts is variably attenuated, to degenerate or necrotic. Many cells have vacuolated cytoplasm that contains small coccidial meronts with merozoites, macrogametes, microgametes and developing oocysts. The lumens of some bile ducts and sinusoids contain microcolonies of small bacterial rods.

Contributor's Morphologic Diagnoses:
Liver: Necrosuppurative hepatitis, acute, multifocal to coalescing, severe, with severe suppurative cholangitis, lymphoplasmacytic pericholangitis, marked biliary hyperplasia and multiple coccidial stages

Contributor's Comment:
Gross and microscopic findings are consistent with listeriosis, a global disease of humans and other animals caused by the opportunistic, Gram-positive, intracellular bacterium, *Listeria monocytogenes*. Infection was confirmed by positive fluorescent antibody staining of the brain and liver. The environmentally resistant bacterium is widely distributed in soil, vegetation and in animals.[1] Large numbers are present in ruminant feces and it is frequently isolated from normal tissues.[2] In humans, transmission usually occurs through the consumption of contaminated food, rarely from infected animals to humans, between humans, and in utero. In foods, it survives processing technologies relying on acidic or salty conditions and can multiply at low temperatures.[1] Encephalitis in ruminants

is often associated with heavy silage feeding.[2]

Cell mediated immune responses are related to intracellular replication and have been recently reviewed, as have strategies used by the bacterium to exploit host molecular mechanisms, including translocation from cell to cell. [3,4,5] In mammalian hosts, three disease syndromes occur in relation to the bacteria's ability to cross intestinal, feto-placental and blood brain barriers. It can also survive in bile and induce biliary tract infection.[6] Infection of the pregnant uterus results in abortion and frequently lethal neonatal disease. Encephalitis usually occurs in adults and is typified by microabscess formation in the brainstem. Ascending infection of the trigeminal nerve follows trauma to the oral mucosa. Septicemia, with coagulative necrosis and abscess formation occurs mainly in the livers of neonates and young juveniles following disruption of the intestinal mucosal barrier and translocation of bacteria to the submucosa and vasculature. Focal infections can include the conjunctiva, skin, mammary glands, heart, arteries, spleen, lymph nodes, joints, bone, and fascia.[1,2]

In addition to its biliary presence, severe intestinal coccidiosis, a common cause of diarrhea in confined young goats, may have compromised the intestinal mucosa, providing a portal of entry for *L. monocytogenes* into the portal circulation. Similar changes, including hepatic necrosis, biliary hyperplasia, periportal fibrosis and lymphocytic inflammation have been reported in association with hepatic coccidiosis, caused by an *Eimeria* sp., in a goat.[7]

Contributing Institution:
www.vet.uga.edu/VPP

JPC Diagnosis:
Liver: Cholangiohepatitis, suppurative and lymphoplasmacytic, chronic, diffuse, marked, with biliary hyperplasia and numerous apicomplexan meronts, gamonts, and oocysts.

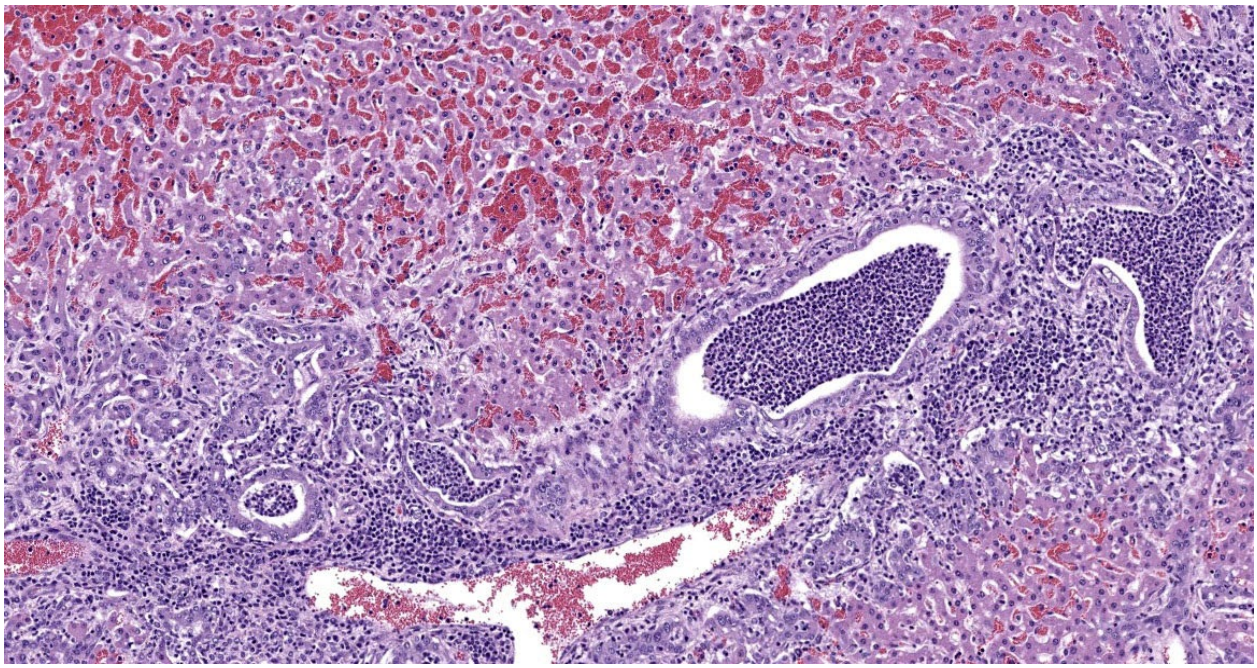

Figure 2-3. Liver, goat. Within portal areas, the lumen of bile ducts are filled with numerous viable and degenerate neutrophils and cellular debris but lining epithelium is intact. There are numerous lymphocytes and plasma cells surrounding ducts. Profound biliary hyperplasia extends well beyond the limiting plate. (HE, 130X)

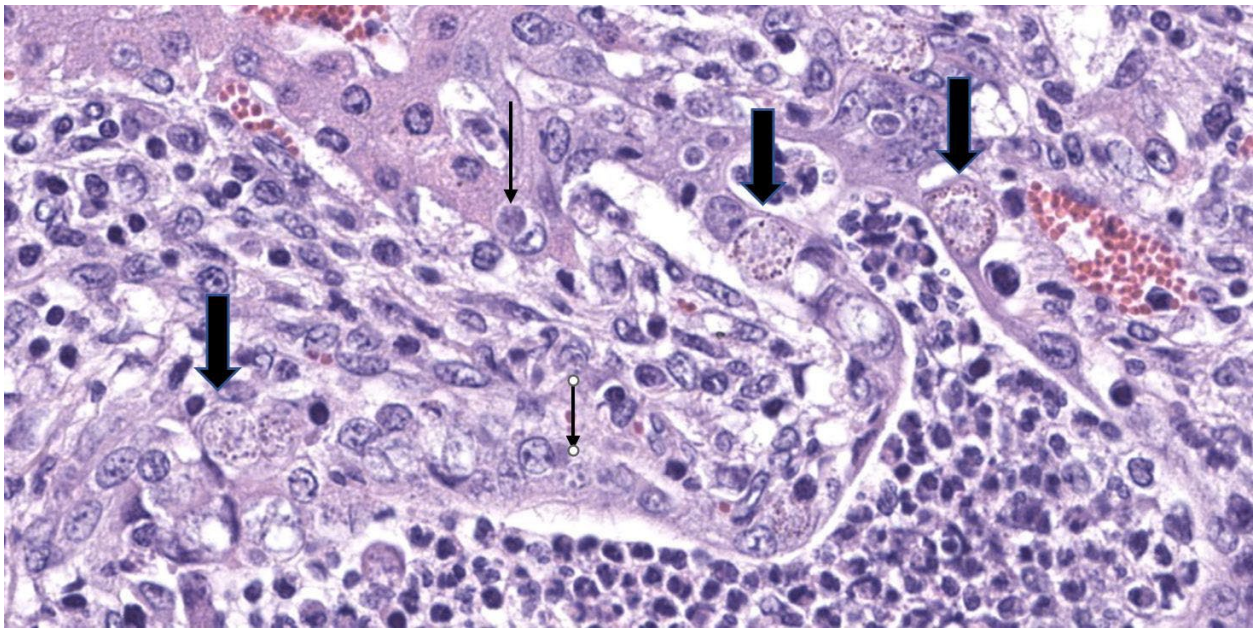

Figure 2-4. Liver, goat. Coccidial meronts, schizonts (small arrows) and gamonts (large arrows) are present within he biliary epithelium. (HE, 623X)

JPC Comment:

Listeria monocytogenes was first described 101 years ago in a human patient and in animal species a few years later. There are 13 serovars, with three serovars (1/2a, 1/2b, 4b) being the most commonly isolated in clinical disease. As the contributor mentions, the bacterium is ubiquitous in the environment, but pathogenic serotypes have frequently been isolated from wild animals, suggesting a possible wild reservoir.[2]

At physiologic temperatures, *L. monocytogenes* actively expresses positive regulatory factor A (PrfA), allowing it to switch from an environmental to an infective lifestyle. A number of virulence factors are then expressed, including internalin A and B (InlA and InlB). These bind to non-phagocytic cell membrane receptors such as E-cadherin and induce receptor-mediated endocytosis. Alternatively, *L. monocytogenes* can be directly phagocytosed by phagocytic cells. Within the cytoplasmic vacuole, listeriolysin O, phospholipase A, and phospholipase B create membrane pores which allow the bacteria to escape in the cytoplasm and begin replicating. Recent evidence has also shown that the bacteria can actually remain and replicate slowly within the vacuoles. Once in the cytosol, the actin assembly-inducing protein causes the cytoskeleton to rearrange, forming actin comet-tails which propel the bacteria around the cytoplasm or into an adjacent cell.[2]

While ocular, cutaneous, and many rhombencephalic infections are initiated via traumatic inoculation, the method with which *L. monocytogenes* establishes enteric infection in ruminants is still somewhat a mystery. In mice, InlA, InlB, and *Listeria* adhesion protein (LAP) enable the bacteria to translocate the mucosa with minimal host reaction. Ruminants are frequently asymptomatically infected, but in clinically affected animals bacterial infection of myocytes leads to neutrophilic inflammation focused on the muscularis mucosa. In subsequent bacteremia, *L. monocytogenes* spreads to the spleen and liver, causing random hepatocellular necrosis, pyogranulomas, and periportal inflammation. Bacteremic spread can also spread to the placenta, causing fetoplacental infection, or to the udder, causing mastitis (though masti-

tis may also occur through direct inoculation). [2] The moderator and conference participants discussed the possibility that this case originated as a fetoplacental infection in which the fetus survived.

In monogastrics, blood-borne bacteria (either extracellular or in leukocytes) can cross the blood-brain barrier, causing meningitis or meningoencephalitis. In ruminants, the bacteria follows a different route of infection, crossing oral mucosa or skin, traveling up the axons of the cranial nerves, and establishing infection in the brainstem, causing rhombencephalitis.[2] For additional information on the neurologic manifestation of *L. monocytogenes* in ruminants, we recommend reviewing WSC 21-22, Conference 12, Case 2, which featured listerial rhombencephalitis in a lamb.

The moderator and conference participants considered the distribution of hepatocellular necrosis in this case and agreed the majority of necrosis is affecting the periportal region, thus included under the umbrella of suppurative cholangiohepatitis.

References:

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CASE III:

Signalment:
67-day old, male, Holstein, bovine (*Bos taurus*)

History:
The calf was transferred to a farm for fattening at 7-days old. The calf did not receive any vaccinations and developed respiratory signs, including coughing; the condition worsened despite antibacterial treatment with oxytetracycline. The calf was euthanized at the age of 67-day old due to anorexia and respiratory distress.

Gross Pathology:
At necropsy, the liver was swollen and faded in color, with multiple, micro-yellow-white foci on the cut surface. The lungs showed hepatization in the anterior and middle lobes and regression failure in the posterior lobes.

Multiple petechiae and white foci were detected in the renal cortex. The spleen was congested and swollen. The growth of rumen papillae was poor, and the contents were muddy with an acidic odor. The pulmonary hilum, hepatic hilum, and mesenteric lymph nodes were edematous and swollen.

Laboratory Results:
Bacterial colonies morphologically and biochemically consistent with *Salmonella enterica* were isolated from the liver, spleen, kidney, lung, pulmonary hilar, and mesenteric lymph nodes by direct culture, and formed the whole blood, rumen, and cecal contents in an enriched culture. The isolates were identified as *S. enterica* subsp. *enterica* by 16S rDNA sequencing and serotyped as *Salmonella* Dublin (serotype O9: g, p :-). The isolates contained the *invA* and *spvC* genes. No virological examination was performed.

Microscopic Description:
Severe multifocal hepatic necrosis and paratyphoid nodules were observed and characterized by histiocytic, lymphocytic, and neutrophil infiltration. Infiltration of lymphocytes and plasma cells was also detected in Glisson's capsule. A hyaline thrombus was observed in the central vein. In the kidney, severe multifocal pyogranulomatous interstitial nephritis was detected with hemorrhage. Similar lesions were found in the spleen and lung interstitium, as well as in the submucosa of the rumen, ileum, and cecum. Immunostaining for anti-*Salmonella* O9 rabbit serum showed positivity in the cytoplasm of macrophages in the hepatic lesions.

Contributor's Morphologic Diagnoses:
Liver: hepatitis, multifocal, necrotizing, histiocytic, lymphocytic and neutrophilic, paratyphoid nodules, *Salmonella enterica* subsp. *enterica* Dublin, *Bos taurus*, bovine

Contributor's Comment:
Salmonellosis is a zoonotic, enteric, or multisystemic disease, distributed worldwide.[1, 3, 6,7,9-11] This infection causes huge economic losses to the food animal industry. *Salmonella* spp. are rod-shaped, gram-negative, facultatively anaerobic bacteria belonging to the *Enterobacteriaceae* family.[4] The genus *Salmonella* includes more than 2,500 serotypes within two species: *S. enterica* (more than 2,400 serotypes) and *Salmonella bongori* (20 serotypes).[4,10] *S. enterica* is a major pathogen that can infect numerous animal species, in addition to humans.10

Some *Salmonella* serotypes have particular host predilections. *S.* Dublin, *Salmonella* Choleraesuis, and *Salmonella* Gallinarum preferentially infect cattle, pigs, and chickens, respectively.[4,9,10] *Salmonella* Typhi and *Salmonella* Paratyphi infect only humans, causing typhoid fever. In contrast, *Salmonella* Typhimurium and *Salmonella* Enteritidis can infect a wide range of host species.

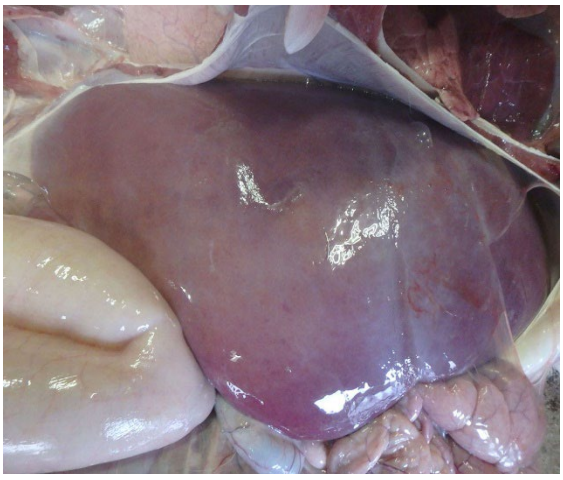

Figure 3-1. Liver, calf. There is diffuse hepatomegaly with rounded edges. (Photo courtesy of: National Institute of Animal Health, National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5Kannondai, Tsukuba, Ibaraki 3050856, Japan, (WSC ID95), http://www.naro.affrc.go.jp/english/niah/index.html)

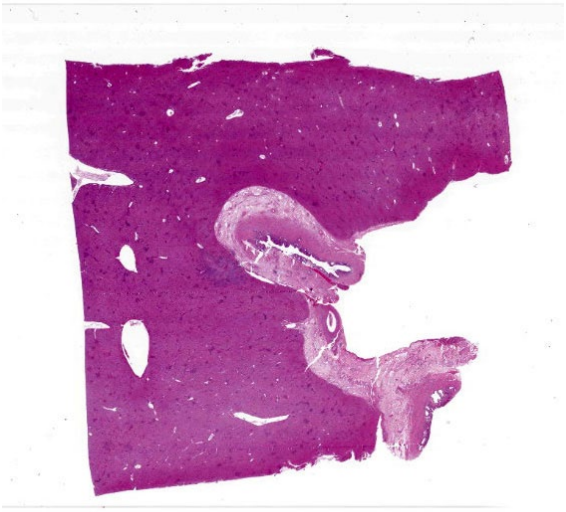

Figure 3-2. Liver, calf. One section of liver and gallbladder is submitted for examination. At subgross magnification, there is moderate edema of the connective tissue surrounding the gallbladder. (HE, 8X)

Bovine salmonellosis is caused predominantly by *S.* Dublin and *S.* Typhimurium;[1,4] however, other serotypes are also capable of causing infection in cattle.[1,4] In adult cattle, *S.* Dublin infection is common, and can be asymptomatic or characterized by abortion in pregnant cows. *S.* Dublin infection is also associated with fever, reduced milk production, and mild-to-moderate diarrhea. The affected animals shed *S.* Dublin intermittently, leading to sporadic or repeated outbreaks of disease in the herd.[10] In calves, *S.* Dublin is associated with systemic infections, which can result in meningoencephalitis, polyarthritis, hepatitis, cholecystitis, pneumonia, splenitis, and lymphadenitis occasionally in the absence of diarrhea.[4,10,11] Recently, pyelonephritis, urocystitis, ureteritis, and gangrene of the distal extremities have also been reported in calves.[3,7,12]

Conversely, *S.* Typhimurium causes enteritis and marked acute exudative diarrhea in young calves less than 2 months of age.[4] Fever, anorexia, prominent diarrhea, and dehydration, which are secondary to acute necrotizing enteritis, are found in bovine *S.* Typhimurium infections. Disease severity and lethality were inversely proportional to the age of the affected calves. The feces are often watery, with variable amounts of mucus, fragments of the intestinal mucosa, or blood clots. Abortion is uncommon in *S.* Typhimurium infections.[10]

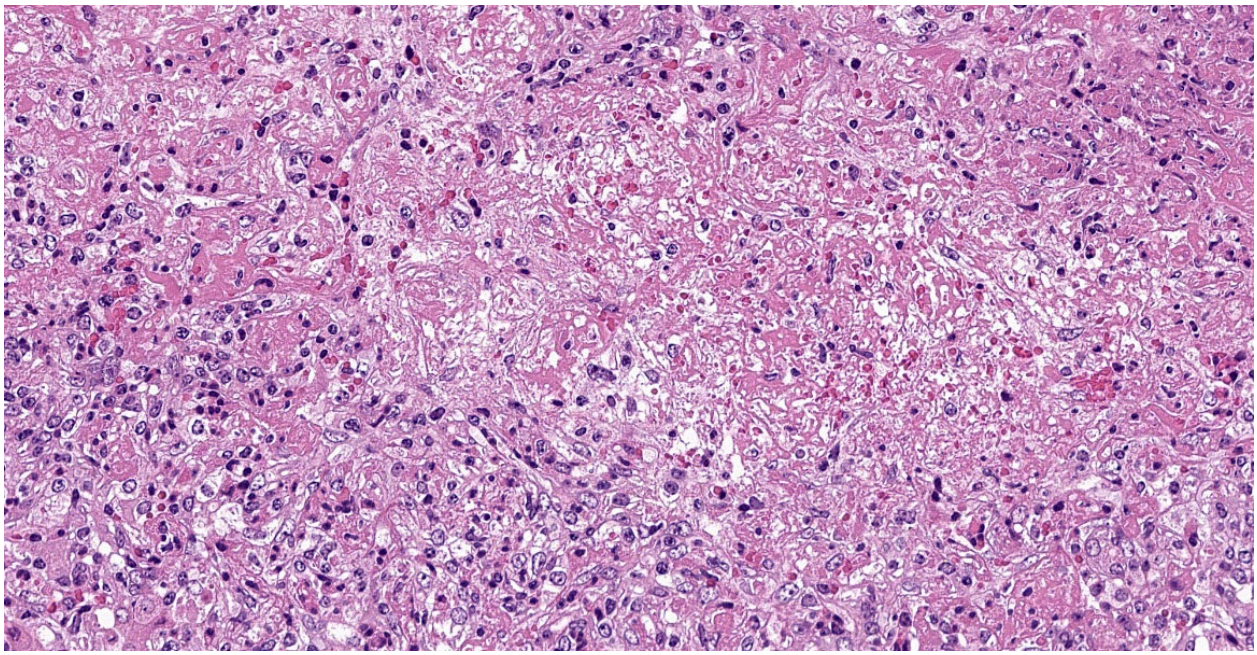

Figure 3-3. Liver, calf. In proximity to the gallbladder, there is a large focus of hepatocellular necrosis and loss with stromal collapse. There is infiltration of macrophages peripherally and fewer lymphocytes. (HE, 367X)

The diagnosis of septicemic salmonellosis is based on clinical and pathological findings and confirmed through microbiological culture and identification of *S.* Dublin by the polymerase chain reaction technique.[6] *S.* Dublin was identified by culture, 16S rDNA sequencing and serotyping. On immunohistochemical examination, *Salmonella* O9 antigen was detected in the lesions. The pathological and bacterial findings in this case were consistent with septicemic salmonellosis.[11]

Oral transmission can occur via the feces, contaminated food, water, or milk/colostrum. The emergence of multidrug-resistant strains is beginning to limit treatment options.[1,5,15]

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JPC Diagnosis:
1. Liver: Hepatitis, necrotizing, multifocal, random, marked (paratyphoid nodules), with vasculitis and thrombosis.
2. Bile duct, adventitia: Edema, diffuse, moderate.

JPC Comment:
It is hypothesized that there were three major steps in the evolution of *Salmonella enterica* subsp. *enterica* from its common ancestor with *Escherichia coli.*[1] The first step involved acquisition of *Salmonella* pathogenicity island (SPI-1), which is present in all *Salmonella* species and encodes virulence factors important in establishing an intestinal infection. [1] The second step involved acquisition of *Salmonella* pathogenicity island 2 (SPI-2), which is present in *S. enterica* but lacking in *S. bongori.* [1] The last jump involved expansion of the host range from ectothermic vertebrates to bird and mammals, and the bacteria may have developed the ability to survive within macrophages as a mechanism to overcome the robust gastrointestinal defense mechanisms in this new host range.[1]

As the contributor describes, different serotypes of *Salmonella enterica* subsp. *enterica* have different host ranges and spectra of disease. *Unrestricted* serotypes, including Typhimurium and Enteritidis, tend to cause enteritis in young animals in a wide range of species. *Host-adapted* serotypes, such as Dublin and Cholerasuis, can cause either enteritis or systemic disease in their specific species.[14] *Host-restricted* serotypes, including Typhi, Gallinarum, and Abortisuis, tend to cause systemic infection with minimal to no enteritis in their specific species.[14]

Table 3-1. Examples of host adapted and restricted *Salmonella enterica* subsp. *enterica* serotypes. Adapted from Uzzau et al, 2000.

	Serotype	Natural host	Other hosts (rare)
Host-adapted	Cholerasuis	Swine	Human
Host-adapted	Dublin	Bovine	Human, ovine
Host-restricted	Typhi	Human	-
Host-restricted	Paratyphi A, C	Human	-
Host-restricted	Sendai	Human	-
Host-restricted	Abortusovis	Ovine	-
Host-restricted	Gallinarum	Poultry	-
Host-restricted	Typhisuis	Swine	-
Host-restricted	Abortisequi	Equine	-

Typically, bacteria which are highly adapted to a host have evolved to cause a minimal to tolerable level of disease, allowing for host survival and spread of bacterial infection.[1] *S. enterica* is unique in that high adaptation corresponds to higher virulence within the host, which mathematic models suggest has favored the development of the carrier state.[1] The success of this strategy is evidenced by the proverbial disease carrier, Typhoid Mary, who harbored *S.* Typhi in a gallstone and spread the infection to numerous individuals while working as a cook in the early 1900s.[8]

Salmonella possesses flagella which enable motility and uses fimbriae to adhere to the mucosal epithelial cells.[13] A type III secretion system allows injection of effector proteins into the cytoplasm. [13] The bacteria is engulfed into a vacuole via receptor-mediated endocytosis or by entering through the intercellular junction complex. [13] LPS on the cell wall resists host defense mechanisms (such as opsonization) and stimulates prostaglandin synthesis. In cases of enteritis, diarrhea occurs as a result of enterocyte loss, prostaglandin E2-induced hypersecretion, and leakage from damaged mucosa. [13]

In systemic salmonellosis, the bacteria invades and survives within macrophages, producing bacteremia and septicemia.[13] *S.* Dublin is unique in that it can also survive extracellularly and spread free within lymphatic

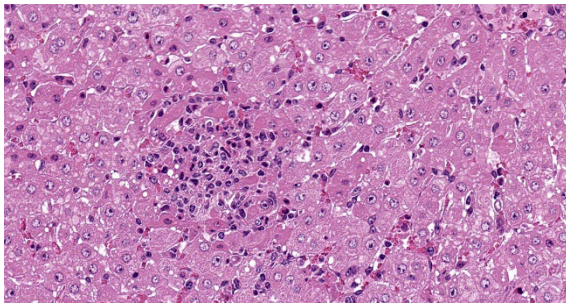

Figure 3-4. Liver, calf. Smaller foci of necrosis are infiltrated by macrophages (paratyphoid nodules). (HE, 459X)

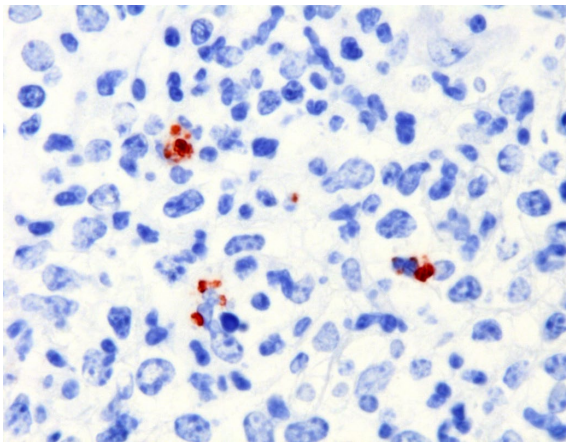

Figure 3-5. Liver, calf. Macrophages within necrotic lesions demonstrate immunopositivity with antisera against Salmonella sp. (anti-Salmonella, 400X) (Photo courtesy of: National Institute of Animal Health, National Institute of Animal Health, National Agriculture and Food Research Organization (NARO), 3-1-5Kannondai, Tsukuba, Ibaraki 3050856, Japan, (WSC ID95), http://www.naro.affrc.go.jp/english/niah/index.html)

fluid.[13] Once in systemic circulation, bacteria are removed by fixed macrophages throughout the body, including in the liver, spleen, kidney, and bone marrow, producing paratyphoid granulomas as seen in this case.[13] In severe cases, septicemia may be fatal. [13]

As the contributor describes, *S.* Dublin causes severe acute disease in calves characterized by endotoxemia with depression, respiratory distress, and death. In a recent review of 57 infected Holstein calves less than 6 months of age, the most consistent lesions were neutrophilic infiltrates in the alveolar capillaries and septa and necrosuppurative and histiocytic hepatitis with paratyphoid granulomas.[11] In approximately half of the cases, neutrophilic infiltrates were observed in the lymph nodes and marginal zone of the spleen.[11] Key features which differentiated *S.* Dublin from systemic *E. coli* infection were paratyphoid nodules in the liver and age, with *E. coli* endotoxemia primarily affecting calves less than two weeks of age and *S.* Dublin affecting older calves.[11]

The moderator provided a general rule of thumb for two gross necropsy findings highly suggestive of *S.* Dublin infection in calves: icterus and gall bladder edema with mucosal pseudomembrane formation.

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tional antimicrobial resistance monitoring system between 1996 and 2016. *Front Microbiol.* 2019; **10**:985.

CASE IV:

Signalment:
4 day-old, female, Belgian blue x Holstein, Bos Taurus, cattle/bovine.

History:
This calf was from a 100 head dairy farm with a few weeks history of diarrhea in neonatal calves; diarrheal episodes were also observed in a few older calves and heifers. As the diarrhea resolved within a few days with no or only supportive treatment, the cause was not thoroughly investigated, although coprological examinations revealed *Cryptosporidium*. However, as four diarrheic calves had died in the previous week, the veterinarian submitted an untreated dead calf to our diagnostic laboratory. The same herd had experienced *Salmonella Dublin*-associated disease 5 years before.

Gross Pathology:
The calf was submitted partially frozen. Body condition was assessed as good. The perineal region was covered with abundant feces. There was marked bilateral enophtalmos and the subcutaneous tissues were "sticky", consistent with dehydration. The thymus was moderately atrophied. Intestinal contents in the distal half of the small intestine, the cecum and spiral colon were not abundant, but they were liquid, pinkish to brownish with small clumps of fibrin-like material. The intestinal mucosa was hard to assess due to freezing/thawing, but there was no evidence of hyperemia, necrosis or fibrinous pseudomembranes. There was some milk in the rumen ("ruminal drinking"), but contents and mucosa of the gastric compartments were otherwise normal.

Laboratory Results:
Bacteriology: ileum and colon: ++++ non-hemolytic *E. coli* that were eae+, but F5-, STa-, Stx1- and Stx2- (3/3 colonies tested → EPEC). Negative for *Salmonella*. Virology: PCR negative for BCoV (coronavirus), type A rotavirus and BVDV. Parasitology: only a few *Cryptosporidium* oocysts were detected.

Microscopic Description:
This is a section of ileum; there is variability among slides (either one of two sections) and moderate post-mortem changes, mostly desquamation. Multifocally, short bacilli are adherent to the villous enterocytes' brush border, sometimes intimately with basophilia (bacteria) and palisading, and/or a scalloped epithelial surface. A Gram stain showed the bacilli to be Gram-negative (slide not provided). Similar lesions were present in the middle and distal jejunum and rarely in the colon. Associated changes vary from a few scattered luminal neutrophils to a luminal mass of fibrin, neutrophils and debris; in the latter case, there are a few capillaries with fibrin thrombi in the lamina propria. There is moderate lymphoid depletion in Peyer's patches.

Contributor's Morphologic Diagnoses:
Ileum, villous epithelium: intimately adherent bacilli with attaching and effacing morphology and minimal enteritis (consistent with EPEC infection)

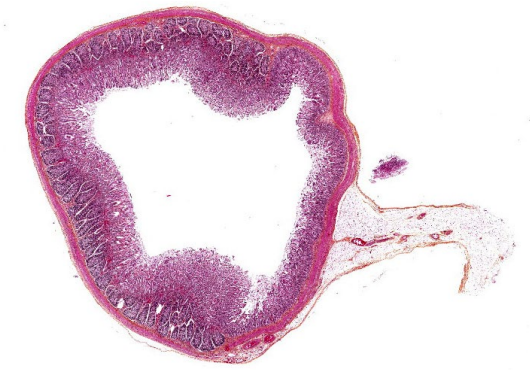

Figure 4-1. Ileum, calf. A single section of ileum is submitted for examination. (HE, 5X)

Contributor's Comment:
Based on the typical attaching and effacing intestinal lesions, the bacteriological results, and the absence of another cause, the diarrhea in this case was attributed to EPEC (*enteropathogenic E. coli*) infection, which is unusual as although EPEC infection is not infrequent in calves, it is rarely diagnosed as the main or sole cause of diarrhea. Although not conspicuous in examined sections, it was assumed that microulcerations were present and responsible for the fibrinosuppurative exudate seen grossly and in one of the sections. The Gram stain was done to be sure the bacteria were not enteroadherent cocci (e.g. *Enterococcus durans*), a rare cause of diarrhea in calves and piglets.

In humans, diarrheagenic *E. coli* (DEC) can be classified into six major classes, or pathotypes: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E.coli* (EPEC), enterohemorrhagic E. coli (EHEC), enteroinvasive E.coli (EIEC), enteroaggregative E. coli (EAEC) and diffusely adherent *E. coli* (DAEC).[1,4,9] Another pathotype, adherent invasive *E.coli* (AIEC), has been proposed to be involved in inflammatory bowel disease (Crohn's disease; ulcerative colitis).[1,7] Of these pathotypes, only ETEC is a significant cause of diarrhea in domestic animals, especially in piglets and calves,[9,11] except in rabbits in which EPEC is a major cause of diarrhea;[14] EPECs in humans primarily involves children, especially infants, in developing countries.[4] Domestic animals, especially ruminants, are mostly reservoirs for EHECs, but EHECs can occasionally cause diarrhea in some species. The other pathotypes (EIEC, EAEC and DAEC) have not to our knowledge been reported in natural disease in domestic animals and will not be discussed further. ETECs, EPECs and EHECs are defined by virulence factors, have histopathologic characteristics (type and localization), and are associated with typical clinical signs; these categories

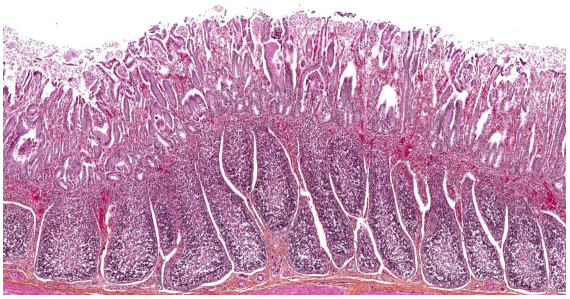

Figure 4-2. Ileum, calf. There is mild autolysis of this section, hampering evaluation of villar length. There is mild depletion of the underlying Peyer's patch. (HE, 38X)

are not mutually exclusive and some strains overlap pathotypes.[4]

ETECs are able to colonize the small intestinal villous enterocytes through one or more fimbriae and produce one or more enterotoxin that cause secretory diarrhea, which is watery and profuse.[1,11] On light microscopy, bacilli are seen close to the apical portion of the villous enterocytes and are typically not associated with significant morphologic or inflammatory changes except in piglets in which mostly F4+ strains can occasionally cause a hemorrhagic gastroenteritis, possibly due to endotoxic shock.[2,11]

EPECs and the vast majority of EHECs cause a characteristic lesion known as "attaching and effacing" (AE) and are thus collectively known as attaching and effacing *E. coli* (AEEC).[1, 9,11,12] AEEC lesions have described in humans/primates, rabbits, pigs, small ruminants, dogs, cats and birds (broiler chickens and turkeys).[6,12]This lesion is characterized at the light microscopic level by a colonization that is more intimate than for ETECs and associated with morphologic/degenerative changes in colonized enterocytes; bacteria in well-established lesions can be seen as coccobacilli, which are often more basophilic, in the brush border. Villous and cryptal epithelium in the small and/or large intestine can be involved. Colonized enterocytes eventually appear rounded-up, giving

the epithelium a scalloped surface, and ultimately die and slough; if enterocyte loss is marked, villous atrophy/fusion, erosions and superficial ulcerations may be observed, with secondary inflammatory changes.[1,11,12] Electron microscopic AEEC lesions are characterized by intimate attachment of the bacteria to the enterocytes' apical cytoplasmic membrane, often with a cup-shaped actin "pedestal" in the underlying cytoplasm, and effacement of the microvilli.[12] The genes responsible for the attaching and effacing lesion are located on the "locus of enterocyte attachment" (LEE), a large pathogenicity island; the LEE includes the eae (intimin) and, except in EHECs and "atypical" EPECs, bfp (bundle-forming pili) genes; detection of the eae gene is used for molecular identification of AEEC strains. The genesis of AE lesions essentially goes through four stages: 1) initial non-intimate attachment (bfp in typical EPECs; other fimbriae in atypical EPECs and in EHECs), 2) translocation of bacterial proteins, including Tir (translocated intimin receptor), into the enterocyte's cytosol, 3) intimate attachment mediated by intimin, which binds to Tir, with underlying cytoskeletal reorganization (actin pedestal) and 4) invasion of bacteria into the cytoplasm (free or within vacuoles).[4, 12] Attaching and effacing lesions are not restricted to AEEC. *Citrobacter rodentium* causes transmissible murine colonic hyperplasia in mice (one strain of *Citrobacter rodentium* had previously been called mouse-pathogenic *E.coli*), and *Escherichia albertii* is emerging as a new human diarrheagenic bacterium (including some misidentified as atypical EPECs) and avian pathogen; both produce AE lesions via the LEE genes.[1,8,13]

In contrast to EPECs, EHECs produce shigatoxins from either or both Stx1 and Stx2 families/groups; although EHECs have other potential virulence factors, this is the defining characteristic that separates EHECs from EPECs.[1,4,9] EHECs are included in the shigatoxin-producing *E. coli* pathotype (STEC),[1] and STEC and EHEC are sometimes used interchangeably, which is not correct as some STEC strains are neither AE nor diarrheagenic (e.g. Stx2e-producing in porcine edema disease).[9] Some STEC strains are LEE-negative (and thus not AE), but produce

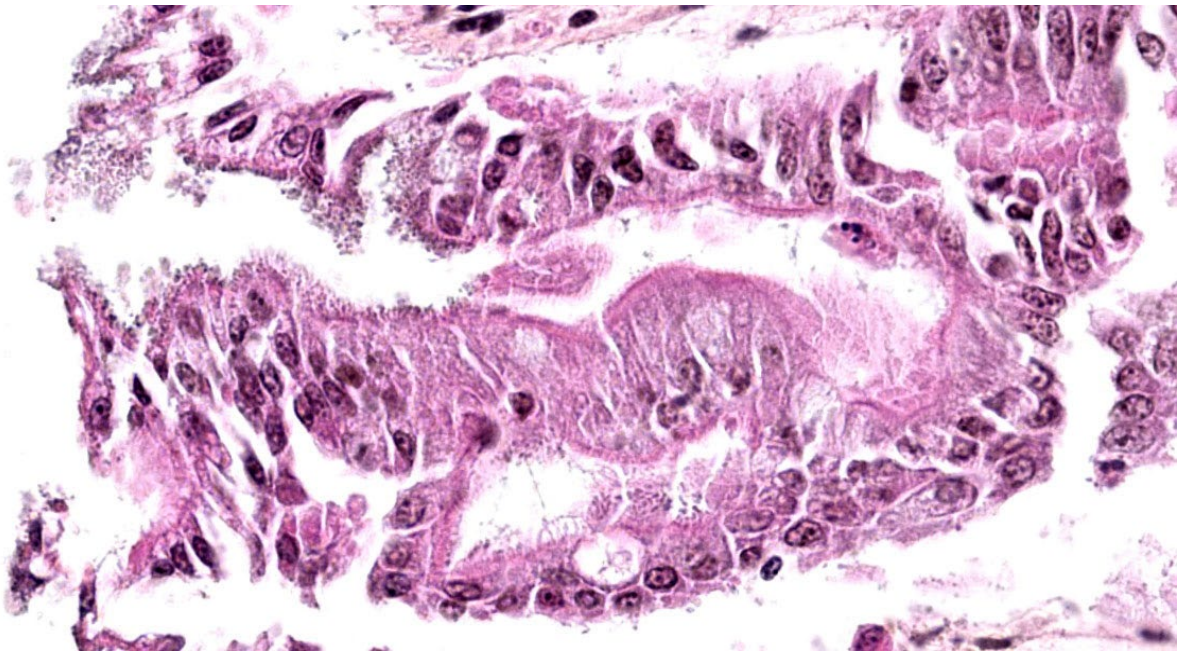

Figure 4-3. Ileum, calf. Numerous short bacilli line the apical surface of enterocytes lining the villar enterocytes.

hemorrhagic colitis and HUS, and are thus considered EHECs.[1,9] Shigatoxins bind and cause damage to enterocytes and vascular endothelial cells (mostly intestinal and renal). Damage to renal endothelial cells is the basis of a thrombotic microangiopathy known as hemolytic uremic syndrome (HUS), which is a potentially lethal complication of EHEC infection in humans;[1] HUS-like lesions have been reported in animals, but were not associated with EHEC-induced hemorrhagic colitis. EPEC-induced diarrhea in humans and other animals varies from mild to marked and may be variably blood-tinged if epithelial damage is extensive. The pathogenesis of EPEC-induced diarrhea does not involve toxin production and the exact mechanism is uncertain. Diarrhea is assumed to be due mainly to maldigestion (loss of brush border enzymes) and malabsorption (loss of absorptive surface), thus osmotic in nature, and dependent on lesion extent; alteration of water and ion channels due to the AE lesion may also be involved.[1,11] EHEC-induced diarrhea in humans varies from mild, with only AE lesions, to severe with hemorrhagic colitis (or ileocolitis) and variably HUS; the best known and studied serotype associated with the latter is O157:H7.[1] In other animals, EHEC-induced diarrhea has only been reported, to our knowledge, in calves and a goat.[9,11,12] In calves, lesions are essentially similar to those in humans, but are generally less severe and not associated with HUS.[9,11,12] This has been ascribed to the lack of Stx receptors in bovine endothelial cells; however, Stx does bind to bovine enterocytes and causes epithelial damage.[9]

The main causes of diarrhea in young calves are *E. coli*, type A rotavirus, bovine coronavirus, *Cryptosporidium parvum* and *Salmonella enterica* subsp. *enterica* (mainly serovars Typhimurium and Dublin).[11] With regards to *E. coli*, the overwhelming majority of cases are due to F5+ Sta+ ETECs and are seen as a profuse watery diarrhea in the first 7-10 days of life.[9,10] EPECs and EHECs have been isolated from feces of diarrheic and normal calves, usually 1-5 weeks of age (i.e. older than for ETECs);[9,11] their importance in calf diarrhea is not clear. In autopsy cases, both EPECs and EHECs have been shown to cause diarrhea/enterocolitis in calves, but are much less frequently involved than ETECs and are often associated with other diarrheagenic agents. Clinically, the disease ranges from mild diarrhea to, mostly with EHECs, dysenteric (variably hemorrhagic). In our laboratory, EPECs is rarely diagnosed as the main or sole cause of diarrhea in calves and piglets, usually in immunocompromised animals.

Contributing Institution:
Faculty of veterinary medicine, Université de Montréal. Website: http://www.med-vet.umontreal.ca

JPC Diagnosis:
Small intestine: Enterocyte degeneration, acute, multifocal, moderate, with villar blunting and numerous attached apical coccobacilli.

JPC Comment:
The contributor provides an outstanding explanation of attaching and effacing pathogens, *E. coli* classifications, and differential diagnoses for this case. At the heart of the attaching and effacing activity is a type 3 secretion system (T3SS) that allows the bacteria to inject virulence proteins (effectors) into the host cell cytoplasm. The T3SS is encoded in the LEE, as described by the contributor, which is highly conserved in all attaching-effacing pathogens.[3] Also known as an injectisome, the T3SS contains a syringe shaped structure with a central channel which accommodates translocation of unfolded proteins, and each EPEC cell is estimated to have

twelve needle complexes.[3] Injection of effectors into the host cytosol enables the bacteria to modify host actin cytoskeleton, interfere with ion transport, and prevent formation of microtubules.[3]

Gram-negative bacteria have six dedicated secretion systems, numbered I through VI, which vary in structure and substances which can be transported. T1SS, found in bacteria such as *Vibrio cholerae* and *Serratia marcescens,* can transport substances across two membranes, the inner and outer bacterial membranes, in one step.[5] Types IV and VI can transfer substances across three membranes: both bacterial membranes and one additional host membrane.[5] T4SS is capable of transporting DNA and proteins, and is found in *Brucella suis, Helicobacter pylori*, and *Neisseria gonorrheae,* among others.[5] T6SS, found in *V. cholerea* and *Pseudomonas aeruginosa,* may transfer effectors into host cells but may also be used to secrete substrates into the environment for bacterial communication and competition.[5]

T2SS and T5SS are isolated to the outer bacterial membrane. T2SS is found in most Gram-negative bacteria and transfers folded proteins from the periplasm to the extracellular space.[5] T5SS is unique in that the substrates are auotransporters, forming their own channels in the outer membrane.[5] This system requires proteins in the periplasm to be unfolded prior to secretion, and most of the substrates are virulence factors such as toxins or receptor-binding proteins.[5] Examples include YadA from *Yersinia enterocolitica* and IcsA of *Shigella flexneri.*[5] From assisting with adhesion to secreting toxic effectors, these secretion systems play important roles in pathogenesis of various gram-negative infections.

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