Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2012-2013

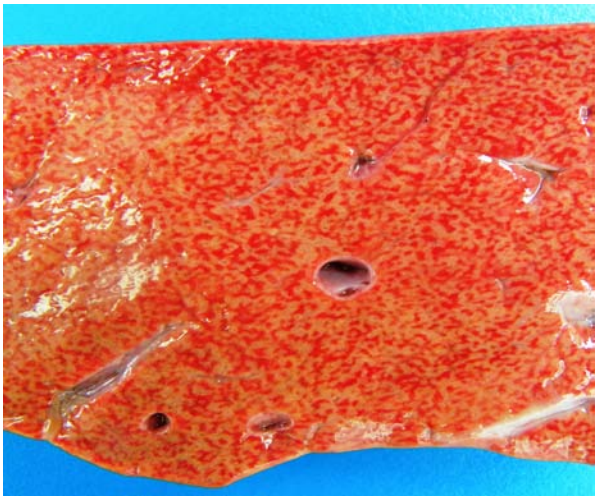
Conference 15

20 February 2013

CASE I: UFSM1 (JPC 4021297).

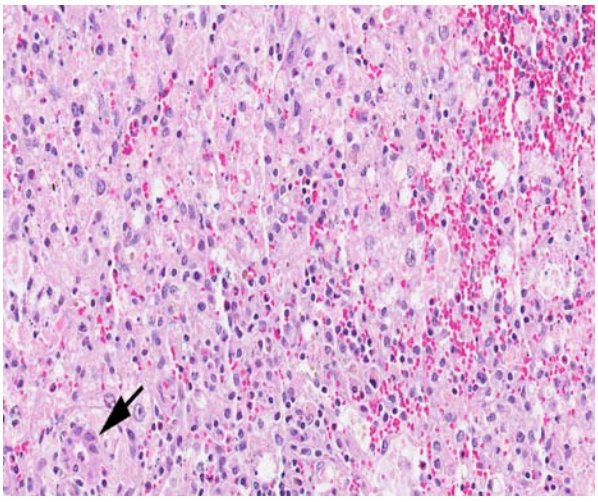
Signalment: 18-month-old, female, mixed breed, bovine (*Bos taurus*).

History: In a farm in southern Brazil there were 200 yearling calves (approximately 18-month-olds) of both sexes. During winter (July-August) 16 of those yearlings died within a one-month period (morbidity rate of 0.8%, lethality rate of 100%). Affected cattle had weakness, muscular tremors, apathy and death within 1-5 days of the onset of clinical signs. Some animals became very agitated and aggressive and those surviving for longer periods had icterus and occasionally photodermatitis. Very large numbers of 2-2.5 cm in length, black insect larvae were found at the pasture.

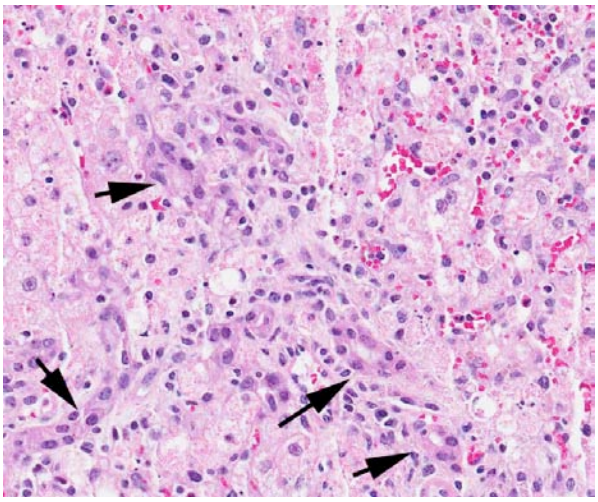


1-1. Liver, ox: The liver was enlarged with rounded edges. The cut surface showed an accentuated lobular patter with -2 mm in diameter bright red depressed irregular areas surrounded by lighter (beige or tan) areas. Photo courtesy of: Departamento de Patologia, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil. http://www.ufsm.br/lpv)

1-2. Very large numbers of 2-2.5 cm in length, black insect larvae were found at the pasture. The larvae formed closely packed masses on the ground and were observed crawling over the grass forming an orderly column of 10-15 cm long. (Photo courtesy of: Departamento de Patologia, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil. http://www.ufsm.br/lpv)



1-3. Liver, ox: Classic massive necrosis, with necrosis of hepatocytes in all regions of the lobule. (HE 360X)



1-4. Liver, o: The few surviving hepatocytes are adjacent to portal triads, in which there is moderate ductular reaction (biliary hyperplasia). (HE 400X)

The larvae formed closely packed masses on the ground and were observed crawling over the grass forming an orderly column of 10-15 cm long. Large numbers of these larvae were collected from this farm and subsequently identified as *Perreyia flavipes* Konow, 1899 (Hymenoptera: Pergidae).

Gross Pathology: One out of the 16 dead calves was necropsied. This calf died within 24 hours after the onset of clinical signs. Gross findings were consistent with an outbreak cause by an hepatotoxin and included ascites, petechiae and suffusion over the serosal surfaces of thoracic and abdominal cavities; the liver was somewhat enlarged (round edges) and mottled with an accentuation of the lobular pattern; this was best seen at cut surface as 1-2 mm in diameter bright red depressed irregular areas surrounded by lighter (beige or tan) areas. (The red areas would later be identified on histological examination as being centrilobular [periacinar]). There was edema of the gall bladder wall. Subendocardial and supepicardial petechiae and ecchymosis were also observed. Sawfly (*P. flavipes*) larval body fragments and heads were found in the rumen.

Note: The larvae collected from the pasture where deaths occurred were ground and orally fed to an unrelated calf of the same age (18-month-old) in one single dose of 20 g/kg/body weight. The calf developed clinical signs similar to the ones described above within 3 days after the administration of the larvae and died after a short period (approximately 24 hours) of the onset of clinical disease. The serum activity of liver enzymes was increased as measured before administration of the larvae and just prior the death of the experimental calf. Results of the two evaluated samples are within parenthesis (first value is from the pre-experimental sample and the second value is from the blood sampled just prior the death of the calf). Aspartate aminotransferase (73-804 U/L); gamma-glutamyl transferase (10-89 U/L); and alkaline phosphatase (54-404 U/L). The levels of total bilirubin were also elevated (3.46 mg/dL) and direct bilirubin elevation (2.98 mg/dL) accounted for this increase. Necropsy and histopathological findings on this experimental calf were similar to the ones observed in the spontaneous outbreak but were included in this submission.

Histopathologic Description: The main microscopic lesion was restricted to the liver and consisted of centrilobular (periacinar) to massive hepatocellular necrosis. In most lobules necrotic areas extended up to the portal triads where only a few viable hepatocytes remained. The lesion appeared diffusely and no preference for any hepatic lobe could be noticed. Hemorrhage occurred in centrilobular areas. Mild to moderate lymphocyte necrosis was seen in the splenic white pulp.

Contributor's Morphologic Diagnosis: Liver, centrilobular to massive hepatocellular necrosis, diffuse, acute, and severe.
Etiologic diagnosis: Toxic hepatopathy.
Etiology: ingestion of sawfly (*Perreyia flavipes*).

Contributor's Comment: Sawfly larval poisoning (SLP) is an acute hepatotoxicity of cattle caused by

the ingestion of larval stage of insects of the suborder *Symphyta*, order *Hymenoptera*, commonly known as "sawfly." SLP has been described in cattle,[2,5,8,10,15,17,19,22] sheep,[5,14,21] and pigs[6]; there is one isolated report of SLP in a dog.[1] It is a remarkable disease since the toxin is present in the larvae of the insects.

In Australia, SLP is caused by the ingestion of the larvae of the sawfly *Lophyrotoma interrupta* (*Pergidae*, *Hymenoptera*), formerly referred also as *Pterigophorus analis*, *interrupts interrupta* and *Platysectra interrupta*.[8,17] The poisoning of cattle by eating *L. interrupta* (commonly referred to as Australian sawfly larva) has been studied for over 70 years, since it was first described in the Maranoa district of southern Queensland in 1911[2,8,17,22] where it reportedly is responsible for an annual loss of 1 million Australian dollars (1981 values).[8] During 1972-1981 in Australia, 37 farms experienced 5,254 deaths during July to September and 1,800 deaths in cattle occurring in just one year were attributed to the intoxication.[4] Cattle were introduced in this area between 1862 and 1866 and the first suspected cases occurred in 1887 but the definite occurrence was only established in 1911.[22] Outbreaks in Australia are confined to districts where there are large forests of the silver leaf ironbark tree (*Eucalyptus melanoplhoia*), the main host for *L. interrupta*[2,8,11,12] and most deaths occurred between July and October and some deaths may occur after the dried-out remains of dead larvae are moistened by rain during summer.[4]

In Denmark, SLP was reported in sheep caused by the ingestion of the larvae of sawfly *Arge pullata* (Argidae), commonly referred as Danish sawfly larva which feed on birch trees (*Betula pendula*). This outbreak of SLP have been reported from the Danish Island of Sjelland where 50 sheep from a flock of 250 died 3 days after they were moved to the area infested by *A. pullata* larvae.[21] At this occasion, the disease was reproduced experimentally in goats that were fed larvae from this outbreak.[21]

The sawfly *Perreyia flavipes* Konow, 1899 (*Pergidae*: *Perreyiinae*) also referred as *Lophyroides flavipes* and *Brachytoma flavipes* have been reported from Argentina, Uruguay and Brazil in cattle,[5,15,19] sheep[5,14] and pigs[6] and the disease was reproduced in cattle, sheep[5] and pigs.[18] *P. flavipes* is commonly referred to as South American sawfly larva.

The economical importance of sawfly (*P. flavipes*) larval poisoning in South America can be evaluated if one considers that within a three-year period (1993-1995) at least 40 outbreaks of this intoxication occurred in Uruguay and that during just one year (1995) cattle losses exceeded 1,000 heads.[5] Mortality rates vary and are reported as 1.6%, 7.0% and 1.38% from one study[5] and 0.8%, 6.2% and 33% from another.[19]

D-amino acid-containing peptides have been found to the toxic principle in each sawfly involved in farm animal poisonings.[7,9,10] The octapeptide lophyrotomin is the major toxin in the larvae of Australian and Danish sawflies and is present in small amounts in the larvae of South American sawfly. The heptadecapeptide pergidin is the main toxin in the South American sawfly while small amounts of pergidin have been found in the other two species of toxic sawfly.[9]

One interesting environmental phenomenon related to sawfly occurred in Florida. The broad-leaf paper bark tree *Melaneuca quinquenervia* was introduced from Australia into Florida, USA, early in the 1900's, and has since then proliferated to such an extent as to be found in all 10 counties of Southern Florida in an area over 200,000 hectares where it causes considerable environmental and economic damage. Therefore the sawfly *Lophyrotoma zonalis* was introduced, again from Australia, as a possible biological control agent for this tree due to its ability to defoliate *M. quinquenervia*. However, this may turn out to be a dangerous practice since, although no cases of spontaneous poisonings in animals attributable to the ingestion of *L. zonalis* larvae have been detected either from Florida or Australia, the toxins lophyrotomin and a mixture of pergidin and val[4]- pergidin were demonstrated in *L. zonalis* larvae[12], making the larvae a possible threat to livestock. Furthermore, the toxic substances in *L. zonalis* larvae proved toxic to mice.[10]

The biological cycle of *P. flavipes* was studied in the laboratory[18] and it was determined that it develops along the whole year. Larvae appear in pasture from March on, when they are bright black and small, 1 mm in length, thus being not promptly observed. From March to August (autumn and winter in the south hemisphere) they measure 17-22 mm in length and are promptly detected. Under normal conditions full growth is reached in the late winter and early spring. They are ingested by cattle during this period. The reason that cattle eat the sawfly larvae is

unknown. It has been suggested that this behavior reflects some form of nutritional deficiency (Roberts 1932), however, this remains unproven. Alternatively it has been suggested that some property of the sawfly may be involved in causing the animals to seek out and eat the larvae.[10] The nature of such attraction has not been determined, but it may also explain the existence of "toxic areas."[10] Larvae of *P. flavipes* feed on decomposing plant material, dry leaves and dried cattle manure. Larvae go through a series of changes until they pupate, when the insect penetrates 3-10 cm into the ground and form a bright black, leathery-like, 1x0.5 cm ovoid cocoon. Within the cocoon the larvae becomes white and remains in this stage from August to January when they emerge as adults. The adult insects are bright black and have a short life span: only 18-36 hours for females and 24-48 hours for males, time sufficient to start a new cycle. Females are 8-10 mm and males are 7.5-10 mm in length.[18]

The SLP is an acute condition in all affected species. The cattle most affected have weakness, muscular tremors, apathy, stupor and death within 2-5 days of the onset of clinical signs.[4,5,8,15] Some animals become very agitated and aggressive[5] and those cattle surviving for longer periods may show hepatogenous photodermatitis.[2,5] Some less affected cattle may recover.[2,5,17]

Necropsy findings include accentuation of the lobular hepatic pattern, edema of the gall bladder and serosal hemorrhages. Fragments of larvae (*P. flavipes*) can be found in the rumen and omasum. The main histological lesion consists of centrilobular to massive liver necrosis and necrosis of lymphoid tissue.[5,15] Reportedly in cattle, necrosis of hepatocytes was most severe in the right lobe.[5] In our experience this is not an unusual finding in acute hepatotoxicosis in cattle, and we think it may be related to differences in the input of blood supply to different regions of the liver.

The acute centrilobular necrosis as seen associated to SLP is not specific of this condition and occurs in association with several other hepatotoxins, mainly phytotoxins, in farm animals.[16] Hepatocytes in the center of the lobule (zone 3) are more vulnerable to a toxic insult as compared with peripheral (zone 1) located hepatocytes because centrilobular hepatocytes have more abundant enzymes which act to transform liposoluble compounds in toxic substances and because centrilobular hepatocytes have lower levels of oxygen and glutathione peroxidase. Hepatocytes in the periphery of the lobule (zone 1) are more vulnerable to toxins of direct action due to the proximity of these periportal hepatocytes to the blood flow that arrives, but the portal vein and hepatic artery branches at the portal triads.[20] Interestingly, when the toxic principles of sawfly were administered to mice treated with lophyrotomin they developed periportal (zone 1) necrosis, and mice treated with pergidin developed centrilobular (zone 3) necrosis.[12] Regarding this aspect it is interesting to notice that a reduction in the sawfly-induced liver pathology associated as a consequence of the concurrent *Fasciola hepatica* infection was experimentally observed in lambs. A possible explanation for this phenomenon is as an inhibitory action of *F. hepatica* on the microsomal oxidative enzymes responsible by the biotransformation of the active principles within the larvae in toxic metabolites.[13] Other hepatotoxins that affect livestock in Brazil are in the included table.

The diagnosis in our case was based primarily in the clinical signs and pathological findings and on some epidemiological aspects. The aspects of the outbreak reported here are remarkably similar to SLP reported in cattle from Australia caused by *L. interrupta*[2,8,17] in sheep from Denmark caused by *Arge Pullata*[21] and in cattle and sheep from Uruguay[5] and cattle,[15,19] sheep,[14] and pigs[6] from Brazil caused by *P. flavipes*. Further evidence include the finding of fragments of the larvae in the fore- stomachs of the necropsied calf and the reproduction of the disease by feeding ground *P. flavipes* larvae collected from the site where the spontaneous outbreak occurred to a susceptible calf.

The neurologic clinical signs presented by cattle in this report affected by SLP are typical of hepatic encephalopathy. Cattle poisoned by the larvae *L. interrupta*[8] and sheep fed *Arge pulllata*[21] also show signs of hepatic encephalopathy, and the fatally poisoned calves presented increased plasma ammonia sufficient to account for the clinical signs.[8] Hepatic encephalopathy is common in ruminants and horses with hepatic failure.[3] Undetermined as yet are the specific metabolites that cause the neurologic dysfunction in hepatic encephalopathy, but increased concentrations of plasma ammonia derived from amines absorbed from the gastrointestinal tract may be responsible.[3] Normally, amines are absorbed from the intestines into the portal blood and metabolized by the liver. The toxic

Table1. Acute hepatotoxicosis in farm animals in Brazil

Hepatoxin	Affected species	Toxic principle	Main lesion	Comments
Plants				
Xanthium spp.	Cattle, sheep, pigs	Carboxyatractylosíde	Periacinar to massive necrosis	Poisoning in swine is associated with hypoglycemia and ascites
Cestrum parqui	Cattle	Carboxyatractylosíde	Periacinar necrosis	
Cestrum corymbossum var. *hirsutum*	Cattle	Not determined	Periacinar necrosis	Serosal hemorrhages, edema of the gall bladder wall. In natural conditions only causes acute poisoning.
Cestrum intermedium	Cattle	Not determined	Periacinar necrosis	Serosal hemorrhages, edema of the gall bladder wall. In natural conditions only causes acute poisoning.
Sessea brasiliensis	Cattle	Not determined	Periacinar necrosis	Serosal hemorrhages, edema of the gall bladder wall. In natural conditions only causes acute. Experimental small repeatedly administered doses can cause hepatic cirrhosis.
Dodonea viscosa	Cattle	Not determined	Periacinar necrosis	Serosal hemorrhages, edema of the gall bladder wall. In natural conditions only causes acute poisoning.
Myoporum laetum	Sheep	Furanosesquiterpenoid oils (ngaione)	Usually periacinar necrosis but variable zonal necrosis can occcur.	Other species can be affected but the in Brazil was only recognized in sheep.
Cestrum intermedium	Cattle	Not determined	Periacinar necrosis	Serosal hemorrhages, edema of the gall bladder wall. In natural conditions only causes acute poisoning.
Cestrum laevigatum	Cattle	Saponins, cestrumide	Periacinar necrosis	Serosal hemorrhages, edema of the gall bladder wall. In natural conditions only causes acute poisoning. Experimental small repeatedly administered doses can cause hepatic cirrhosis.
Dodonea viscosa	Cattle	Not determined	Periacinar necrosis	Serosal hemorrhages, edema of the gall bladder wall. In natural conditions only causes acute poisoning.
Trema micanthra	Goats and sheep	Not determined	Periacinar necrosis	Serosal hemorrhages, edema of the gall bladder wall. In natural conditions only causes acute poisoning.
Vernonia molissima	Catle and sheep	Not determined	Periacinar necrosis	There is also degeneration of renal tubular epithelium
Vernonia rubricaulis	Catle	Not determined	Necrose centrolobular	Outbreaks occur in the dry season
Bacteria				
Microcystis aeruginosa	Cattle, sheep, horses, goats	Microcystins and oithers	Periacinar to massive necrosis	Multiple toxins present. Can also cause death by neuromuscular disturbances. This poisoning was not doccumented in farm animals in Brazil but there are evidences that it occurs
Inscect larvae				
Perreyia flavipeds (sawfly)	Cattle, sheep and pigs	Pergidin and lophyrotomin	Periacinar to massive necrosis	Serosal hemorrhages, edema of the gall bladder wall.
Mycotoxins				
Aflatoxin	Pigs, cattle	Bisfuranocoumarin compounds	Cnetrolobular necrosis (lipidosis)	Hemorrhages. Other species can be affected but the listed two are so more often in the country. Cattle are affecter as young and develop a chronic form with fibrosis, megalocytosis and bile duct hyperplasia

products may not be fully eliminated by severely damaged liver. However, abnormal ammonia concentrations are not the only possible cause of hepatic encephalopathy. An imbalance between inhibitory and excitatory amino acid neurotransmitters, -aminobutyric acid, and L-glutamate, respectively, and increased brain concentrations of endogenous benzodiazepines are other possible explanations.[3] Alternatively a low blood sugar can account for the neurological signs, and a fall in glucose was noticed in the fatally poisoned calves by *L. interrupta*.[8]

Widespread hemorrhages are prominent in some field cases of SLP, but they were not invariably present and their severity varied. Lengthened thrombin, activated thromboplastin times and reduced fibrinogen concentration have been reported in calves experimentally poisoned with the larvae of *Lophyrotoma interrupta*.[8] Hemorrhagic diathesis occurs terminally in animals with severe liver necrosis.[3] In these cases bleeding tendencies associated with hepatic failure may be due to impaired synthesis of clotting factors, reduced clearance of the products of the clotting process, and metabolic abnormalities affecting platelet function that affect normal clotting, individually or in combination. In acute liver failure (as is the case of liver failure in SLP) diminished synthesis of clotting factors with a short half-life, such as factors V; VII, IX, and X, impairs the ability of blood to coagulate. Diminished clearance of fibrin degradation products,

activated coagulation factors, and plasminogen factors by the damaged liver also perturbs clotting. Metabolic disturbances resulting from liver failure can affect platelet function and lead to synthesis of abnormal fibrinogen, a condition termed dysfibrinogenemia.[3]

Due to the short course of the disease, jaundice and photosensitization were not common findings. An increased concentration of serum bilirubin has been seen in experimental sawfly larval poisoning of cattle and sheep.[8,21]

Tubular and degeneration of the renal epithelium tubular as described in cattle,[2,19] sheep,[14] and pigs[18] were not observed in our cases.

JPC Diagnosis: Liver: Necrosis, massive, diffuse.

Conference Comment: The contributor provides a very interesting and thorough discussion of sawfly larval poisoning. Conference participants discussed characteristics of the histological patterns of necrosis observed in acute toxicities such as this, noting that the patterns are very repetitive. Discussion focused on the extent of necrosis (centrilobular to massive) that is often a function of dose, as well as the presence of early ductular reaction that often occurs in response to hepatic damage. Ductular reaction is the phenomenon in which biprogenitor cells (cells that have the propensity to differentiate into either biliary epithelial cells or hepatocytes) proliferate in response to severe hepatic injury or nutritional deficits to form islands or small, crude tubules of small basophilic cells at the margin of the limiting plate. Ductular reaction is considered to be the hallmark of severe injury and may occur as early as 2-3 days after the toxic insult.[3]

Additionally, participants discussed the various toxins that can result in similar lesions of acute hepatic necrosis. The moderator provides the included table of agents involved in acute hepatic toxicity in cattle.

Name	**Toxic Principle**
Blue-green algae	Microcystin-LR
Mushrooms: Amanita, Phalloides and others	Amatoxins
Cycads (Zamia sp.)	Methylazoxymethanol
Solanacae (Cestrum sp.)	Atractyloside
Compositae (Xanthium-cockleburr)	Carboxyatractyloside
Ulmaceae (Trema sp.-Poison peach)	Trematoxin
Myoporacae (Myoporum)	Ngaione (periportal)
Iron	
Sawfly larvae (Lophyrotoma sp.)	Lophyrotomin/pergidin

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CASE II: 01019006 (JPC 4017217).

Signalment: 8-day-old, male, miniature horse (foal), *(Equus ferus caballus)*.

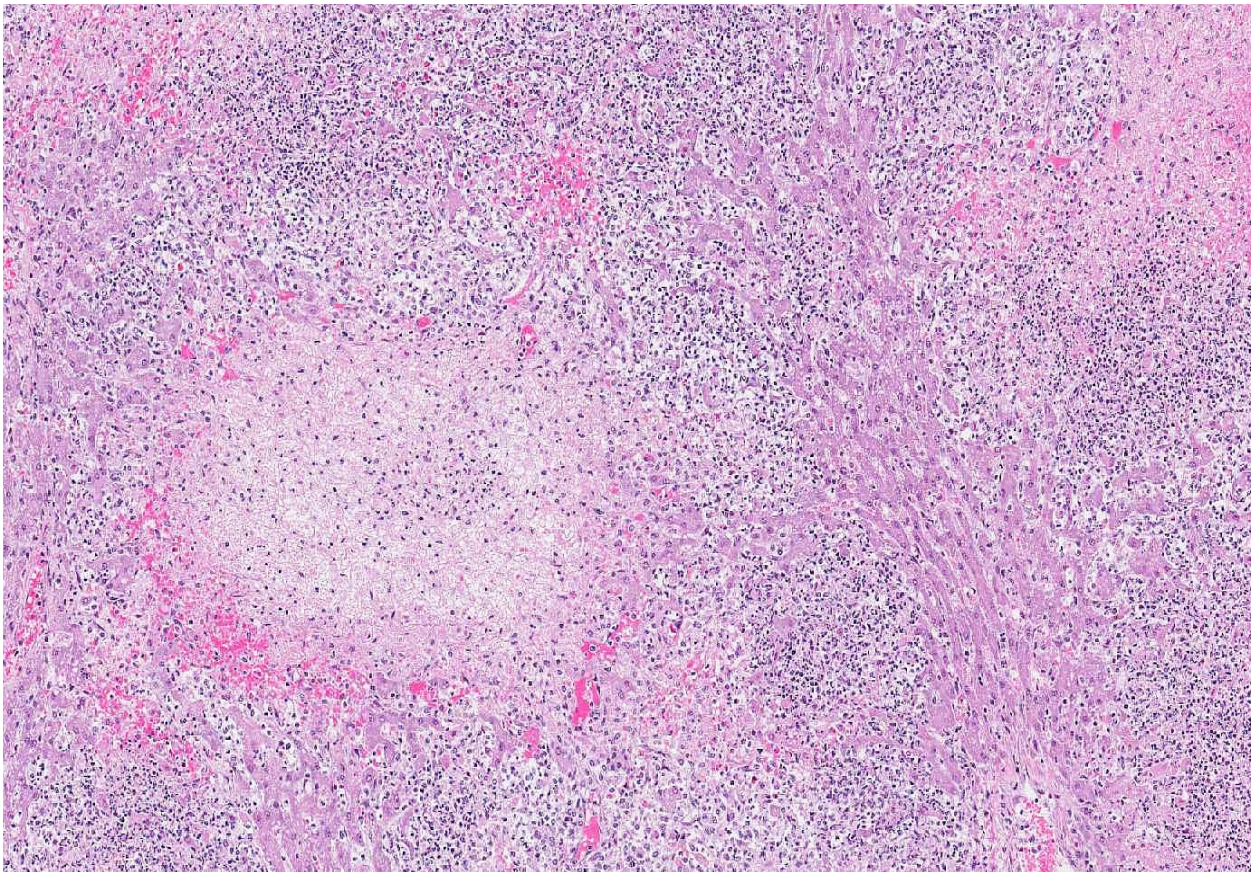
History: Presented for complete necropsy following natural death. It had a reported clinical history of impaction.

Gross Pathology: The foal had a body condition score of 3/5 and was in fair post-mortem condition. The adipose tissue throughout the carcass was pale yellow (icterus). The liver contained numerous pinpoint pale tan and red-black foci throughout. Upon opening the elbow joints, minimal amounts of yellow, strand-like material was adherent to the cartilaginous surface of the humeral condyles and the radial fossa (fibrinosuppurative arthritis). The stomach contained a moderate amount of pale yellow, pasty material (digested milk), while the small intestines and cecum contained mild to moderate amounts of dark gray, opaque, viscous digesta. The large and small colon contained moderate amounts of yellow pasty feces throughout.

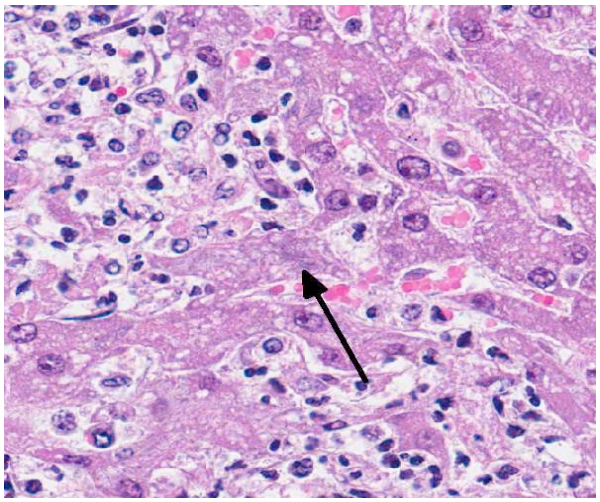
Histopathologic Description: Liver: The hepatic parenchyma is replaced by multifocal to coalescing round to oval aggregates of hepatic necrosis and inflammation. These areas are characterized by a central area of hepatocytes undergoing coagulative and liquifactive necrosis admixed with homogenous eosinophilic fibrillar material (fibrin) and neutrophils undergoing necrosis. The areas of hepatocellular necrosis are often surrounded by a dense layer of karyorrhectic and karyolytic nuclear debris from necrotic neutrophils. Hepatocytes can be observed at the periphery of these lesions that contain intracytoplasmic aggregates of rod-shaped bacteria.

Contributor's Morphologic Diagnosis: Marked acute multifocal random necrosuppurative hepatitis with intracytoplasmic bacilli.

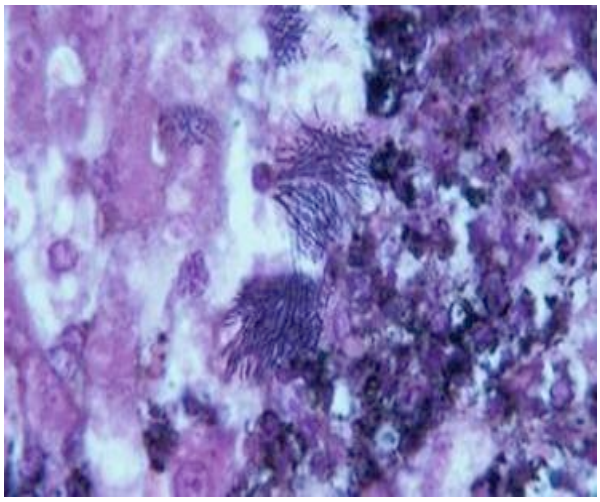
Contributor's Comment: Tyzzer's disease was first described in laboratory rodents, but has been reported in a number of species as a naturally occurring disease.[3] The causative organism is *Clostridium piliforme*, formerly known as *Bacillus piliformis*. The organism is a gram-negative,



2-1. Liver, miniature horse: Scattered throughout the liver are areas of coagulative necrosis, rimmed by hypercellular areas of lytic necrosis, and most peripheral, by intact hepatocytes. (HE 120X)



2-2. Liver, miniature horse: At the periphery of necrotic areas, faintly stained outlines of filamentous rod-shaped bacilli (arrow) in a characteristic "haystack" or "pickup-stick" arranged may be seen in the cytoplasm of intact hepatocytes. (HE 120X)



2-3. Clostridium piliforme is best demonstrated with silver stains, such as Warthin-Starry stains, which or GMS with HE counterstains, as seen here. (GMS/HE 600X)

obligate intracellular, spore-forming rod. Exposure is likely through the fecal-oral route with systemic dissemination taking place after infection establishes in the intestines. The liver and myocardium are primarily affected. Necrotizing enteritis can be seen in some laboratory animal species, most notably rodents and rabbits, with the distal small intestine primarily affected. Silver stains (e.g. Warthin-Starry or Gomori's methanamine silver) are useful in highlighting the bacteria.[3] Immunohistochemical or immunofluorescence staining can also be used to confirm the diagnosis.[1]

Foals from 7 to 42 days of age are primarily affected[1] and immunocompromised animals are predisposed to infection. The organism is not considered highly contagious and animals are typically affected sporadically in a given population. Infected animals can be found dead or may present for acute onset of non-specific clinical signs such as pyrexia, lethargy, anorexia and icterus. Typically elevations of hepatocellular leakage enzymes and hyperbilirubinemia take place.[3] Treatment of animals with confirmed disease is typically unrewarding.

Organism	**Species affected**
Listeria monocytogenes	Neonates: ruminants, foals, piglets
Campylobacter fetus	Lambs, foals, piglets
Actinobacillus equilli/suis	Foals/piglets
Yersinia pseudotuberculosis	Lambs, dogs, cats
Francisella tularensis	Lambs, cats
Mannheimia hemolytica/ Histophilius somni	Lambs
Salmonella spp.	Various
Clostridium piliforme	Foals, dogs
Nocardia/Mycobacterium	Various

JPC Diagnosis: Liver: Hepatitis, necrotizing and suppurative, multifocal to coalescing, severe, with intracytoplasmic filamentous bacilli.

Conference Comment: The contributor provides a good summary of *C. piliforme*, an atypical member of the genus *Clostridium*. Other members of the clostridia are large, Gram positive spore forming bacteria with straight or slightly curved morphology, in contrast to the filamentous, Gram negative spore forming *C. piliforme*.[2] Further differentiating *C. piliforme* from other clostridia is the fact that it does not possess characteristics that allow its inclusion into one of the three general categories of the other pathogenic members of the genus. These categories of clostridia are neurotoxic (*C. tetani, C. botulinum* types A-G), histotoxic (*C. chauvoei, C. septicum, C. novyi* types A and B, *C. perfringens* type A, *C. sordellii, C. hemolyticum)*, and enteropathogenic/ enterotoxemia-producing (*C. perfringens* types A-E, *C. difficile, C. colinum, C. spiroforme*).[2]

Conference participants discussed the importance of recognizing both the pattern of necrosis (i.e. multifocal, random) as well as the inflammatory

component in this section, and discussed the differential diagnosis for such lesions. The conference moderator provided the included table of common causes of bacterial hepatitis in various domestic animal species.

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CASE III: MS1104670 (JPC 4019870).

Signalment: Four-month old, female, athymic nude, mouse (*Mus musculus*).

History: Second mouse in cage to develop spontaneous emaciation and paresis.

Gross Pathology: Prior to euthanasia the animal is depressed. Mild splenomegaly and lymph node enlargement is present. The mesenteric lymph node is white and firm. Heart, lung, liver, GI and reproductive tract are grossly normal.

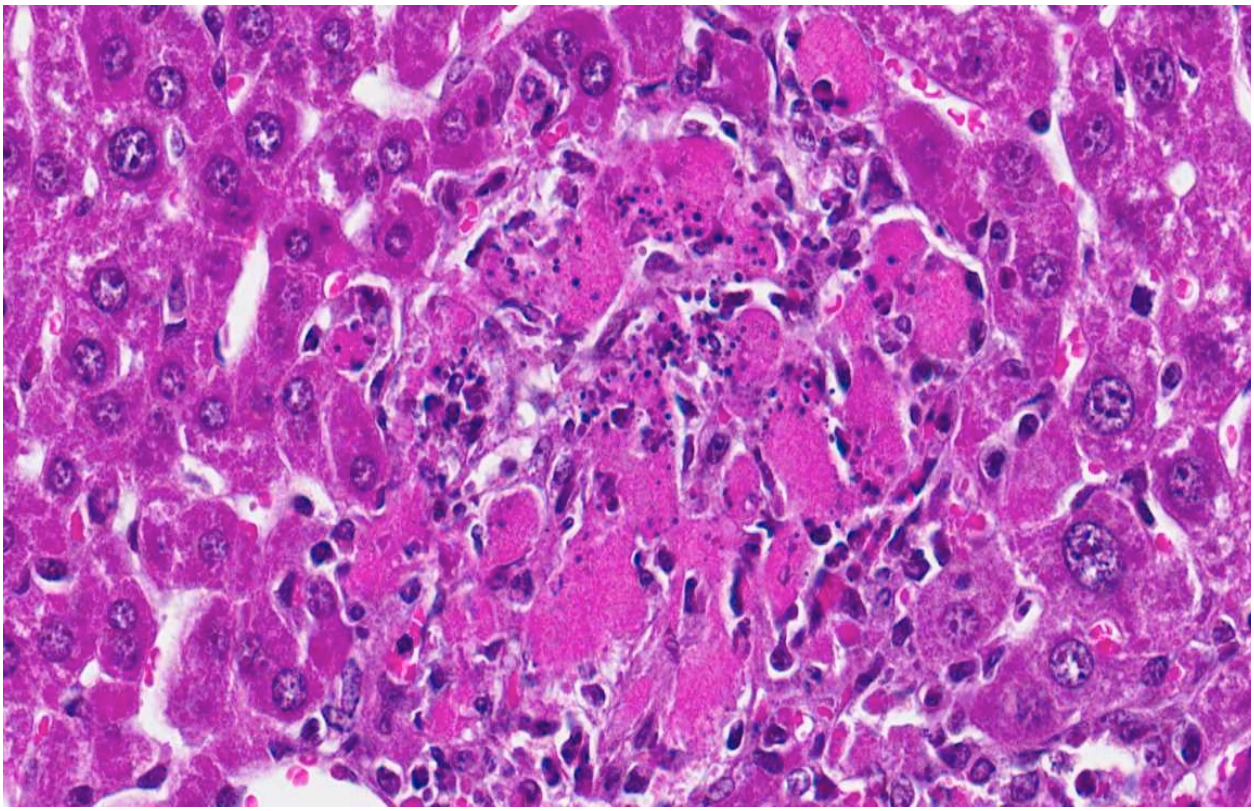
Laboratory Results: The fecal, kidney and mesenteric lymph node tissue samples are negative for MPV via PCR. The liver and mesenteric lymph node tissue samples are positive for MHV via RT-PCR.

Histopathologic Description: The liver has moderate, multifocal necrotizing and suppurative hepatitis. Some necrotic foci contain syncytial cells. Findings in other (not submitted) tissues include necrotizing splenitis and necrosis, variable suppuration and syncytial cells in the brain, bone marrow and lymph nodes, and the nasal cavity has necrosuppurative rhinitis.

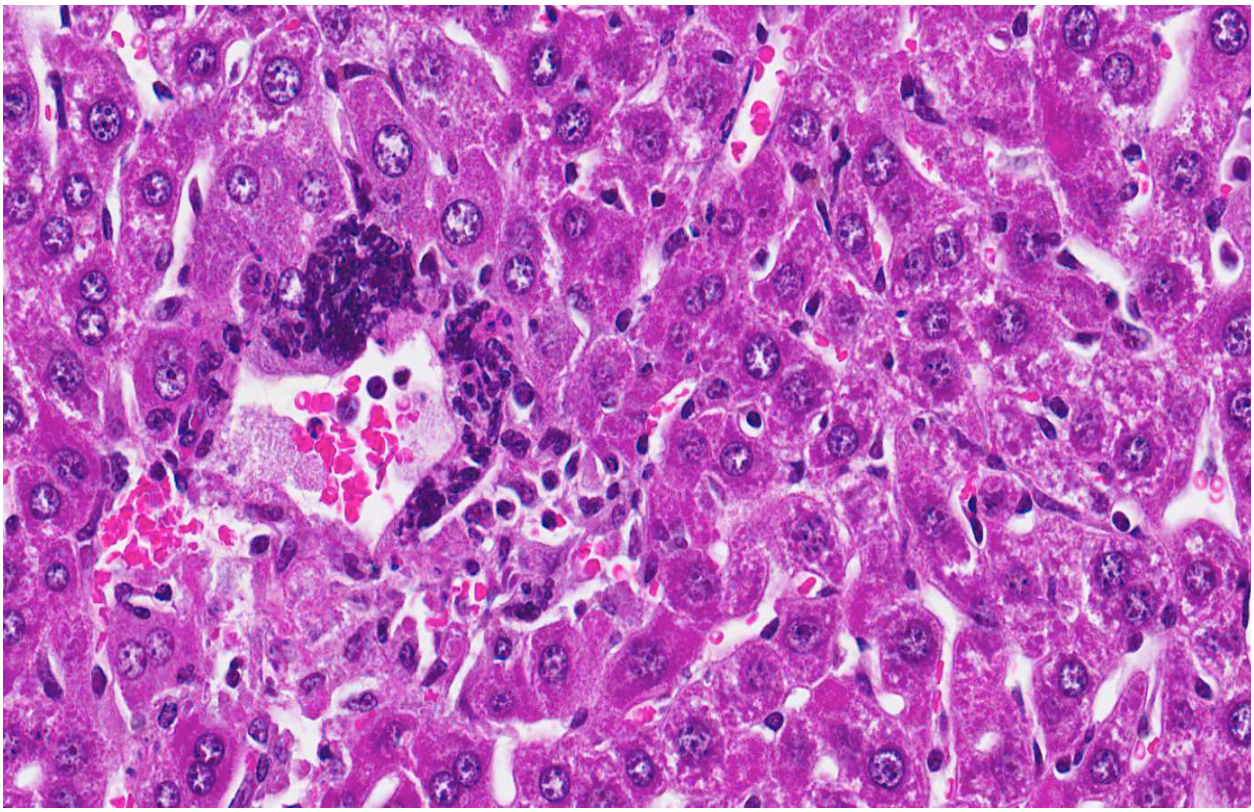
Contributor's Morphologic Diagnosis: Hepatitis, necrotizing, moderate, multifocal with syncytial cells.

Contributor's Comment: This mouse is one in a group of other nude mice housed separately but near a room known to be positive for MHV and MPV. Of the known strains of MHV, this mouse likely presented with a polytropic strain, most notably with neurotropic characteristics. This mouse had characteristic necrotizing lesions with syncytial cells in multiple tissues, suggesting a polytropic strain of MHV.

Mouse hepatitis virus is a single-stranded, enveloped RNA virus of the family *Coronaviridae*. Approximately 25 strains or isolates of MHV have been described. Isolates can be divided into two biotypes with varying degrees of overlap: the respiratory (polytropic) and enterotropic strains.[3] Respiratory strains of MHV are extremely contagious and are transmitted primarily via aerosol, direct contact, and fomites. Once inhaled, the virus replicates in the nasal mucosa and subsequently



3-1. Liver, mouse: Randomly scattered throughout the section of liver are numerous small foci of necrotic hepatocytes. (HE 400X)



3-2. Liver, mouse: Multinucleated hepatocellular viral syncytia are characteristic of infection by mouse polytropic coronavirus (mouse hepatitis virus). Necrotic syncytia are still recognizable in areas of hepatocellular necrosis. (HE 400X)

spreads via the blood and lymphatics through cervical and mesenteric lymph nodes to multiple tissues.[1] Dissemination is more likely with more virulent strains of virus in infant mice without maternal antibody and in immunocompromised (particularly nude) mice. MHV has high morbidity and mortality in these groups. Immunocompetent mice may clear the infection in five to seven days post-infection with no carrier status. BALB/c mice are generally quite susceptible to MHV, whereas SJL mice are remarkably resistant. The spike protein (SP), responsible for viral entry, is a major determinant of tropism and virulence.[2] SP binds carcinoembryonic antigen-related cell adhesion molecule 1, CEACAM- 1 on either the cell surface or in endosomes. SP also mediates cell-to-cell fusion. The allelic form of CEACAM in a particular strain of mouse is a determinate of susceptibility. Strains that express CEACAM-1α such as Balb/C are susceptible. Strains that express CEACAM -1β such as SJL are resistant.[2] Disease severity is determined by thrombosis and coagulation necrosis due to induction of procoagulant activity by macrophages in susceptible mice.[5] T cell function is important for viral clearance depending on both CD8 activity and antibody and cytokine induction.[2]

Enterotropic strains of MHV selectively infect intestinal mucosal epithelium, with minimal to no dissemination to other organs, even in immunodeficient mice.[4] All stages and strains of mice are susceptible to enterotropic MHV infection, including SJL mice, which are resistant to polytropic MHV.[5] The severity of intestinal disease is associated with age-related intestinal mucosal proliferative kinetics, thus severe disease occurs only in infant mice.[4] Even in nude or SCID mice, disease can be minimal; however, hyperplastic colitis has been reported in nude mice.[5] Recovery from enterotropic MHV is T-cell dependent; persistent infections can occur in immunodeficient mice.[3]

Diagnosis is usually made via serology using an ELISA, but MHV may also be isolated in susceptible tissue culture cells. Differential diagnoses include Salmonellosis, Tyzzer's disease and reovirus infection.[5] Respiratory syncytial virus forms syncytial cells similar to MHV, but these cells are restricted to the pulmonary parenchyma.

JPC Diagnosis: Liver: Hepatitis, necrotizing, multifocal and random, moderate, with viral syncytia.

Conference Comment: The contributor provides a very good summary of mouse hepatitis virus (MHV). Conference participants discussed the polytropic nature of the respiratory strains of MHV. Despite being called a "hepatitis" virus, these viruses actually have a secondary tropism for a variety of cells and tissues other than the liver to include vascular endothelium, lymphoid tissue, hemopoietic tissue, and the central nervous system.[6] The majority of natural polytropic MHV infections are subclinical in immunocompetent mice; whereas infections in immunodeficient mice often result in wasting disease, neurologic signs and mortality. Interferon-gamma deficient mice infected with polytropic MHV develop a unique clinical presentation of granulomatous polyserositis and subsequent abdominal distention.[5,6] Participants also discussed the significance of MHV in laboratory mouse colonies, where its effects on research resulting from immunomodulation can be particularly devastating.[5,6]

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CASE IV: V11-30618 (JPC 4019134).

Signalment: Adult, male, sugar glider, (*Petaurus breviceps).*

History: The owner had seven sugar gliders. Two sugar gliders, one female and one male, became acutely lethargic, dehydrated, and were vomiting. The sugar gliders died and the male was presented for postmortem examination. The diet did contain cantaloupe that had been previously frozen and raw organic soybeans.

Gross Pathology: The liver was slightly enlarged with multiple pinpoint tan foci of necrosis. The lungs were mildly congested.

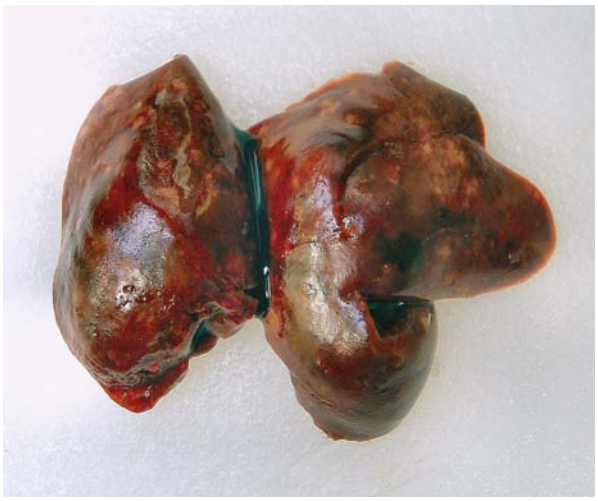
Laboratory Results: *Listeria monocytogenes* was isolated on bacterial culture of the liver.

Histopathologic Description: The liver contains numerous random multifocal to coalescing foci of necrosis filled with necrotic cellular debris and variable numbers of degenerate neutrophils. The margins of the necrotic foci contain variable numbers of gram-positive intracellular and extracellular rod-shaped bacteria. The necrotic foci occasionally extend into portal veins resulting in necrosis of the vascular wall and fibrin thrombi within the lumen. Throughout the liver, there are moderate numbers of hepatocytes that contain clear distinct cytoplasmic vacuoles consistent with lipid. There are moderate numbers of hepatocytes that contain golden-brown to yellow-green cytoplasmic granular pigment that is consistent with hemosiderin and bile.

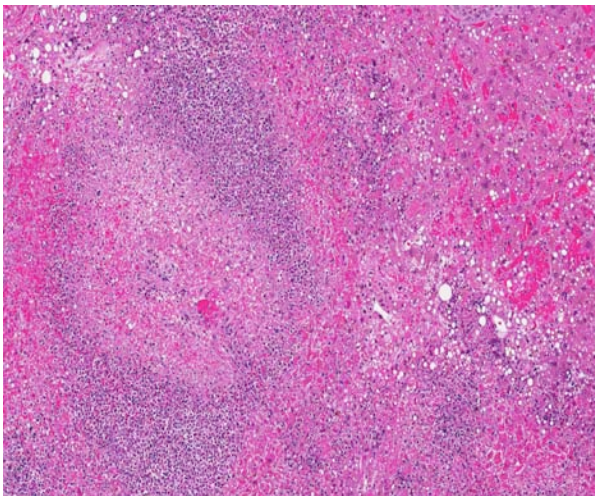
Contributor's Morphologic Diagnosis: Liver: Severe multifocal and random necrotizing suppurative hepatitis with rare vascular necrosis and thrombosis and intracellular and extracellular gram-positive rod-shaped bacteria consistent with *Listeria monocytogenes.*

Contributor's Comment: *Listeria monocytogenes* is a gram-positive, intracellular, non-spore forming, facultative anaerobic, rod-shaped bacterium that causes the disease listeriosis.[2,5,6] It has the ability to infect and cause disease in multiple species including but not limited to humans, ruminants, pigs, horses, rabbits, chinchillas, and birds.[1,2,4,5,6,7,8,9] The bacterium is believed to be distributed worldwide with many animals and people (estimated up to 70% of people in some areas are carriers) being asymptomatic carriers.[2,6,7] In addition, the bacterium has been isolated from sewage, stream water, silage, and soil.[6]

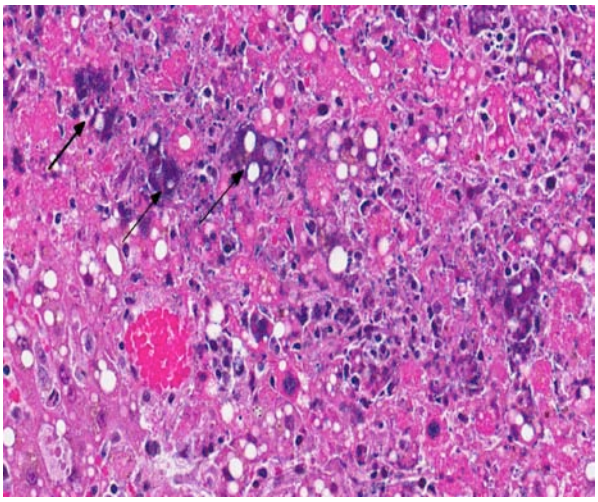
Most incidents of listeriosis occur when the host ingests contaminated soil, water or food.[1,2,5,6,7] The bacterium is internalized into the host cell using the surface protein internalin, which interacts with E-cadherin on the host cells allowing the bacterium to cross the intestine, placenta, and blood-brain barrier.[1,2,5,7,9] Once inside the host cell, the bacterium lyse the phagocytic cell phagolysosome using a pore-forming protein called listeriolysin O and two phospholipases allowing it to gain access to the host



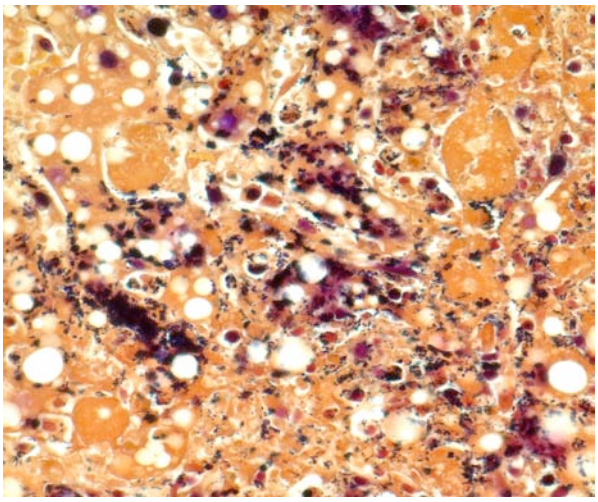
4-1. Liver, sugar glider: The liver was slightly enlarged with multiple pinpoint tan foci of necrosis. (Photo courtesy of: The New Mexico Department of Agriculture Veterinary Diagnostic Services http://www.nmda.nmsu.edu/vds/)



4-2. Liver, sugar glider: There are numerous foci of coagulative necrosis bounded by areas of lytic necrosis scattered throughout the section. (HE 80X)



4-3. Liver: Aggregates of 2-3 µm rod-shaped bacilli s are scattered throughout areas of lytic necrosis. (HE 320X)



4-4. In areas of lytic necrosis, a tissue Gram stain reveals numerous intra- and extracellular rod-shaped bacilli consistent with Listeria monocytogenes. (Gram 400X). (Photo courtesy of: The New Mexico Department of Agriculture Veterinary Diagnostic Services http://www.nmda.nmsu.edu/vds/)

cell cytoplasm. The bacterium then replicates within the host cell cytoplasm. *Listeria* has the ability to co-opt the host cell actin filaments using the bacterial surface protein ActA to help it migrate to the host cell membrane to induce pseudopod-like protrusions that can be transferred to another host cell. The host protection against *Listeria* appears to be mediated by IFN-γ production by NK cells and T-lymphocytes as host macrophages stimulated by IFN-γ phagocytose and kill *Listeria* while host macrophages that internalize *Listeria* coated by C3 become infected.[1]

Severe disease caused by *Listeria monocytogenes* tends to manifest as three distinct syndromes in both animals and humans: abortion after infection of the pregnant uterus, septicemia with visceral abscesses, and meningoencephalitis.[1,2,9] The syndromes, particularly the neurologic and genital forms of listeriosis, rarely occur at the same time.[2] Abortions occur most commonly in cattle and sheep, but have also been reported in women and other species.[1,2] Encephalitic listeriosis occurs most commonly in ruminants while people tend to develop suppurative meningitis.[1,2] All species seem to be susceptible to septicemia with *Listeria*.[5]

In abortions, *Listeria* crosses the placenta and most likely results in fetal septicemia.[6] The gross lesions in the fetus often include severe placentitis and multiple pinpoint yellow foci in multiple organs, which are most prominent in the liver. However, the gross fetal visceral lesions are easily obscured by postmortem decomposition. The microscopic lesions in the fetal organs consist of foci of necrosis filled with degenerate neutrophils and macrophages surrounded by bacteria. Necrotizing enterocolitis with intralesional bacteria may be present. Meningitis can also be seen in the fetus. The microscopic placental lesions include necrosis of the cotyledonary villi with a suppurative exudate and many bacteria.

The neurologic form of listeriosis in ruminants is most often the result of feeding contaminated silage.[2,5,7,9] The bacteria travel via the trigeminal nerve and other sensory nerves to the brainstem possibly gaining entry to the nerves through wounds in the oral cavity. There usually are no gross lesions associated with neurologic listeriosis in ruminants. The microscopic lesions are limited to the brainstem. The microscopic lesions can range from small early lesions of accumulations of glial cells to severe encephalitis with macrophages, lymphocytes, plasma cells, neutrophils, and the formation of the characteristic microabscesses. Bacteria can be seen in some lesions.

The septicemic form of listeriosis most often occurs in neonates and young animals, but can occur in adult animals.[1,2,8,9] The gross and microscopic lesions consist of multifocal necrosis and microabscesses that can contain bacteria.[2] These lesions occur most frequently in the liver. Although the source of the bacterium is often not identified in septicemic cases, potential sources include contamination of the pregnant uterus by *Listeria* during gestation and the bacterium crossing the

intestine after the animal ingesting contaminated soil, water or food.

In the fall of 2011, in multiple states including Colorado and New Mexico, a total of 146 persons were sickened, 30 people died, and one woman miscarried after eating cantaloupe contaminated with *Listeria monocytogenes*.[3] The sugar glider in this case was known to eat cantaloupe in its diet. *Listeria monocytogenes* has 11 different serotypes that can be distinguished from one another using molecular techniques. Using pulse field gel electrophoresis, the serotype of the *L. monocytogenes* isolated from the sugar glider was not the same as the serotype of the *L. monocytogenes* isolated from the cantaloupe associated with the listeriosis outbreak in people. Nevertheless, this case illustrates the "One Health" concept of the potential of infectious agents to infect veterinary species and humans that are sharing the same environment and ingesting the same food and water.

JPC Diagnosis: Liver: Hepatitis, necrotizing and suppurative, multifocal, severe, with vascular necrosis, thrombosis, and numerous intra and extracellular bacilli.

Conference Comment: The contributor provides an excellent review of listeriosis. Conference participants discussed the vascular lesions and described them as vascular necrosis due to local inflammation rather than a true vasculitis. Participants also commented on the necrotic areas, noting the core of lytic necrosis and inflammation surrounded by a corona-like ring of coagulative necrosis. There was speculation that such lesions could be due to either the local effects of listeriolysin O and phospholipase facilitating transfer of the bacteria across cell membranes resulting in coagulative necrosis in advance of inflammation; or to a locally released toxin directly resulting in cell death.

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