Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

C o n f e r e n c e 5

25 September 2019

CASE I: V17-00724 (JPC 4121052).

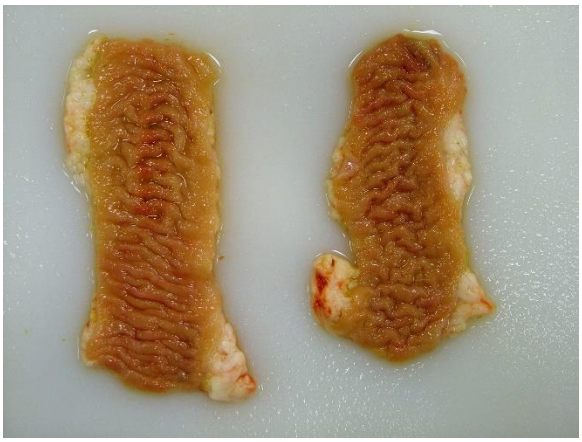
Signalment: Three years old, female, breed not specified, *Ovis aries*, sheep

History: The ewe had poor weight gain especially in the winter with periodic loose, nonpelleted feces. The ewe had subcutaneous edema in the skin of the ventral mandibular area (bottle jaw).

Gross Pathology: There were segments of the small intestine that were thick and corrugated. The mesenteric lymph nodes were enlarged with numerous variably sized tan foci that effaced the lymph node parenchyma.

Laboratory results: The mesenteric lymph node was PCR positive for *Mycobacterium avium* subspecies *paratuberculosis* (MAP). MAP was not isolated on culture of the mesenteric lymph node.

Microscopic Description:
The lamina propria of multiple segments of the small intestine is markedly thickened by diffuse infiltrates of numerous macrophages, which are mixed with lesser numbers of lymphocytes. The infiltrates of macrophages result in blunting and widening of the



Intestine, sheep. Multifocally, the intestinal mucosa was thickened and rugose. (Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services, www.nmda.nmsu.edu/vds)

intestinal villi. The macrophages in the lamina propria contain numerous intracellular acid fast bacilli. There are occasional lymphatic vessels in the mesentery that are surrounded by and infiltrated by numerous macrophages with lesser numbers of lymphocytes and neutrophils (not present in all sections).

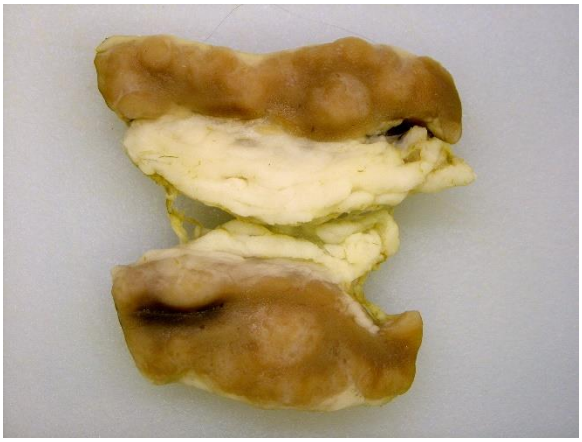
Contributor's Morphologic Diagnoses: Small intestine. Enteritis, granulomatous, diffuse, severe with numerous intracellular acid fast bacilli; etiology consistent with

Mycobacterium avium subspecies *paratuberculosis* (Johne's disease)

Contributor's Comment: Mycobacteria are thin rods that can vary in length from 0.2 to 10.0 µm.[7] Mycobacteria are acid-fast and are gram-positive, but the lipid in the cell wall typically prevents staining with a Gram stain. They are aerobic, oxidative, non-motile and do not form spores. Mycobacteria are typically slow-growing with a range of generation times of 2-20 hours.

Johne's disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP).[6,7,8] Johne's disease occurs most commonly in domestic ruminants, but nondomestic ruminant species as well as non-ruminant species can be infected with MAP. MAP is shed in the feces of infected animals and is spread by fecal-oral transmission. Transmission of MAP to naïve animals typically occurs in young animals while they are still nursing while older animals are more resistant to infection. There are two strains of MAP: type I or S strain and type II or C strain. The type I strain of MAP was first isolated from sheep and is fairly species specific for sheep with only rare reports of cattle being infected with this strain. The type II strain of MAP was first isolated from cattle and can infect a multitude of species including cattle, sheep and goats. The type II strain of MAP is the most common.

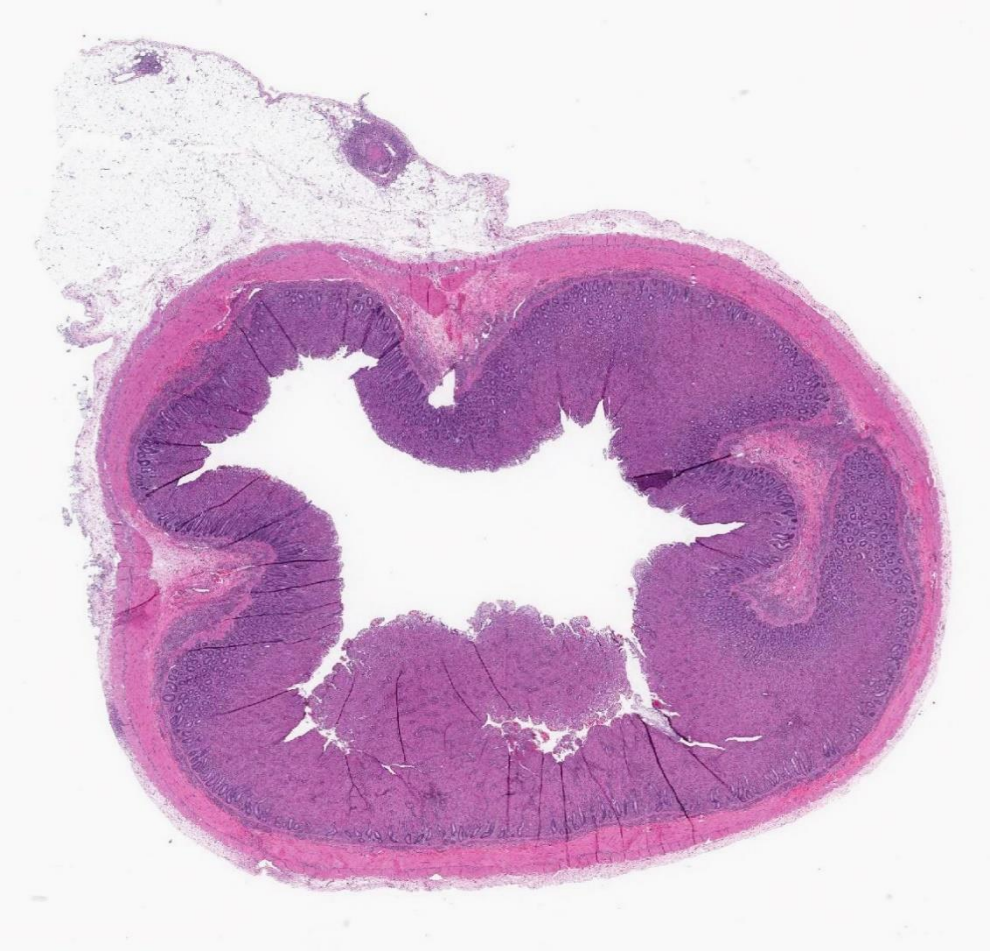
Unlike in cattle where Johne's disease causes severe diarrhea, Johne's disease in sheep manifests as a wasting disease in the majority of sheep.[3,6,7,8] The intestinal thickening caused by granulomatous enteritis can involve the jejunum, ileum, cecum and colon, but are most common in the ileum.[2,3,6,7,8] The intestinal lesions of Johne's disease in sheep are typically mild, can be multifocal and easily missed at necropsy. Sheep with Johne's disease can also develop



Mesenteric lymph node, sheep. Mesenteric nodes were enlarged with variably-sized tan foci of inflammation. (Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services, www.nmda.nmsu.edu/vds)

granulomatous lymphangitis of lymphatic vessels in the mesentery as well as lymphadenopathy and granulomatous lymphadenitis of the mesenteric lymph nodes. Tubercle-like caseating mineralized granulomas are more common in the intestine, lymph vessels and mesenteric lymph nodes of sheep than they are in cattle. Lymph nodes other than mesenteric lymph nodes, the liver, the lungs and the spleen can also have small granulomas with MAP in sheep. The microscopic lesions of Johne's disease in sheep occur in two forms. One form consists of dense infiltrates of numerous macrophages in the mucosal epithelium of the intestine with numerous bacteria (the multibacillary form associated with a strong humoral immune response). The other form consists of focal to multifocal lymphocyte-rich infiltrates of macrophages in the mucosal epithelium of the intestine with few bacteria (the paucibacillary form associated with a cell-mediated immune response).

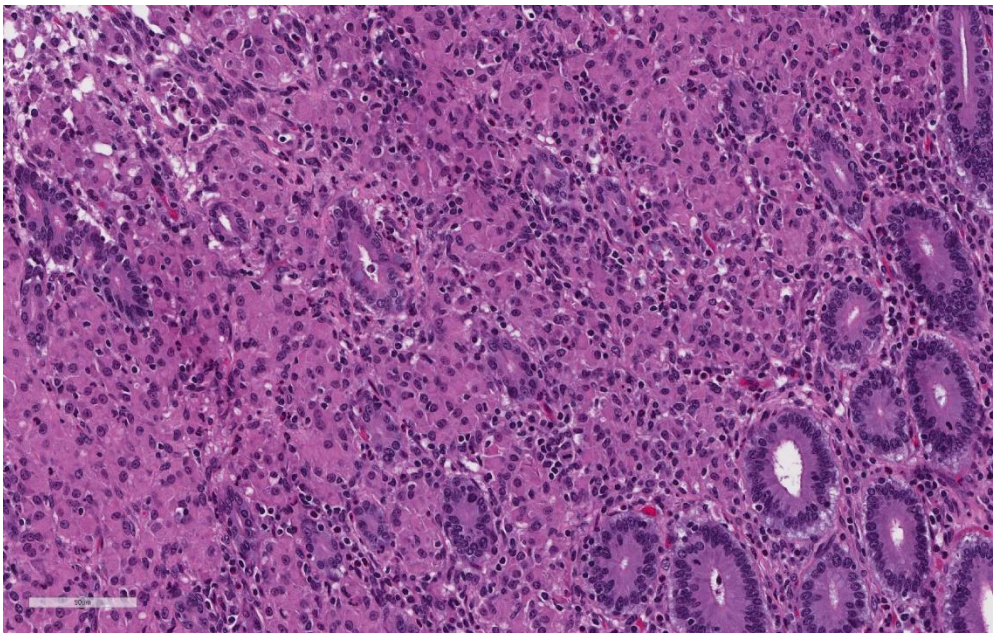
After ingestion of MAP, the mycobacteria gain access to the small intestine through M cells or epithelial cells over the submucosal Peyer's patches.[2,6,9] The mycobacteria then infect and survive in macrophages in the



Intestine, sheep. Subgross magnification demonstrates circumferential loss of villi with expansion of the lamina propria by a prominent cellular exudate. (HE, 7X)

intestinal mucosal epithelium and the mesenteric lymph nodes. In sheep experimentally infected with MAP, infection could be identified in most sheep by 18 months postinfection.[4] Infection was typically identified first in the mesenteric lymph nodes. Microscopic lesions could be identified 6-12 months after the identification of infection with MAP with the lesions usually being identified in the mesenteric lymph nodes first. The microscopic lesions and the disease in the sheep experimentally infected with MAP developed at variable rates with clinical signs first apparent at 24 months usually corresponding to the development of severe intestinal lesions. Although the intestinal lesions may be less severe in subclinical sheep, there is evidence of continuous fecal shedding of MAP in some subclinically infected sheep potentially resulting in a continuously infected environment.[10]

Diagnosis of Johne's disease in sheep, particularly of an individual sheep, can be more difficult than it is in cattle.[6,8,9] Isolation of mycobacterium from the feces or tissue from sheep is much more difficult than it is from cattle as the type I strain is difficult to isolate.[3,6,8,9] PCR can be used to detect MAP in feces and tissue of sheep, but molecular techniques are most likely useful on an individual animal basis and not a herd basis due to the cost of testing.[8,9] Serologic testing (ELISA and AGID) can be used for antemortem diagnosis of Johne's disease, but there are studies that indicate the usefulness of serologic testing depends on whether the sheep has the multibacillary form (a strong humoral immune response and more likely to be serologically positive) or the paucibacillary form (a cell-mediated immune response and more likely to be serologically negative).[8] In addition, the serologic tests can also cross react with *Corynebacterium pseudotuberculosis* (caseous lymphadenitis)



Intestine, sheep. Crypts are separated and replaced by sheets of histiocytes with abundant eosinophilic cytoplasm.

another wasting disease of sheep. The diagnosis of Johne's disease using histopathology collected postmortem or from rectal biopsy can also be difficult with the paucibacillary form of Johne's disease because it can have a multifocal distribution.[4,9]

Contributing Institution:
New Mexico Department of Agriculture
Veterinary Diagnostic Services
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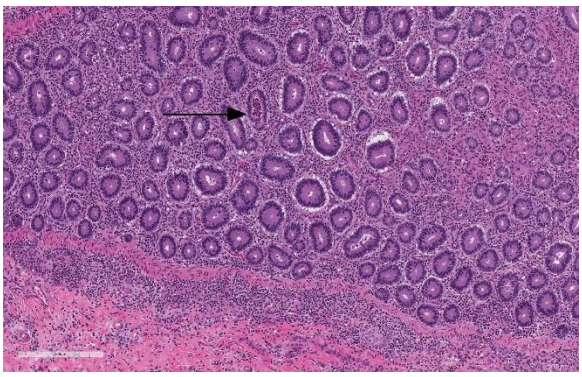
JPC Diagnosis: Small intestine: Enteritis, histiocytic, diffuse, severe with marked villar and crypt loss, villar blunting and fusion, crypt abscesses and hyperplasia, and edema (with multifocal lymphohistiocytic lymphangitis).

JPC Comment: The contributor has provided a concise review of *Mycobacterium avium* susbsp. *paratuberculosis* (MAP) in ruminants with a focus on its peculiarities in sheep.

The economic importance of Johne's disease in sheep cannot be ignored. While reports in small ruminants, as compared to affected cattle are sketchy, annual mortality in affected flocks in Australia have averaged 6-7% annually, with some reaching up to 20%. Economic losses to the British sheep industry range up to 20 million pounds annually if replacement strategies are factored in. In Italy, MAP infection decreases profit efficiency by 20% in affected farms. [10] These are direct losses only; potential indirect losses arising from trade restrictions at the international and national levels are difficult to estimate. Unlike many other highly contagious diseases of serious economic import, the OIE as of yet considers this a non-

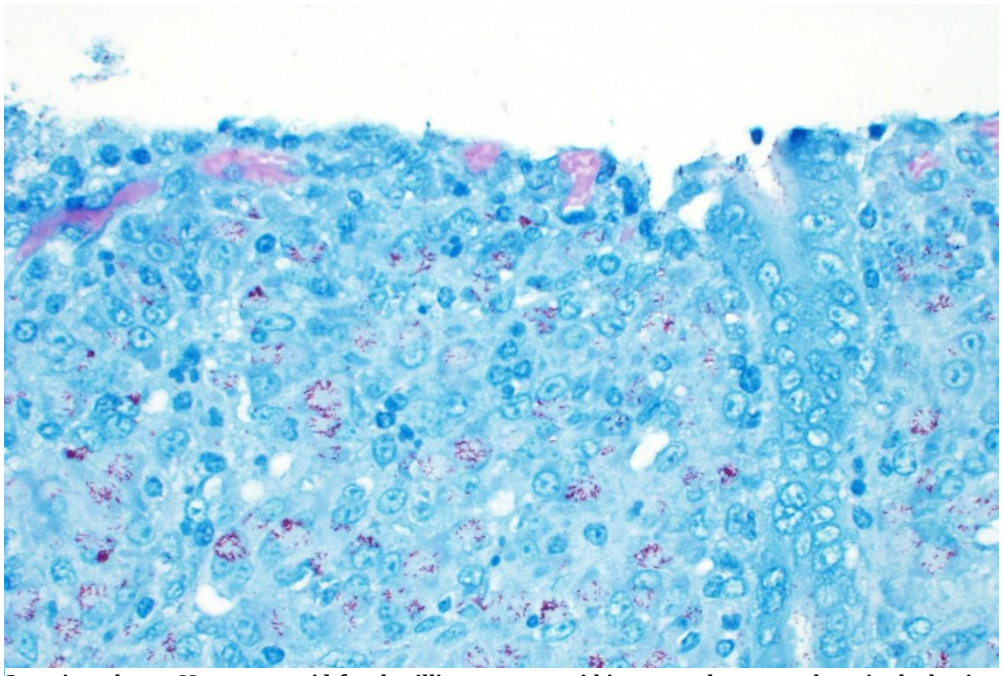


Intestine, sheep. The inflammatory infiltrate is less prominent in lamina propria surrounding the deep crypts and extends into the underlying submucosa. Rare crypt abscesses are present (arrow)

reportable disease and offers no guidance on paratuberculosis. Norway has had no known cases of MAP since 2015, and Sweden has been free of Johne's disease in cattle since 2008, but due to the insidious nature of this disease, all countries remain at risk.[10] Another risk factor is Johne's disease infection of non-domestic ruminant species including wild species of goats and sheep, deer, camelids, bison, rhinoceroses, and some species of marsupials may serve as additional reservoirs.

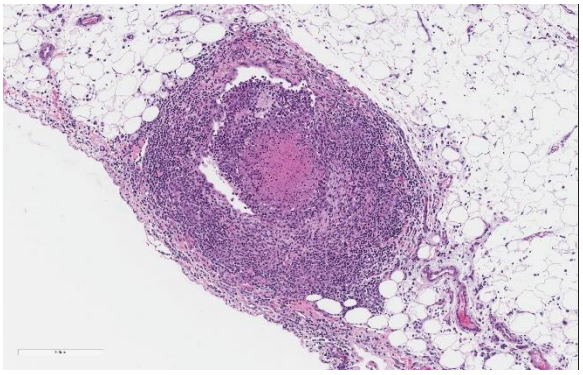
Another determining factor in the potential spread of Johnes's disease appears to be the differential susceptibility of various breeds to infection by MAP. In a recent article by Begg at al.,[1] Merino sheep (prized for their fine wool with an average value of 2-3 times more than mutton sheep) have the highest rate of clinical disease 14 months after oral inoculation at 42%, followed by Merino-Suffolk cross (36%), Border Leicester (12%) and Poll Dorset (11%). The authors were careful to point out that this may simply imply a longer disease duration Border Leicester and Poll Dorset breeds; however, this information is useful when considering control programs that emphasize a rapid decrease in environmental contamination by infected animals.[1]

Over the years, the MAP has been considered as a potential pathogen in humans in a number of conditions, yet never definitively incriminated. Numerous studies have identified potential risks for both waterborne (farm effluent) and foodborne (contaminated milk and meat) pathways for zoonotic transmission to humans, however at present, MAP is still not considered a zoonotic pathogen. One of the most common theories is that MAP may be a cause of IBD in humans, and is supported by circumstantial evidence including similar morphologies between Johne's disease and various forms of IBD including Crohn's disease, increases in IBD in areas in which animals demonstrate a 30% incidence of Johnes's disease and isolation in breast milk from mothers with IBD. However, Koch's postulated have never been fulfilled, and MAP has not yet been isolated with by ELISA-based or PCR in individuals with IBD. MAP has also been theorized to play a role in the generation of Type I diabetes in certain populations due to potential epitope mimicry between islet cell proteins GAD65 and ZNT8 with MAP proteins and HSP65 and MAP3865c,, respectively as well as and



Intestine, sheep. Numerous acid-fast bacilli are present within macrophage cytoplasm in the lamina propria. (Ziehl-Nielsen, 400X)

immune responses to HSP65 have been seen in patients with rheumatoid arthritis and Hashimoto's thyroiditis. [4]



Intestine, sheep. Lymphohistiocytic inflammation is centered on lymphatics in the adjacent mesentery. (HE 95X)

The moderator discussed the difference between Ziehl-Nielsen and Fite-Furaco stains. "Acid- fastness" refers to microorganisms whose cell wall has a high lipid content of mycolic and long chain fatty acids, which cause them to bind the basic dye carbol-fuchsin, a stain which remains after strong decolorization with acid-alcohol (thus "acid-fast") The Fite-Furaco uses peanut oil and xylene to protect the wall of bacteria from decolorization and allows for the visualization of bacteria with less lipid content, such as *M. leprae* and *Rhodococcus equi*. The moderator also mentioned a newly discovered strain of the bacterium, called the type III B strain (as opposed to type I S and type II C strains) which is pathogenic for buffalo, and currently a problem in India.

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CASE II: 160360 (JPC 4085120).

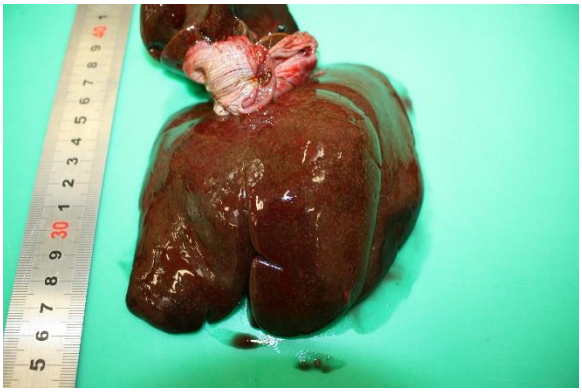
Signalment: Adult, female, European brown hare (*Lepus europaeus*).

History: Found dead in the woods

Gross Pathology: At necropsy the animal presented a severe jaundice. Mesenteric lymph nodes were enlarged and on cut section they presented multiple coalescing foci of caseous necrosis. The spleen was severely enlarged and was characterized by 1 mm white foci also present in the liver and the kidneys.

Laboratory results: Bacteriology**:** *Yersinia pseudotuberculosis* in liver and spleen.

Microscopic Description:
Liver: 60% of hepatic parenchyma is characterized by the presence of multifocal to coalescing, 1mm in size, randomly distributed inflammatory lesions (Fig. 4). These lesions are composed, from the periphery to the center, by a moderate amount of macrophages, epithelioid cells and less multinucleated giant cells (Langhans and foreign body types) admixed with scant heterophils. The inflammatory cells surround some cells (macrophages and hepatocytes) with condensed (pyknosis) or fragmented nuclei (karyorrhexis), nuclear and cellular debris (necrosis). The center of the lesion is occupied by small, 1µm in length, coccobacillar basophilic organisms (bacterial



Liver, hare. Numerous 1mm white foci were present in the liver. (Photo courtesy of Laboratoire d'Histopathologie animale, Vetagro Sup, campus veterinaire, http://www.vetagro-sup.fr/)

colonies) enmeshed within an eosinophilic material (fibrin). Liver parenchyma presents severe and diffuse hydropic degeneration, characterized by enlarged cells with clear granular eosinophilic cytoplasm (glycogenosis). A multifocal single cell necrosis is also present. Within the sinusoids a moderate amount of heterophils and monocytes are present, as well as Kupffer cells (Kupffer cell hyperplasia). An extramedullary hematopoiesis is evident within Disse'space.

Contributor's Morphologic Diagnoses:
Liver: Hepatitis, granulomatous and necrotizing, multifocal to coalescing, severe chronic with bacteria consistent with *Yersinia pseudotuberculosis*.

Kidney (not submitted): Nephritis, granulomatous and necrotic, multifocal, chronic moderate with bacteria consistent with *Yersinia pseudotuberculosis*.

Spleen (not submitted): Splenitis, granulomatous and necrotic, multifocal to coalescing, chronic severe with ibacteria consistent with *Yersinia pseudotuberculosis*.

Spleen, hare. There was marked splenomegaly with coalescing white foci as well. (Photo courtesy of Laboratoire d'Histopathologie animale, Vetagro Sup, campus veterinaire, http://www.vetagro-sup.fr/)

Contributor's Comment: *Yersinia pseudotuberculosis* is a gram-negative, facultative intracellular coccobacillus, which occurs worldwide in wild rodents, lagomorphs and birds. Sporadic infections can occur in domestic animals and humans. It is shed in the feces by infected animals and it can survive and grow in the environment at low temperatures. Infection occurs by ingestion; the organism penetrates the intestinal mucosa through the M cells.[3] Then it migrates to the Payer's patches and by the lymphatic route to the mesenteric lymph nodes. If the organism becomes septicemic/bacteremic, it spreads to other organs, typically liver and spleen.

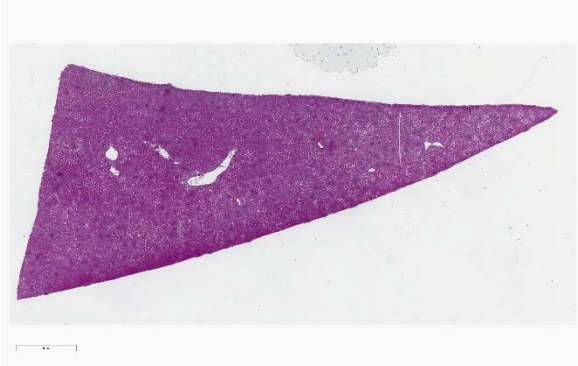
Pathogenic factors include:

- *Invasin*. This protein is expressed at low temperature conditions and it facilitates bacteria translocation through the intestinal epithelium into the lamina propria and Peyer's patches. Invasin binds to β1 integrins on cells, favoring bacterial-cell adhesion.
- *Type III secretion system* (T3SS): it is a virulence mechanism found in several gram-negative bacteria. It is composed of a needle-like syringe that injects into the host cell the effector proteins. After being attached to host cells, *Y. pseudotuberculosis* uses this system to inject the Yersinia outer proteins (Yops) [3]
- *Yersinia outer proteins* (Yops): there are 6 known Yops. These effector proteins alter the actin cytoskeletal structures to inhibit phagocytosis, induce apoptosis and downregulate proinflammatory responses. They are produced at 37°C environmental temperature, which means within the host.[3]

Gross typical lesions are white 1-10 mm foci in affected organs, mainly intestine, liver, spleen and mesenteric lymph nodes. In the large lesions a caseous necrosis is visible. Histologically, the lesions consist in micro abscesses composed of necrotic neutrophils with or without granulomatous reaction. Large bacterial colonies are easily visible.

In hares and rabbits differential diagnosis are *Yersinia enterocolitica* and *Francisella tularensis*. The three bacteria produce similar gross and histologic lesions and bacterial culture is necessary to identify them.

Contributing Institution:
Laboratoire d'Histopathologie animale, Vetagro Sup, campus vétérinaire, http://www.vetagro-sup.fr/

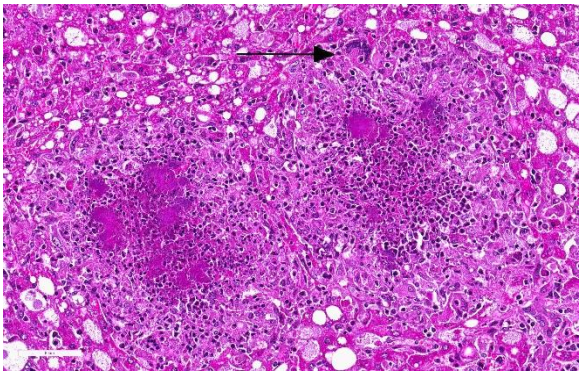


Liver, hare. At subgross magnification, there are numerous occasionally coalescing areas of necrosis. (HE, 7X)

JPC Diagnosis: Liver: Hepatitis, necrotizing, multifocal to coalescing, marked, with numerous colonies of bacilli.

JPC Comment: The genus *Yersinia* is a member of the family Enterobacteriaceae and consists of 18 species of gram-negative bacilli, three of which, *Y. enterocolitica*, *Y. pseudotuberculosis*, and the causative agent of plague, *Yersinia pestis* are pathogenic for animals, Nonhuman primates and man are considered extremely susceptible to infection by these pathogens, although *Y. enterocolitica (Yec)* was not considered as a human or veterinary pathogen until the late 1960s.[1] In contrast, a number of domestic and wildlife species including pigs, rodents, and wild birds, are and important reservoirs of this pathogens and considered significantly less susceptible to their pathogenic effects. (Interestingly, pork chitterlings (intestine) have been often identified as a source of food-borne illness to young children, during the cleaning and preparation phase.)[4] *Y. enterocolitica* has over 60 serotypes, but less than ten are pathogenic in primate hosts. It has six biotypes, of which one is highly pathogenic (1B), one is non-pathogenic (1A) and the remained, biotypes 2-5) are considered mildly pathogenic.[2]

The plasmid of *Yersinia* virulence (pYV) which encodes for many of the virulence factors mentioned by the contributor is the most important factor in pathogenicity. All biotypes are capable of mucosal invasion; however only those strains with the pYV can migrate from Peyer's patches to mesenteric nodes, and onward to establish the necrotic lesions in multiple organs demonstrated in this case. While this would logically mean that identifying plasmids are an easy way to identify pathogenic serotypes, it has been demonstrated that pathogenic bacteria may spontaneously lose this plasmid when



Liver, hare. Areas of lytic necrosis are centered on large bacterial colonies. Multinucleated giant cell macrophages are scattered at the periphery (arrow). (HE, 185X)

exposed to temperatures over 37C, prolonged storage, or frequent passaging.[2]

The primary site of translocation in the intestine is via the M cells. Some strains of *Yec* may cluster on M cells at a density of over 1000X that of the surrounding absorptive villar epithelium.[2] By five days after infection, M cells and Peyer's patches are usually destroyed[2], and the bacteria have migrated to mesenteric lymph nodes.

The contributor mentions several important virulence factors including the Type III secretion system, a unique needle-like system which actively injects yersinial outer proteins (Yops) into cells to aid in evasion of the immune response. A few other virulence factors also bear mentioning: mucoid *Yersiniae* factor, which closely resembles fimbriae of enterotoxigenic *E. coli* and assists in mucosal attachment in early stages of infection, and several heat-stable enterotoxins (which induce diarrhea in infected individuals).[2]

Yersinia enterocolitica has been a popular submission with seven cases in the last decade of the WSC alone (and over 25 since 1975). A list of more recent cases include: WSC 2018-2019 liver and spleen, African green monkey; WSC 2016-2017, Conf 8,

case 4, lung, African Green monkey; WSC 2015-2016, Conf 12, Case 3 liver and cecum, hare; WSC 2013-2014, Conference 1, Case 3, small intestine and lymph node blackbuck; 2011-2012, WSC Conference 3, Case 1, Liver and spleen, guinea pig; and WSC 2010-2011, Conf 15, Case, 1, intestine, Indian macaque.

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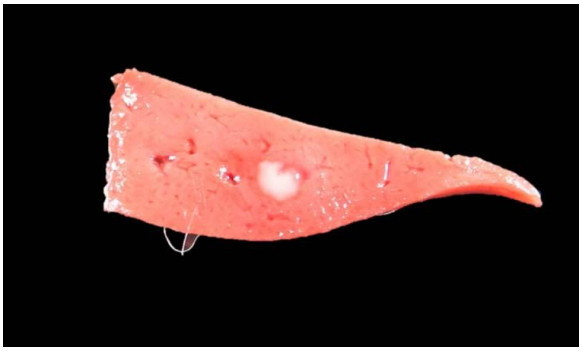
CASE III: Case 1 (JPC 4101226).

Signalment: 8-week-old female Rex cross rabbit, *Oryctolagus cuniculus.*

History: Found dead in the woods

Gross Pathology: The rabbit was one of several young rabbits housed and hand-reared at a rescue organization. Both this rabbit and its litter mate displayed symptoms

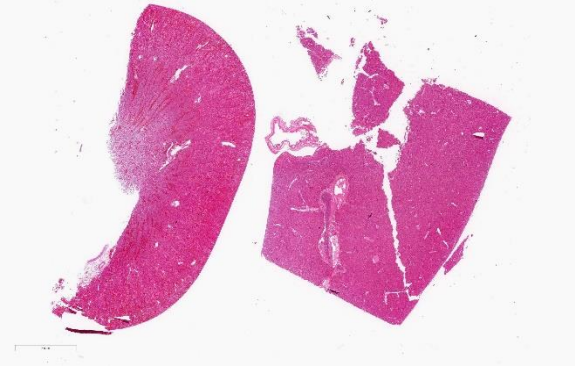


Liver, rabbit. The liver appeared grossly pale and enlarged; three separate white hepatic nodules measuring 3 to 5mm in diameter were present in the parenchyma. (Photo courtesy of: Institute of Veterinary Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand 4442)

of lethargy, anorexia and hypothermia. Within 48 hours of the onset of clinical signs, the rabbit died. The litter mate and several other young rabbits in the organization also died in the days following this death.

Laboratory results: Bacteriology**:** *Yersinia pseudotuberculosis* in liver and spleen.

Microscopic Description:
Despite moderate autolysis of the tissues, the liver has multifocal areas of hepatocytes with reasonable preservation. Scattered amongst these areas, and throughout the hepatic lobule, are swollen eosinophilic hepatocytes with varying degrees of pyknosis, karyolysis and karyorrhexis (necrosis) and marked loss of chord architecture. In addition to these findings, there is marked multifocal distention of bile ducts with periductal fibrosis expanding and compressing the surrounding hepatic parenchyma. The biliary epithelium is hyperplastic and forms papillary projections into the lumen. Numerous epithelial cells contain asexual and sexual developmental stages of coccidia and the biliary duct lumina contain large numbers of oocysts. Moderate numbers of lymphocytes and plasma cells and smaller numbers of heterophils are admixed within the periductal fibrous tissue and the



Kidney, liver, rabbit. Sections of both kidney and liver were submitted for examination. (HE, 5X)

connective tissue stroma of the papillary projections.

The kidney diffusely shows recent vascular congestion and tubular and glomerular hemorrhage. Intracapillary fibrin thrombi are present multifocally in glomeruli. Scattered renal tubular cells exhibit hypereosinophilic cytoplasm, pyknotic nuclei, karyorrhexis and karyolysis (necrosis).

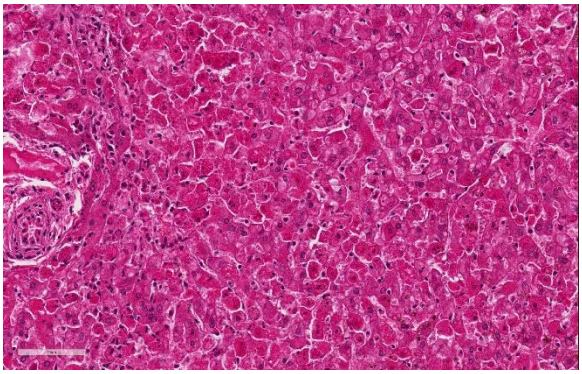
Contributor Morphologic Diagnoses:
Liver: Hepatitis, acute, moderate to severe, random with hepatocellular necrosis
Liver: Cholangitis, proliferative, chronic severe with intraepithelial coccidia
Kidney: Nephritis, haemorrhagic, severe, acute, with multifocal glomerular thrombosis

Contributor Comment: In this case, the rabbit had two concurrent disease processes: hepatic coccidiosis (*Eimeria stiedae*) and rabbit hemorrhagic disease (RHD) caused by rabbit calicivirus. RHD was the cause of death, with hepatic coccidiosis as an incidental finding.

Rabbit hemorrhagic disease or viral hemorrhagic disease is caused by a calicivirus of the genus Lagovirus, which rapidly infects wild and domestic rabbits (*Oryctolagus cuniculi*). The virus was first identified in 1984 in China where it killed 140 million rabbits within months.[21] It was released in Australia and New Zealand as a method of pest control of wild rabbits and is endemic in the populations in those countries.[6,27]

The RHD virus is highly infectious, with a greater than 80% mortality rate, and it usually produces death in affected individuals within 48 to 72 hours of infection.[1,21] Death is due to acute liver damage and disseminated intravascular coagulation (DIC).[24] No clinical signs may be observed if the infection is peracute. Alternatively, they may manifest as anorexia, lethargy, pyrexia, conjunctival congestion and neurological signs such as ataxia, opisthotonos or paralysis in acute infections.[13,29] Other signs such as dyspnea, cyanosis, or hemorrhagic epistaxis may also be seen. Subacute infections result in milder clinical signs, with some rabbits surviving. Many rabbits with chronic infections will die within 1 to 3 weeks after a period of jaundice, weight loss and lethargy.[11]

The primary findings at necropsy include hepatomegaly with an enhanced lobular pattern, splenomegaly, renomegaly, pulmonary hemorrhage and oedema, blood-tinged nasal discharge or bloody foam in the tracheal lumen.[1,14] Additionally, hyperemia or sub-serosal hemorrhages may be found on

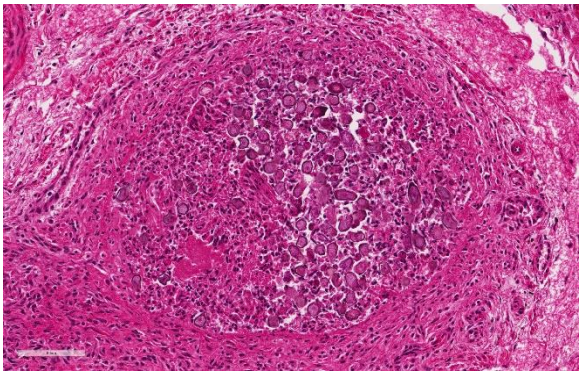


Liver, rabbit. Throughout the entire lobule, the majority (up to 80%) of hepatocytes are individualized, hypereosinophilic, with nuclear changes including peripheralized and crescentic chromatin or karyorrhexis. (HE, 321X)

multiple organs such as the intestine, pericardium, kidney and lungs.[1]

The primary histopathological lesion is an acute necrotizing hepatitis.[2] In adult rabbits, the virus has tropism for hepatocytes and can be detected in the liver a few hours post-infection.[2] Viral replication occurs primarily in hepatocytes in the centrilobular areas but also in Kupffer cells.[2,8-10,22] Additionally, viral antigen has been detected in macrophages of the spleen, alveoli, kidneys and small intestine.[22,23] The virus induces apoptosis in these cells, releasing viral progeny to infect other cells.[7] In younger rabbits, viral antigen has been detected only in rabbits greater than 4 weeks of age.[18,22] Younger rabbits appear to be resistant to the RHD virus. Rabbits less than 3 weeks of age are fully resistant, and this resistance decreases as rabbits increase in age to 8 weeks old, where mortality rates are the same as adult rabbits.[1]

The mechanism of resistance is unclear. It is thought to relate to viral attachment to the carbohydrate group of host-cell histo-blood group antigens (HBGA).[24,25] RHD attaches to HBGA H type 2, A type 2 and B type 2 oligosaccharides which are found on the surface of epithelial cells of the upper respiratory and digestive tracts. The expression of HBGA H type 2 seems to be mostly lacking in the upper respiratory and gastrointestinal tracts of resistant rabbits.[17] However, other factors must be involved in viral attachment to cells as hepatocytes, which are the main cell involved in viral replication, do not contain these HBGA receptors.[25] Additionally, in young rabbits, viral replication occurs only in a small fraction of hepatocytes, indicating other factors are involved in the resistance to this virus.[1]



Liver, rabbit. Bile ductules contain numerous luminal oocysts. The lining epithelium is hyperplastic, but disorganized as a result of autolysis. (HE, 282X)

In this rabbit, hepatic coccidiosis, was an incidental finding that likely contributed to its poor condition. *Eimeria stiedae* is a common cause of morbidity and mortality in rabbits. Ten other species of *Eimeria* spp. infect the domestic rabbits but specifically infect the gastrointestinal tract.[15,17] Clinical signs for hepatic coccidiosis include a thin body condition, diarrhoea, a pot-bellied appearance and, in severe cases, icterus.[21]

Young, weanling rabbits are most often infected when ingestion of the sporulated oocyst from the environment occurs. The oocysts are shed after a prepatent period of 15 to 18 days, and once in the environment, are extremely resistant to disinfectants.[21]

Sporulated oocysts are ingested, where sporozoites are released to invade the duodenal mucosa and lamina propria.[18] It is possible that sporozoites are then transported to the liver via either lymphatic or hematogenous spread. Organisms have been found in macrophages in the lymphatics and in regional lymph nodes within 12 hours of exposure, in bone marrow within 24 hours and in the liver within 48 hours.[18,21] Once in the liver, sporozoites invade the epithelial cells of bile ducts to become trophozoites. Trophozoites undergo the asexual division of schizogony and merogony over several

generations to eventually form the macro- and microgametocytes involved in sexual division. Fertilization of a macrogametocyte by a microgametocyte to form an oocyst which is then shed in the feces.[18,21]

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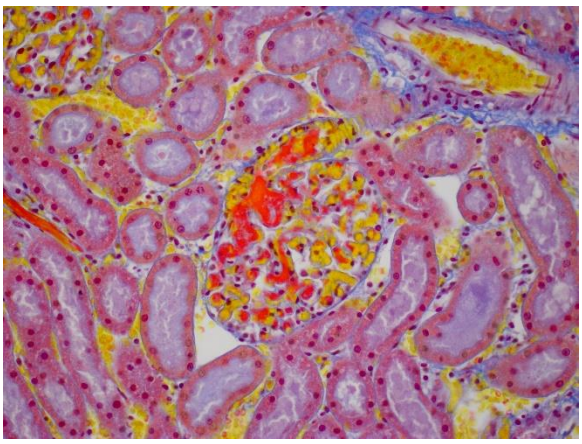
JPC Diagnosis: Liver: Hepatitis, necrotizing (it's really apoptosis), massive, diffuse, severe.
2. Liver, bile ducts: Epithelial hyperplasia, diffuse, mild to moderate, with intraluminal apicomplexan oocysts.

JPC Comment: The contributor has done an excellent review of a virus of global import in this species, as well as well-known common parasite of young rabbits.

The Czech v351 strain of rabbit lagovirus has been used in Australia and New Zealand (following illegal release on the South island) to control pest rabbits for a number of years.[14] However, even before this virus was released into a naïve population, wild rabbits demonstrated cross-reacting antibodies suggesting other similar viruses had previously circulated within this population. The first non-pathogenic rabbit calicivirus was identified in Italy, following seroconversion of animals in a rabbitry with no history of clinical disease. Shortly thereafter, a non-pathogenic strain of rabbit calicivirus with 88% was identified in Australia, and New Zealand. One common factor was that these viruses appeared to prevail in cool high-rainfall areas.[14] Additional "benign" viruses have also been identified in European hares in Australia, with evidence of previous recombination, confirming previous hypotheses of the origin of genetic diversity within this genus of virus.[12] In 2014, a recombinant strain of RHDV was identified in Australia which contained capsid and non-structural genes of non-pathogenic RHDV variants.[12]

Other interesting changes have been noted in RHDV since its release thirty years ago in Australia. In contrast to myxoma virus, which was also released into the wild to control pest rabbits and rapidly attenuated in virulence over time as local rabbits developed genetic resistance, pathogenic genotypes of Australian RHDV appear to have increased in virulence.[7] A comparison study of deaths in a closed population experiencing outbreaks back to the original release of RHDV noted that in outbreaks from 2007-2009 (as compared to the 1990's), more recent outbreaks demonstrated elevation in case fatality rates, disease duration (time to death), as well as the amount of virus produced in infected animals.[7]

The first lagovirus other than RHVD infecting rabbits in the United States was identified by Bergin et al in 2009. This virus, referred to as Michigan rabbit calicivirus



Kidney, rabbit. Fibrin thrombi within glomerular capillaries stains dark red. (Martius scarlet blue trichrome, 400X) (Photo courtesy of: Institute of Veterinary Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand 4442)

occurred in a closed rabbitry with an approximately 32% case mortality and clinical and necropsy findings of epistaxis, vulvar hemorrhage, diarrhea, and ocular discharge. This virus averaged 79% homology with the RNA genome of RHDV virus. The rabbitry was ultimately depopulated.[5]

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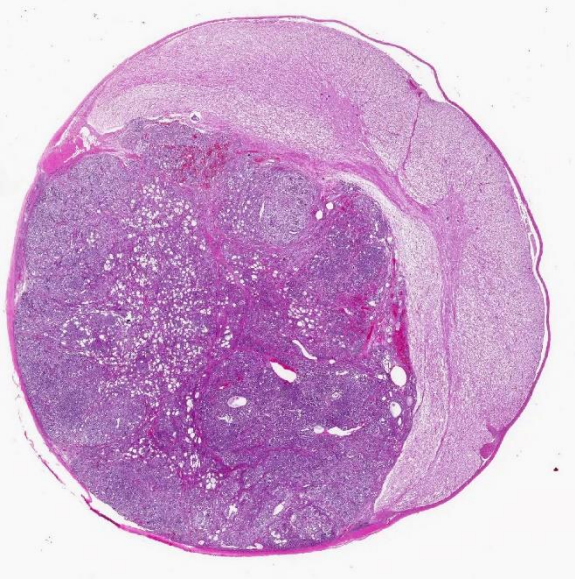
CASE IV: 15-0463 (JPC 4119789)

Signalment: 9-month-old, male-entire, Rottweiler cross canine (*Canis familiaris*).

History: A deceased, male-entire, 9-month-old Rottweiler cross canine was submitted to the Murdoch University Anatomic Pathology diagnostic service for necropsy examination following barbiturate euthanasia. The dog had a progressive 5-week history of non-ambulatory bilateral hindlimb paresis progressing to paralysis and loss of deep pain; there was no history of prior trauma, clinical signs were non-responsive to carprofen. Otherwise the patient appeared healthy and well, and had been eating and drinking normally. At the time of euthanasia it had developed fecal and urinary incontinence and no pain was elicited on



Spinal cord, dog. The spinal cord is compressed by a well-demarcated intradural neoplasm. (HE, 5X)

spinal palpation. A neurological examination carried out prior to euthanasia found the following:

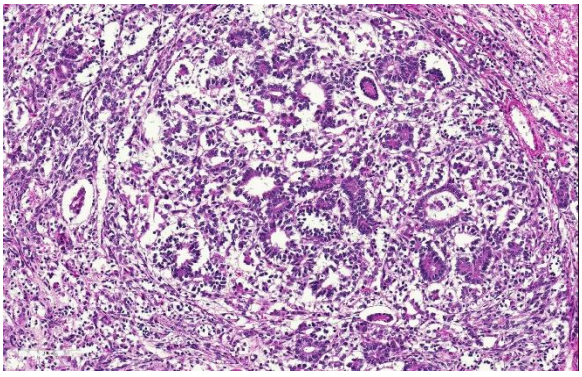
- The panniculus reflex was absent caudal to L4 bilaterally, with a very sharp demarcation.
- Hind limbs: tonic-clonic patellar reflex bilaterally, cranial tibial clonic-tonic on left and intermittently tonic clonic on right. Withdrawal reflex bilaterally intact, sciatic unconvincingly present, absence of deep pain bilaterally on all digits. Negative Babinsky. Negative crossed extensor. Negative superficial sensation. No motor function exhibited.
- Anal tone: decreased to virtually absent.

The cadaver was unfortunately frozen and thawed prior to necropsy examination.

Gross Pathology: A focal, 10mm diameter, well-demarcated, mottled grey to pink-red nodule was identified within the spinal cord at the level of the T13/L1 intervertebral disc; its cut surface bulged slightly. Depending upon the level at which it was sectioned, the nodule was intradural and extramedullary, or intramedullary in location. The dorsal surfaces of the metatarsal regions bilaterally exhibited moderate, multifocal, chronic dermal erosions and ulcerations. The hindlimb musculature was mildly symmetrically atrophied.

Laboratory results: None performed.

Microscopic Description:
Thoracolumbar spinal cord: Effacing the grey matter, extending into the adjacent white matter and compressing the remaining adjacent spinal cord is a non-encapsulated, moderately well-demarcated, mildly infiltrative, moderately cellular neoplasm measuring 10mm in diameter. The neoplasm is composed of three distinct neoplastic cell populations. The first, epithelial, population forms tubules and acini lined by cuboidal, low columnar, to pseudostratified epithelium; frequently, the tubules form papillary projections (glomeruloid structures). 8 mitotic figures are seen in 10 HPF (400x) within this epithelial population. The second population consists of polygonal cells forming dense clumps or occasionally palisading along basement membranes; they possess indistinct cell borders, and a small amount of pink granular cytoplasm (primitive blastemal population). 12 mitotic figures are seen in 10 HPF (400x) within the blastemal population. The third and least abundant, mesenchymal, population is composed of



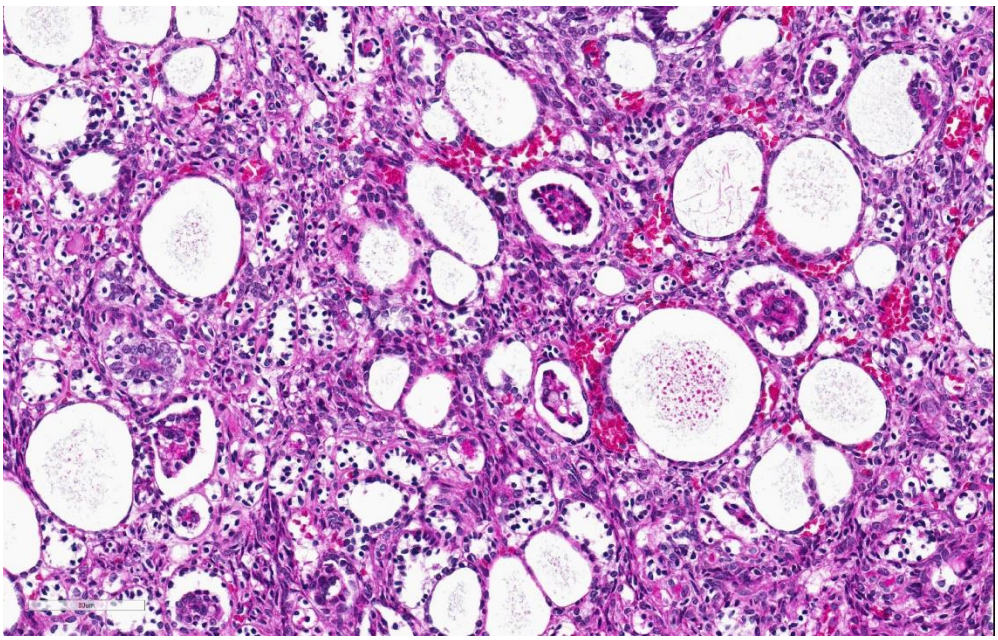
Spinal cord, dog. Neoplastic cells often form well-differentiated tubules, lined by columnar epithelium. (HE, 210X)

spindloid cells, forming loose streams which ramify throughout the other two populations, and occasional whorls. Spindloid cell nuclei are round to ovoid with a single indistinct nucleolus and finely stippled to lacy chromatin; their cytoplasm is eosinophilic with indistinct cell borders. 5 mitotic figures are seen in 10 HPF (400x) within the mesenchymal population. Blood vessels within the neoplasm are distended with erythrocytes (hyperaemia). The remaining spinal cord parenchyma contains large numbers of variably sized clear spaces (rarefaction, freeze-thaw post-mortem artefact).

Contributor's Morphologic Diagnoses: Spinal cord T13/L1: ectopic nephroblastoma.

Contributor's Comment: Microscopic evaluation of the mass found within the spinal cord at the level of T13/L1 reveals a neoplastic infiltrate, which, given the lack of a renal lesion, is consistent with a primary spinal ectopic nephroblastoma.

Unlike primary renal nephroblastomas (Wilms' tumor), which are reported in a wide range of domestic companion and production animal species, including dogs, primary spinal ectopic nephroblastomas are rarely reported neoplasms that occur in young dogs between 5 months to 4 years, the median age being 14 months.[1,4] Whilst they are rare, comprising merely approximately 1% of all canine primary CNS tumors, they are an important differential in the aforementioned age group.[4] Of course, secondary spinal nephroblastomas can arise as a result of metastasis from a primary renal nephroblastoma; however the lungs and liver are more common secondary sites, with over 50% of canine cases of primary renal nephroblastoma exhibiting widespread pulmonary and hepatic metastases.[6] Primary spinal ectopic nephroblastomas typically arise as a single mass, or sometimes multiple masses, at the level of the thoracolumbar junction between T10-L3[3,4] and exhibit both intramedullary and extramedullary intradural growth; extradural growth is also reported.[1,11]

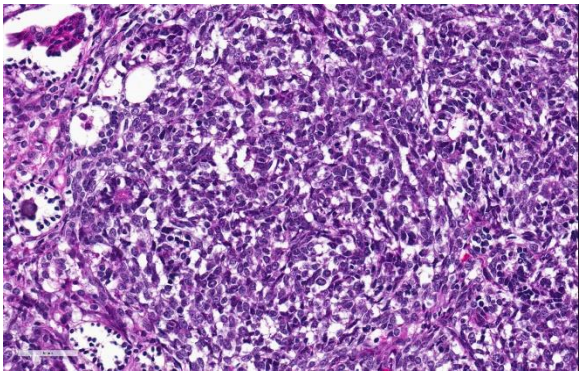


Spinal cord, dog. Ectatic tubules occasionally contain a papillary projection with peripheralized nuclei, resembling a fetal glomerulus. (HE, 300X)

Whilst historically German Shepherds and female dogs were thought to be predisposed[1], a breed and sex predisposition is not supported in more recent reports, with dogs of variable sizes and breeds reported.[3,4,11] Affected dogs exhibit clinical signs of a compressive myelopathy, i.e. progressive

unilateral or bilateral paraparesis, paraplegia, and/or ataxia; the duration of clinical signs is reported to range from 2 to 60 days, with the median being 14 days.[1,4] The prognosis appears variable, with one study involving 11 dogs having found the post-surgical resection median survival time to be 70.5 days;[1] whilst another reported a median survival time of 374 days post-surgery (6 dogs) or radiation (1 dog), and 55 days in 3 dogs only receiving palliative prednisone and gabapentin.[5,11] The latter study also found that tumor location was important for prognostication – dogs with intramedullary nephroblastoma (n = 4) had a much shorter median survival time (140 days) compared to 380 days in the 6 dogs with intradural but extramedullary nephroblastoma;[5] however, the 10 dogs in the study received varied treatment regimens. Previously it was not thought to metastasize,[10] however multifocal spinal nephroblastoma is reported in 2 dogs, consistent with intraspinal metastasis through the cerebrospinal fluid.[3,10] Metastasis to the caudal vena cava, adrenal glands, hepatic and mediastinal lymph nodes, pulmonary interstitium, bone marrow, and periosteum as well as extradural space of two vertebral bodies is reported in a single canine case with a dorsal retroperitoneal mass contiguous with a right renal tumor.[2] However, it is difficult to ascertain whether the primary neoplasm was of renal or spinal origin in the reported case.

Their histogenic origin remains unconfirmed and controversial, given the complexity of nephrogenesis; it is believed they originate from (a) ectopic metanephric blastema or, (b) mesonephric rest tissue, either of which become entrapped between the dura mater and the spinal cord during embryogenesis.[2,9] Nephrogenesis involves two embryologically distinct tissues - nephrogenic and ductogenic; the former developing from the intermediate mesoderm and progressing through the



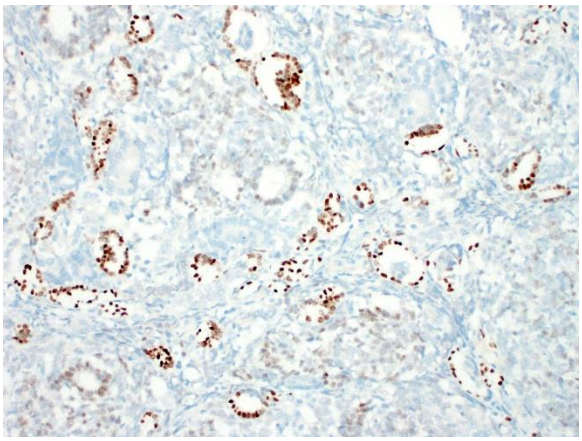
Spinal cord, dog. Less differentiated areas of the neoplasm are composed of nests of polygonal to spindle cells. (HE, 400X)

pronephros, mesonephros and metanephros phases.[12] In humans, this progression normally ceases after 36 weeks gestation, whereupon the renal metanephric blastema disappear.[12] Occasionally, the nephrogenic blastema fail to develop into normal mature renal parenchyma (however, it is not known whether arrest occurs at the mesonephros or metanephros phase in any given case[8]); persistent blastemal tissue is then referred to as nephrogenic rest tissue, which may undergo oncogenesis resulting in nephroblastoma[12] (either primary renal in the case of renal nephrogenic rests, or primary extrarenal in the case of ectopic rests). In humans, extrarenal nephroblastomatosis, i.e. ectopic immature renal tissue, has been reported in such ectopic sites as the inguinal canal, testis, lumbosacral area, adrenal gland, thorax, colon, and heart;[8,12] however, primary extrarenal nephroblastoma is rare, with few cases reported in the retroperitoneum and inguinal canal.[8]

Previously in the canine literature, this tumor was mistakenly thought to be of neuroectodermal origin, leading to its frequent misdiagnosis as one of the other main differentials for intradural spinal cord tumors in young dogs (i.e. ependymoma, medulloepithelioma, neuroepithelioma); it has also been erroneously classified as spinal

cord blastoma, poorly differentiated astrocytoma, and hamartoma.[4,5,10] Teratoma can be excluded due to the lack of other non-nephrogenic tissues within the neoplasm.[12] As seen in this case, ectopic nephroblastomas are characterized by a triphasic neoplastic population, comprised of epithelial, blastemal, and stromal cells, redolent of Wilms' tumor.[3,4,6] Distinctive histological features aiding diagnosis are the presence of tubules and acini, as well as glomerulus-like structures that resemble fetal glomeruli.[3,4] Metaplastic differentiation towards muscle, bone or cartilage is frequently reported in renal nephroblastomas,[6] however is not a feature of spinal ectopic nephroblastomas, with only 1 case reporting metaplastic cartilage formation.[3] Wallerian degeneration (multifocal myelin sheath distension, swollen axons, and myelomacrophages) attributable to a compressive myelopathy is commonly seen; however it was felt in this case post-mortem change due to freeze-thaw artefact obscured ability to observe such changes.

Immunohistochemistry can aid diagnosis, and also supports their ectopic renal origin.[10] The blastemal and mesenchymal (stromal) populations are immunoreactive for vimentin, whilst the epithelial population forming tubules/acini shows immunoreactivity for cytokeratin.[10] Immunoreactivity of polysialic acid to the blastemal nuclei is seen;[4,10] however the definitive antigenic marker is Wilms' tumor gene product (WT-1) – the blastemal nuclei in the glomerulus-like structures are typically strongly immunopositive.[4] There are, however, reports of canine cases that are negative for this marker; presumably due to abnormal antigen expression by tumour cells.[1,6] In human nephroblastoma, a poorer prognosis is seen in cases in which stronger blastemal WT-1 expression is observed,[1] however this correlation has not been found in canines. Primary spinal ectopic



Spinal cord, dog. There is strong intranuclear staining of cells lining differentiated tubules as well as glomeruloid structures (arrow). (anti WT1, 200X)

nephroblastomas lack immunoreactivity with routine neuronal (SYN, NeuN, and TNF) and glial (Olig2, GFAP) markers.[4] Nephroblastomas lack immunoreactivity for GFAP and neurofilaments, yet their epithelial population is immunopositive for cytokeratin, which rules out the main differential diagnoses, the primitive neuroectodermal tumors;[4] in particular, ependymomas, given the perivascular pseudorosettes and true rosettes typical of ependymomas are similar to the tubules found in nephroblastomas.[10]

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JPC Diagnosis: Spinal cord: Ectopic nephroblastoma (spinal nephroblastoma)

JPC Comment: The contributor has done an excellent job summarizing this uncommon and very unique tumor of young dogs.

The Wilms tumor antigen is a tumor suppressor gene that it humans is found at 11p13. It functions as a regulator of transcription in the inner layer of intermediate mesoderm and inhibits transcription of several growth-promoting genes, and plays a critical role in the development of the genitourinary tract, spleen and mesothelium. First identified as a tumor suppressor gene in association with Wilm's tumors in humans, the name WT-1 would lead one to believe that the immunohistochemical stain targeting this gene product would be specific for Wilm's tumor. However, it is also present in a number of normal tissues, including CD-34 positive stem cells, glomerular podocytes and mesangial cells, Sertoli and granulosa cells, ovarian stroma and surface epithelium, uterine endometrial stroma and myometrium, and mesothelium.[7] It is expressed in a wide range of neoplasms in humans, including nephroblastoma, mesothelioma, metanephric adenoma, ovarian carcinoma, transitional cell carcinomas, among others.[7]

While nephroblastomas have been rarely identified as intra- and extradural masses in humans, it has almost always been a consequence of metastasis (10% of Wilm's tumor present with metastatic foci), and less commonly invasive growth through nerve root foramina. Development from rests of ectopic nephrogenic tissue has not yet been documented. Contrarily, there appears to be only one report of spinal nephroblastoma in the dog attributable to metastasis of a renal primary, as opposed to growth of ectopic metanephric blastema.[9]

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