Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE
2020-2021

Conference 13

6 January, 2021

Joint Pathology Center
Silver Spring, Maryland

CASE 1: B-5125-19 (4156736-00)

Signalment:
3-year-old, gender unknown, domestic bovine, *Bos taurus*

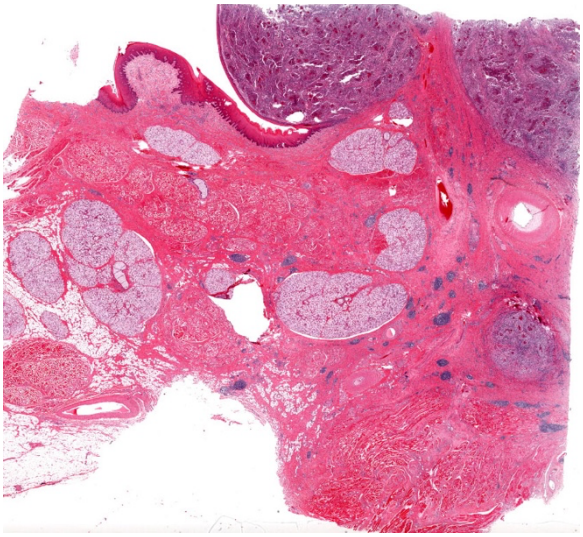
History:
The animal was dyspneic and sent to slaughter. The head and lungs, which appeared abnormal, were collected at the abattoir and submitted to our diagnostic lab for further examination.

Gross Pathology:
The oral mucosa and soft tissues covering most of the hard and soft palate, extending from the rostral maxilla into the pharynx, are extensively ulcerated and markedly thickened by a 2-3 cm thick layer of yellow-tan, solid, slightly firm, homogenous tissue. This tissue often abuts the underlying maxillary bone but does not invade it. Small amounts of cloudy, tan exudate could be expressed from this tissue. Numerous, often coalescing, nodular proliferations of similar tissue, ranging in size from 2-6 cm in greatest diameter are also noted in the buccal mucosa (sections submitted for your examination) and have effaced retropharyngeal and submandibular lymph nodes. Many (> 100) similar, 2-4 cm in greatest diameter, nodules are scattered throughout both the right and left lung lobes.

Oral cavity, ox. View of the hard palate and adjacent maxillary gingiva which are covered and slightly raised by a layer of firm, tissue. The overlying mucosa is extensively ulcerated and red. (Photo courtesy of: Atlantic Veterinary College, University of Prince Edward Island; www.upei.ca/avc)

Laboratory results:
Aerobic culture of samples of affected oral mucosa isolated a heavy growth of *Actinobacillus lignieresii.*

Microscopic description:
The buccal submucosa is raised and contains densely cellular, nodular aggregates of neutrophils and numerous epithelioid macrophages intermingled with fewer plasma cells and lymphocytes. Scattered within inflammatory infiltrates are numerous small clusters of fine, gram negative, coccobacilli which are surrounded by a dense, sometimes undulating layer of hypereosinophilic material composed of tiny, outwardly radiating, blunt,



Oral mucosa, ox. The gingival mucosa is elevated by exophytic inflammatory mass. There is another well-delineated focus of granulomatous inflammation within the submucosa, and numerous lymphoid aggregates. (HE, 5X)

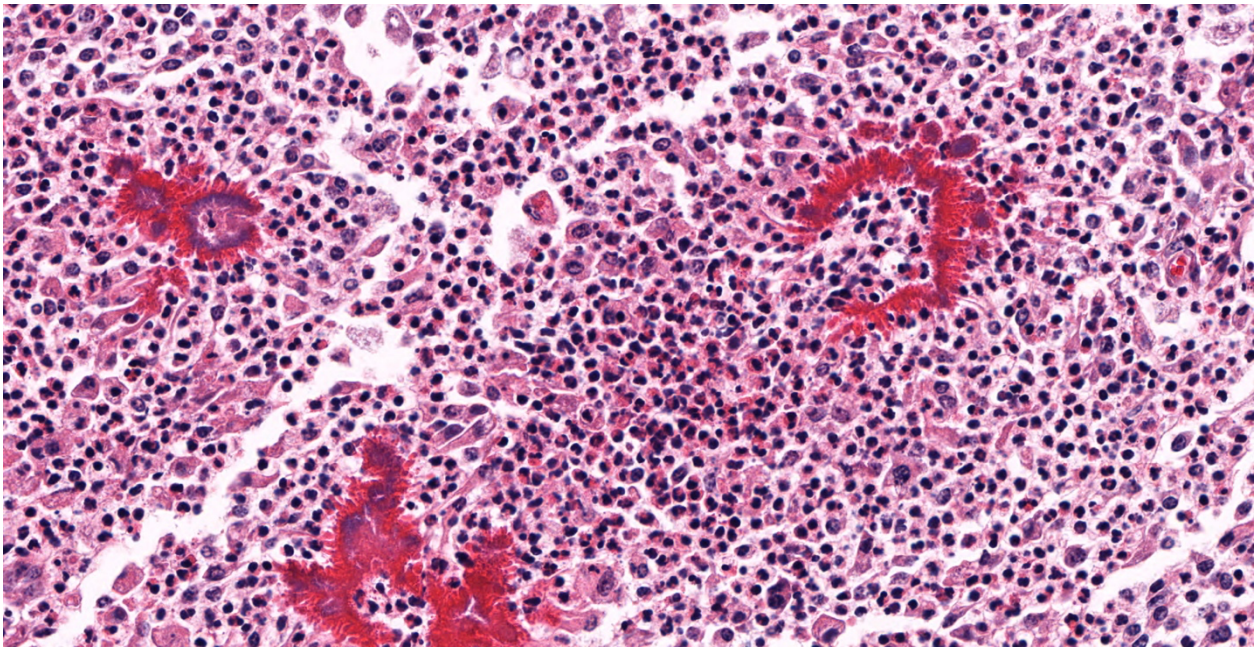
club-like projections (consistent with Splendore-Hoeppli material). The surrounding and deeper submucosa is moderately expanded with large amounts of dense, mature fibrous tissue containing frequent scattered small dense aggregates of lymphocytes and plasma cells. Sections of the regional lymph nodes are largely effaced by similar nodular pyogranulomatous aggregates. The nodules noted in the lung are similar in appearance.

Contributor's morphologic diagnosis:
Oral mucosa: Severe, nodular, pyogranulomatous, stomatitis with intralesional gram negative, coccobacilli surrounded by Splendore-Hoeppli material ("club colonies" or "sulfur granules")

Contributor's comment:
The gross and microscopic appearance of this oral lesion is compatible with a severe presentation of oral actinobacillosis (often referred to as "wooden tongue") due to infection with *Actinobacillus lignieresii*. This gram-negative bacterium is part of the normal oral and rumen flora in cattle and infections have been reported in both beef and dairy herds. Trauma to the glossal and/or oral mucosa, often attributed to the ingestion of rough plants/forages or teeth abrasions, is thought the primary route of bacterial infection in most affected animals. The most common or "typical" presentation of actinobacillosis in cattle is due to bacterial colonization of glossal, oral and/or peri-oral soft tissues resulting in dense foci of pyogranulomatous inflammation or abscesses in which are embedded numerous small dense aggregates of gram-negative bacteria. Bacterial colonies are surrounded by a dense, rim of hypereosinophilic, material consistent with what has been termed Splendore-Hoeppli material. The latter consists of a combination of immune complexes, tissue debris and fibrin and these bacterial aggregates have been referred to as "club colonies" or "sulfur" granules. As in this case, the surrounding soft tissues are often expanded and replaced by marked fibrosis and granulation tissue, which, when involving the tongue, gives it a very firm, immobile, "wooden" appearance .[2,8,10]

Spread of *Actinobacillus lignieresii* infection within soft tissues via lymphatics commonly results in "atypical" forms of the disease characterized by pyogranulomatous inflammation involving regional parotid, retropharyngeal, submandibular, and occasionally, more distant lymph nodes. Infection may extend into the overlying skin which becomes ulcerated and forms draining lesions. Cutaneous, esophageal, lung, and peritoneal pyogranulomas and lesions within the walls of the forestomach, have also rarely been reported in atypical forms of *A. lignieresii* infection, with and without concurrent, oral lesions.[8,9] Hematogenous spread is also likely involved in some cases of disseminated infection. Stress associated with transportation of young cattle was suspected in be a contributing factor in the development of atypical infection in one report.[4] Outbreaks of the disease in cattle have been reported in South America that could not be related to the type of forage fed. Previous viral disease (ie. foot-and-mouth disease) resulting in oral lesions, stress and possibly some degree of immunocompromise was suspected to be a factor in the increased incidence of actinobacillosis noted.[2]

In this case, oral lesions were very severe, involving much of the oral mucosa. Infection was



Oral mucosa, ox. Poorly formed, coalescing pyogranulomas are centered basophilic colonies of 1-2um coccobacilli enmeshed in brightly eosinophilic radiating Splendore-Hoeppli material. (HE, 400X)

also well established in numerous retropharyngeal, parotid and submandibular lymph nodes and myriad embolic pyogranulomas were present throughout the lung. Grossly, these lesions resembled a neoplastic process (such as lymphoma). An underlying cause of such extensive and widespread infection in this case was not identified, but only the head and lungs had been submitted for examination. The dyspnea noted was largely attributed to stenosis of the pharynx due to marked retropharyngeal and pharyngeal lymph node enlargement.

The main differential diagnosis for this oral lesion would be *Actinomyces bovis* infection. The latter is a gram-positive bacteria that also induces oral pyogranulomatous inflammation. This organism typically also invades the underlying bone and causes areas of chronic osteomyelitis ("lumpy jaw" or actinomycosis), findings which were not present in this case. *Nocardia sp*. infection may also produce similar lesions.[2]

Caffarena, et al[2] indicated that *Actinobacillus* sp. infection of lymph nodes of the head of cattle in South America is a relatively common finding in abattoirs that grossly may be mistaken for tuberculosis. Microscopic evaluation, cytologic evaluation of exudates, and/or ancillary bacterial testing would be required to differentiate these conditions.

Although rare, *A. lignieresii* infection is potentially zoonotic. In humans, both acute fatal systemic and chronic localized infections have been reported.[6,9]

Contributing Institution:
Atlantic Veterinary College
University of Prince Edward Island
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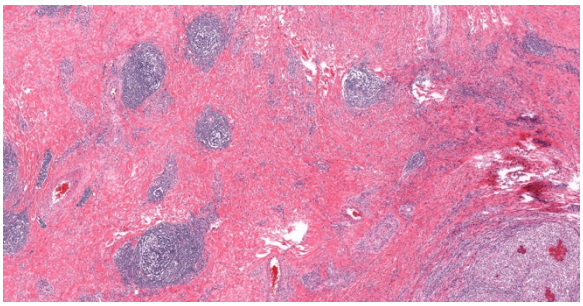
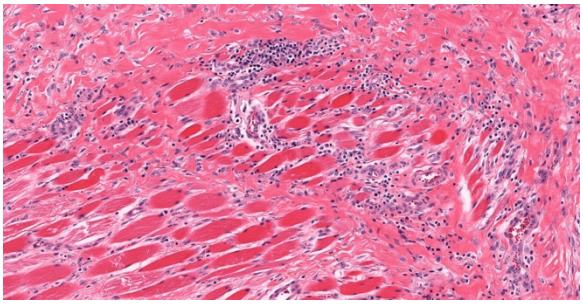


Oral mucosa, ox. There are tertiary lymphoid structures (lymphoid aggregates with nodal attributes) in the submucosa adjacent to a large pyogranuloma (lower right). (HE 41X)



Oral mucosa. The submucosa is markedly fibrotic, with entrapment and atrophy of skeletal muscle fibers. (HE, 182X)

JPC diagnosis:
Oral mucosa: Stomatitis, pyogranulomatous, multifocal to coalescing, chronic, severe, with fibrosis, and colonies of coccobacilli surrounded by Splendore-Hoeppli material**.**

JPC comment:
The moderator reviewed a variety of pathogens most often associated with Splendore-Hoeppli material, which included *Actinobacillus lingnieresii*, *Actinomyces bovis*, *Nocardia* spp, and *Staphylococcus aureus* associated with botryomycosis. The use of gram stains and acid fast stains are most helpful for differentiating between these bacteria.

The contributor provides a succinct summary of this disease of ruminants, and rarely dogs, horses, and rats. *Actinobacillus lignieresii* is a member of the family Pasteurellaceae, and is closely related to *A. pleuropneumoniae*, *A. suis*, and *A. equuli*. While *Actinobacillus* spp are often associated with RTX-type toxins, *A. lignieresii* contains the genes that code for Apx, but lacks a functioning promoter, and so does not elaborate this class of toxin. However, they also produce urease, which releases ammonia from urea. Higher levels of ammonia are chemotactic and activating for neutrophils and macrophages, inhibit phagolysosome fusion, and the increased pH within phagolysosomes reduces the effectiveness of various acid hydrolases. This bacterium also has a capsule that is antiphagocytic and interferes with the membrane attack complex of the complement system, in addition to the lipopolysaccharide (LPS) cell wall.[3]

As noted by the contributor, the Splendore-Hoeppli phenomenon is thought to be an aggregation of protein deposits from antigen-antibody reaction and debris from inflammatory cells. It tends to be arranged in layers of varying thickness but uniform appearance, conferring the radiating attribute to the projecting material.[5] While Splendore-Hoeppli material is most often visualized in histologic sections, it can also be observed in cytologic specimens. In a case of human facial actinomycosis, aspirate smears showed round to ovoid 100-200 μm three-dimensional structures with ill-defined, fuzzy borders. These structures were mats of filamentous *Actinomyces* spp bacteria intermixed with elongated rectangular, rhomboid to rounded, dense, glassy dark green-blue (Diff-Quik) or intensely orange-pink (Papanicoloau stain) crystals, measuring 10-40 x 6-12 μm and found singly or in clusters.[1]

One of the people who first described this host response to antigen was Reinhard Hoeppli, a Swiss-German physician who was a parasitologist by training. He had trained at the Institute of Pathology at Kiel University and then joined the Institut fur Sciffs- und Tropenkraheiten (Institute for Maritime and Tropical Disease) in 1921 as an assistant to Fredrich Fulleborn, the head of the helminthology department. Hoeppli's description of the host response to schistosome eggs embedded in the tissue of a rabbit was the first written in English and was subsequently found to be quite similar to the description of the host reaction elicited by *Sporothrix schenckii* by Splendore in 1908. He eventually renounced his German citizenship and asserted his Swiss citizenship by right of descent. During World War II, he was named the Swiss Honorary Consul in Beijing, which was under Japanese control. He ultimately became acquainted with an Englishman, Sir Edmond Backhouse, an eccentric but charming conversationalist. Hoeppli encouraged Backhouse to record his bizarre and scandalous recollections into two manuscripts, which Hoeppli held until his death in 1973. Unfortunately for Hoeppli, his estate ultimately sent the manuscripts to an Oxford historian, Hugh Trevor-Roper, who found Backhouse to be a forger, charlatan, and fantasist with the documents holding no historical value.

In the process of dismantling the story of Sir Edmond Backhouse, Trevor-Roper was acerbic and disparaging toward Hoeppli, ultimately tarnishing the reputation of a fascinating scientist.[7]

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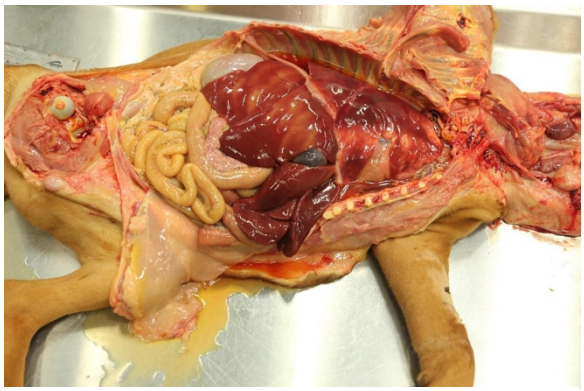
CASE 2: N449-19B (4154915-00)

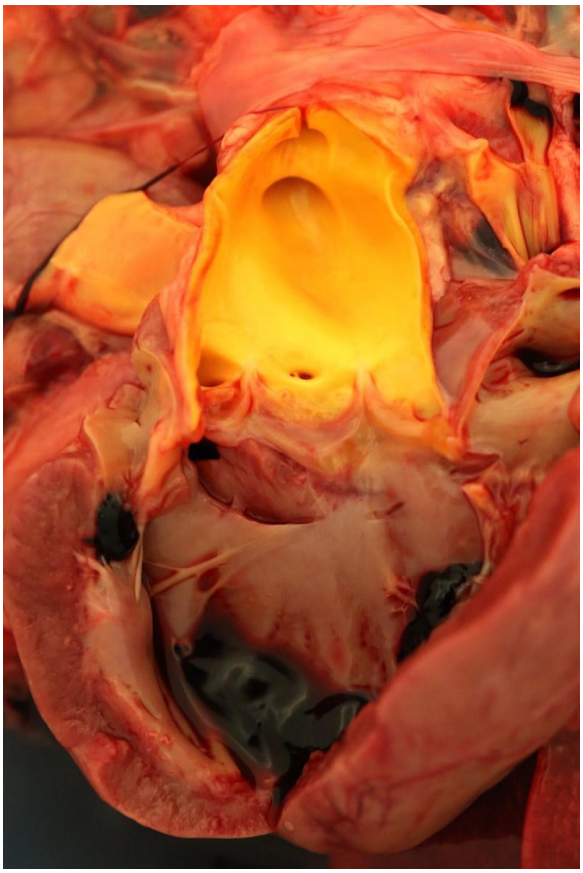
Signalment:
11-week-old, male, Hungarian Vizsla, canine

History:
Died one day following acute onset pyrexia, vomiting, diarrhoea and marked jaundice (icterus). An abdominal scan revealed free-fluid in the peritoneal cavity and bilaterally in the peri-renal regions. The puppy had been given its first vaccination against leptospirosis (*L. canicola* and *L. icterohaemorrhagiae*) and canine parvovirus-2, 2 weeks previously.

Gross Pathology:
Weighed 8.2kg. In good body condition with diffusely yellow mucous membranes, serosal surfaces and sclera. 20ml and 5ml of yellow translucent fluid found in thoracic and pericardial cavities, respectively. Bilaterally the lungs are wet with dark-red mottling. Diffuse yellow staining of pulmonary arterial and aortic endothelia. 30-40ml of yellow translucent fluid in peritoneal cavity. No food in stomach. Gelatinous yellow mucus in small intestinal lumen. Liver diffusely soft featuring pale yellow regions and has a wet glistening capsular surface. Feces scant, mucoid and yellow. Splenomegaly with prominent white pulp on cut section. Sludge-like



Presentation, dog. The cadaver is in good flesh with diffuse icterus. Bilaterally the lungs are wet with dark-red mottling and the liver is flaccid. (Photo courtesy of: Veterinary Sciences Centre, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Irelandhttp://www.ucd.ie/vetmed/)



Aorta, dog. There is prominent yellow discoloration of the aorta. (Photo courtesy of: Veterinary Sciences Centre, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Irelandhttp://www.ucd.ie/vetmed/)

dark green bile distends the gall bladder. Kidneys diffusely pale and tinged yellow with petechiation of capsule. Very small quantity of normal-appearing urine in bladder.

Laboratory results:
Raised alkaline phosphatase, alanine transaminase, total bilirubin and bile acids.

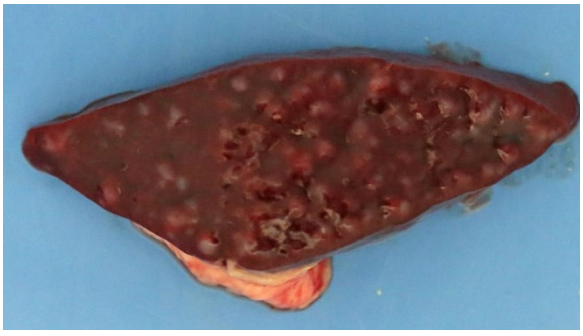
Pathogenic *Leptospira* sp. confirmed by quantitative PCR using primers detecting lipoprotein LipL32 and Taqman probe. Further characterized as *L. icterohaemorrhagiae* by PCR using SYBR green probe to identify species by targeting secY.

Microscopic description:
Liver: diffuse hepatocyte dissociation (loss of inter-cellular connectivity) with approximately 1-2 hepatocytes per HPF exhibiting mitotic activity (hepatic proliferation). Individualized hepatocytes have a more rounded outline and often feature a pyknotic nucleus. Sinusoidal channels are multifocally congested with widespread disruption of their endothelial lining. Diffusely Kupffer cells are prominent (activated). Occasional isolated both necrotic and binucleate hepatocytes noted.

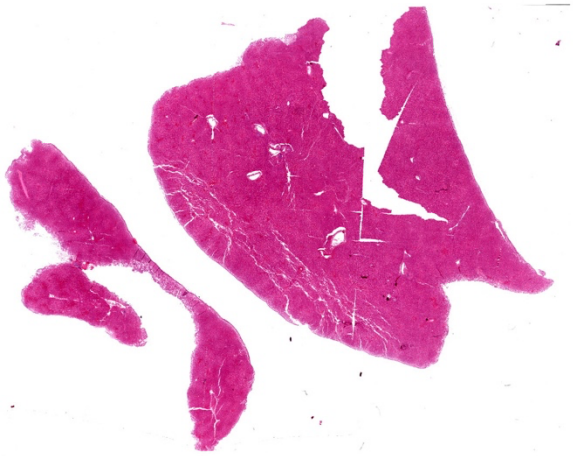
Hyperplasia of periarteriolar lymphoid sheaths noted in spleen along with occasional necrosis of lymphocytes. Glomerular congestion with occasional proteinaceous cast in collecting ducts. Diffuse protein-rich pulmonary oedema with attendant diffuse congestion of alveolar walls and abundant macrophages in alveolar lumens.

Contributor's morphologic diagnosis:
Hepatocyte dissociation, diffuse, marked with occasional individual cell necrosis and attempted regeneration

Contributor's comment:
A diagnosis of leptospirosis was reached based on: signalment, clinical signs, characteristic, microscopically visible hepatic lesions and positive PCR. Acute fatal leptospirosis in dogs presents as fulminant septicemia/hepatic disease largely affecting young animals.[3,9] The often-subtle hepatic changes evident microscopically may present a diagnostic challenge and in many cases the puppy dies prior to the establishment of detectable antibody. In the current case, it would appear that the initial step in the puppy's vaccination protocol did not provide sufficient immunological protection.



Spleen, dog. There is splenomegaly, marked congestion, and prominent white pulp hyperplasia. (Photo courtesy of: Veterinary Sciences Centre, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Irelandhttp://www.ucd.ie/vetmed/)

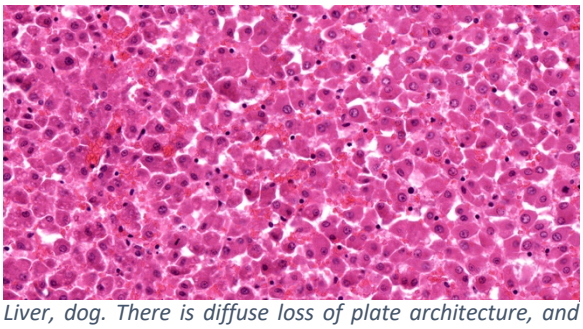


Liver, dog. Several sections of liver are submitted for examination. (HE, 5X)

Canine leptospirosis may occur as peracute, acute, subacute, or chronic disease.[3,9] The peracute/acute form as described here typically results from infection with serovar *Icterohaemorrhagiae*, although clinical disease caused by this serovar (and serovar *Canicola*) are now relatively rare due to widespread vaccination.[10,11] Widespread hemorrhage may be a gross postmortem feature: mucosal hemorrhages, hematemesis, epistaxis, and melena.[3,9] The marked jaundice in this case occurred in the absence of either hemolysis or post-hepatic biliary obstruction. The microscopically visible widespread hepatocyte dissociation described is characteristic and is considered the result of leptospires disrupting inter-cellular tight junctions between hepatocytes. In a hamster infection model leptospires infiltrated the space of Disse, migrated between hepatocytes, detached the intercellular junctions and disrupted bile canaliculi: this disruption coincided with the elevation of conjugated bilirubin, aspartate transaminase and alkaline phosphatase levels in serum.[7] Leptospires have a characteristic spiral shape and corkscrew motion, features considered to facilitate tissue invasion and their ability to bore through highly viscous gel-like media, such as connective tissues, which inhibit the motility of most other bacteria. Bacterial-shaped grooves observed on hepatocyte surfaces are thought to reflect this boring activity which ultimately result in cell from cell detachment.[1] Thus, leptospires are considered invasive but not facultative intracellular pathogens: they can invade but also rapidly exit cells so as to avoid the attention of host leukocytes. How they achieve this high-speed cell translocation remains to be ellucidated.[1] Given a key function of inter-hepatocyte tight junctions is to seal bile canaliculi, their breakdown results in disruption to canalicular structure and thus to bile flow out of the liver: intra-hepatic cholestasis is the consequence. This increased paracellular permeability causes regurgitation of bile constituents into plasma and the attendant hyperbilirubinemia.[2,7]

The fragility of leptospires means they are most effectively transmitted by direct contact. Following penetration of mucosal surfaces or water-softened skin or ingestion of contaminated water, organisms enter the host bloodstream where leptospiremia can last for up to 7 days.[4,10] This persistent leptospiremia would account for the splenic white pulp hyperplasia/activation in this case. In the bloodstream, leptospires evade the host immune response by binding inhibitors of complement on their surface.[10] They invade and multiply particularly well in organs including liver, kidneys, lungs, placenta, and udder. Agglutinating and opsonizing antibody develops at approximately 6 days and clears the organism from most organs except immunologically privileged sites such as the kidney and eye.[3]

Classically, hosts for leptospirosis have been subdivided into 'primary/maintenance' and



Liver, dog. There is diffuse loss of plate architecture, and hepatocytes are disassociated. While there is minimal evidence of degeneration within the cytoplasm, nuclei vary from pyknotic to rrhectic, indicated hepatocellular necrosis. Kupffer cells are activated, as evidenced by prominent elliptical nuclei. (HE, 400X)

'incidental/accidental'.[3] Dogs are the primary host for *Leptospira Canicola* and incidental hosts for *Leptospira Icterohaemorrhagiae* where the primary host is the rat. In dogs that survive acute leptospirosis, the burden of disease shifts from the liver to the kidneys.[3] This is typical of infections caused by serovar *Canicola* and by 'incidental' serovars infecting the dog such as *Grippotyphosa* and *Pomona*. Post-leptospiremic localization of organisms in the kidneys is associated with interstitial nephritis. The organism replicates and persists in the convoluted tubular epithelium, even in the presence of neutralizing antibodies. Acute impairment of renal function may result from decreased glomerular filtration caused by swelling that impairs renal perfusion, along with tubular epithelial swelling and necrosis.[3] Renal function in some dogs that survive acute infections may return to normal within several weeks, or chronic renal failure may develop. Typical subacute renal changes consist of moderate to severe lymphoplasmacytic and neutrophilic tubulointerstitial nephritis, with tubular degeneration, necrosis, and mineralization. In chronic cases interstitial fibrosis and tubular atrophy dominate.[3] Dogs that recover can excrete organisms in their urine intermittently for several months. The WHO consider leptospirosis a neglected but significant zoonosis with global reach.[4,7,10,12]

Dogs with leptospirosis may present with respiratory signs. A similar leptospiral pulmonary hemorrhage syndrome (LPHS) is recognized in human leptospirosis with mortality rates of greater than 50%. The pathogenesis of this syndrome remains to be elucidated but would appear to be mediated by systemic inflammatory and/or immune mechanisms.[10]

Widespread vaccination of dogs against serovars *Canicola* and *Icterohaemorrhagiae* over many decades has resulted in epidemiological shifts to infection with serovars such as *Grippotyphosa* and *Pomona*. This has necessitated the modification of vaccines to provide protection against these and possibly other serovars depending on geographical location.[10,11]

The taxonomy of *Leptospira* remains complex and is based on: (i) serovar - reflecting differences in response of host antibody to the organism's lipopolysaccharide structure; (ii) serogroup - groups of antigenically related serovars; and (iii) genomospecies -organisms grouped based on genomic similarity.[4,11]

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JPC diagnosis:
Liver: Hepatocellular dissociation, diffuse, severe, with random individualized hepatocellular degeneration and necrosis.

JPC comment:
The contributor provides an excellent summary of this interesting disease. This case may be consistent with previous reports of chronic hepatitis caused by *Leptospira* spp, without renal involvement, in kenneled Foxhounds and a colony of Beagles in England, and a second study of 10 dogs of a variety of breeds. The case selection criteria included dogs with granulomatous chronic hepatitis, a definition provided by the World Small Animal Veterinary Association (WSAVA). These dogs were asymptomatic but had elevated hepatocellular enzymes (ALT) and cholestatic enzymes (ALP), and many had elevated bilirubin, bile acids, and cholesterol. Using fluorescence in situ hybridization (FISH) on liver biopsies, clusters of leptospires were found in 8/10 dogs, with PCR confirming bacteria as *L. interrogans* or *L. kirschneri*. Of these 10 dogs studied, 9 had previously been vaccinated against Leptospirosis, highlighting the potential for infection by serovars not present in vaccines.[5]

Because this is a zoonotic disease, it is prudent to understand the prevalence and incidence of Leptospirosis in wild populations of animals, in order to more effectively evaluate the domestic-wild animal interface as a potential risk factor for infection. A recent study investigated 98 animals (domestic and wild) found in areas adjacent to the

Barcelona Metropolitan Area in Spain. While no statistically significant differences were observed between groups comprised of wild carnivores versus domestic carnivores, or between wild carnivores versus free-roaming cats, there was an overall prevalence of approximately 8.5%. This result corroborates previously published results in other studies, where prevalence varied based on infection host, geography, and proximity to water.[6]

Reports of Leptospirosis in cats has been infrequently published, though the prevalence of antileptospiral antibodies varies from 4% to 33.3%, geography dependent. With the consumption of infected rodent prey being implicated in transmission, cats spending time outdoors are at an increased risk of infection. Additionally, cats can be asymptomatic carriers, and continue to shed Leptospira in their urine, contaminating the environment and potentially increasing risk to human populations. The most frequently isolated serovars in infected cats in the United States include Australis, Autumnalis, Grippotyphosa, and Pomona. Interestingly, the serovars most often reported in clinical cases of acute leptospirosis include Autumnalis, Australis, Icterohaemorrhagiae, Grippotyphosa, Pomona, and Sejroe. The incomplete overlap between commonly detected versus clinical disease illustrates our incomplete understanding of this bacterium, and which serovars may cause only incidental infections. Cats are not currently vaccinated against Leptospiral infection, but an effective vaccine would be multivalent to cover many serovars.[8]

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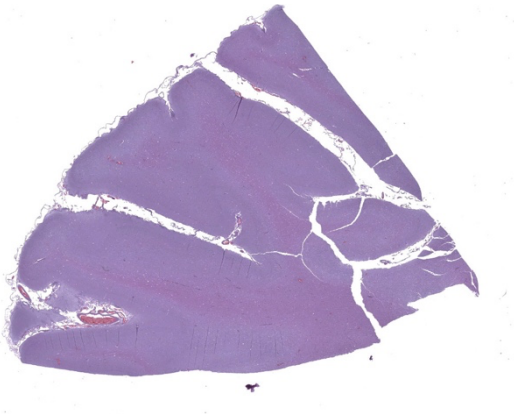
CASE 3: N-361/19 (4156029-00)

Signalment:
Adult, female, striped dolphin (*Stenella coeruleoalba*)

History:
The dolphin was found swimming in circles at 100 meters from the seaside, in Tamariu, Girona (Catalonia). The external examination revealed marked dehydration and increased heart and breathing rates. One hour later, the dolphin started to show seizures, and due to the poor prognosis, she was humanly euthanized and



Cerebrum, striped dolphin. A single section of cerebrum is presented for examination. There are no apparent lesions at subgross magnification. (HE, 5X)

submitted for a complete post-mortem examination.

Gross Pathology:
The dolphin (length: 175 cm, weight: 56.5 Kg) was well preserved and had a moderate loss of body condition. External parasites were not present. No ingesta was found in the forestomach, and the secretory stomach was empty. No other relevant gross lesions were found. Common endoparasites of the species, such as *Phyllobothrium delphini* and *Monorygma grimaldii* larval forms, were found in low numbers.

Laboratory results:

- RT-PCR Cetacean Morbillivirus (CeMV):
  - CNS (brain cortex) positive.
  - Lymph-node, lung and spleen: negative.

- Immunohistochemistry (IHC) CeMV:
  - brain cortex: positive.
  - Lung, lymph node, spleen: negative.

Microscopic description:
With a patchy pattern throughout the cerebral cortex, and specially the left hemisphere, there is a severe chronic inflammatory process mainly located in the grey matter. Due to this uneven distribution, the microscopic lesions described might not be present in all the slides.

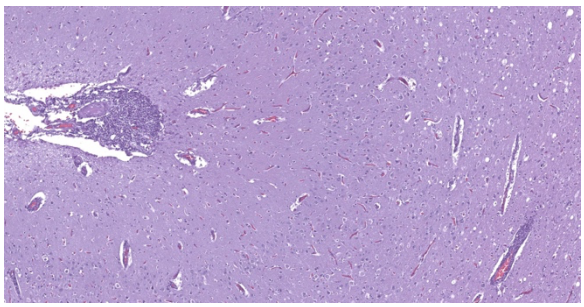
The affected areas of the grey matter show a mild to moderated distortion of the normal texture and architecture of the neuropil due to vacuolization (spongiosis) and loss of neuronal bodies, which are replaced by aggregates of activated astrocytes and microglia (glial foci). Numerous of the remaining neurons show margination of the Nissl granules to the periphery (central chromatolysis) or different features of degeneration and necrosis such as a shrunken and hypereosinophilic cytoplasm, pyknosis and loss of the cell nucleus. These neurons are surrounded by activated phagocyting microglia (neuronophagy) or glial cells (satellitosis). Within the nucleus of many of these neurons, there is a single round, 2 to 7 micrometers in diameter, pale eosinophilic viral inclusion.

Multifocally, the meninge and Virchow-Robin spaces are expanded by up to 10 cells thick mononuclear perivascular cuffing composed of lymphocytes, plasma cells and occasional histiocytes. There is also moderate perivascular edema which extends towards the adjacent neuropil.

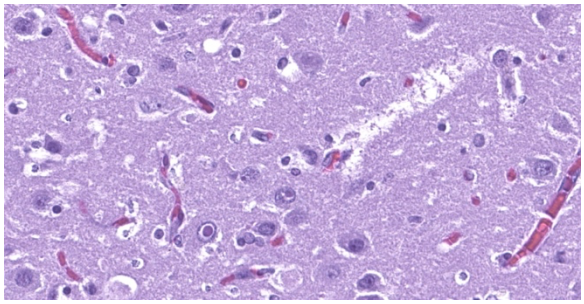
Multifocal areas of spongiosis, astrocytosis and astrogliosis can be seen in the white matter.

Contributor's morphologic diagnosis:
Brain (left cortex). Multifocal, chronic, severe mononuclear (lymphoplasmacytic, histiocytic) meningoencephalitis with nuclear viral inclusions.

Contributor's comment:
Cetacean morbillivirus (CeMV) was first reported in 1990 in the Western Mediterranean causing a mass mortality in striped dolphins.[4]



Cerebrum, striped dolphin. There are aggregates of numerous lymphocytes and plasma cells, and fewer macrophages within the meninges (left) and extending downward along Virchow-Robins spaces. (HE, 94X)



Cerebrum, striped dolphin. Scattered neurons contain a single large eosinophilic viral inclusion which may be surrounded by a clear halo or fill the nucleus, peripheralizing chromatin. (HE, 679X)

There are at least three well characterized strains distributed worldwide, including the porpoise morbillivirus (PMV), dolphin morbillivirus (DMV) and pilot whale morbillivirus (PWMV). Recently, new strains have been also described in a Longman's beaked whale (*Indopacetus pacificus*) from Hawaii, in a Guiana dolphin (*Sotalia guianensis*) from Brazil, and in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) from Western Australia.[1] CeMV is a relevant cause of mortality cetaceans, causing pneumonia, lymphoid depletion, and encephalitis, as well as immunosuppression and facilitation of secondary opportunistic infections.[5] Morbillivirus transmission is thought to occur mainly by inhalation of aerosolized virus shed by infected animals, but transplacental transmission has also been reported. Moreover, transmission between groups is facilitated due to the migratory behavior.

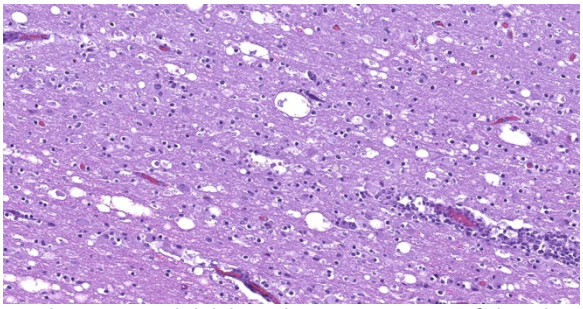
After infection by CeMV, there is an initial replication in lymphoid tissues and a subsequent systemic dissemination, reaching other cell types, mainly epithelia. As a result of lymphoid depletion, there is a severe immunosuppression, hence secondary infections are a common finding in CeMV-affected dolphins. Although CeMV can be pancytopathic, the main damaged cells are those of the respiratory, lymphoid and central nervous system.[13]

In the Mediterranean Sea, CeMV has caused two well-documented mass mortalities in 1990–1992 and 2006–2008, mainly in striped dolphins, but also in long finned pilot whales (*Globicephala melas*).[6,9] During the interepizootic period, all the necropsied animals in the Catalonian coast were immunohistochemically negative for CeMV, hence it was assumed that CeMV was not circulating in that period.[11]

CeMV circulation and death of dolphins also occurred in 2012 and 2016-2017 in the same area, although apparently with a much lower mortality. In the four years after the two main epizootic waves, cases with exclusive affection of the central nervous system were observed and were referred as chronic CNS infections.[12] These cases were interpreted as reactivation of a chronic infection, similarly to subacute sclerosing panencephalitis (SSPE) in humans or old dog encephalitis (ODE) in dogs, but a definitive proof is lacking. Similar encephalitis cases have been observed after a recirculation of CeMV in striped dolphins in the period 2016-2017. The submitted case is one of these cases. What is peculiar of these encephalitis cases of is the absence of lesions and CeMV antigen or its genomic material outside the CNS. Viral antigen, demonstrated with IHC against the N-protein with a MoAb raised against CDV (VMRD) shows accumulation of antigen in cell body and processes of neurons, often without evidence of clear cytopathic effect.

Summarizing, different forms of CeMV infection may be observed:[1]

1. *Acute systemic disease*
   Characterized by severe multifocal to diffuse interstitial pneumonia with necrosis of bronchiolar epithelium, formation of large syncytia and nuclear viral inclusions in syncytia and epithelial



Cerebrum, striped dolphin. There is spongiosis of the white matter, numerous dilated myelin sheaths, swollen axons (arrows) and numerous lymphocytes and plasma cells within Virchow-Robins' spaces. (HE, 242X)

cells of the lung. There is also diffuse lymphoid depletion with germinal center necrosis, with syncytial cells. Multifocal non-suppurative encephalitis may also be present. In such cases, there is a strong immunohistochemical (IHC) staining in the organs affected.

2. *Sub-acute systemic disease*
   Animals that survive the acute infection and present secondary infections such as aspergillosis, *Toxoplasma gondii,* or herpesviruses. The lesions of the acute form may not be present or can be obscured by these secondary processes. Both IHC and RT-PCR are useful for confirmation of the diagnosis.

3. *Chronic, localized encephalitis*
   This form is hypothesized to be the result of clearing the initial systemic infection and of reactivation restricted to the CNS, with absence of lesions and antigen outside of this organ. In these cases, there are occasional nuclear viral inclusion and no syncytia. Besides, there is a strong IHC staining of the neuronal processes with a patchy pattern, mixing areas of high-antigen content with others free of antigen. A cell-to-cell spread of infection is suggested for these cases, rather than a multifocal infection indicative of blood-borne infection. This form shares multiple characteristics with subacute sclerosing panencephalitis (SSPE) and old dog encephalitis (ODE), which are chronic latent localized infections affecting humans and dogs. The most prominent lesions are perivascular cuffing, diffuse gliosis and glial nodules with neuronophagia. Demyelination is less prominent in dolphins compared with ODE. In these cases, IHC and RT-PCR for CeMV are also helpful tools to reach a final diagnosis.

4. *Subclinical infection*
   The existence of a subclinical CeMV infection in cetaceans has been suggested but remains speculative. These subclinical infections would help to disseminate the virus to long distances.

The present case is a florid example of a chronic localized encephalitis, with many evident eosinophilic nuclear viral inclusions. The multifocal pattern is one of the characteristics of this stage, as well as the strong IHC staining of the neuronal processes also observed in this dolphin. As expected in such cases, no other lesions were found in the lymphoid and respiratory system. Furthermore, only the CNS sample resulted positive to CeMV RT-PCR and IHC (lung, lymph-node and spleen were negative).

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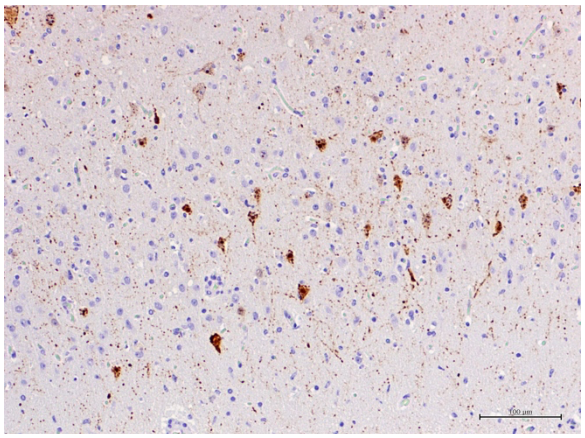
Barcelona, Spain
https://www.uab.cat/web/els-serveis/-servei-diagnostic-de-patologia-veterinaria-1297063220061.html

JPC diagnosis:
Cerebrum: Meningoencephalitis, lymphohistiocytic and necrotizing, multifocal to coalescing, moderate, with gliosis, spongiosis, and intracytoplasmic and intranuclear viral inclusions.

JPC comment:
The contributor provides a good summary of Cetacean morbillivirus as we understand it today.



Cerebrum, striped dolphin. There is abundant immunostaining of the neuronal bodies, dendrites and axons as well as the nuclear viral inclusions. (anti-CeMv, 400X)

There was discussion about which cells contain the viral inclusions. Participants had a reasonable degree of certainty that both neurons and astrocytes contained viral inclusions, and that the perivascular lymphocytes may also have contained viral inclusions.

Morbilliviruses use two cellular receptors, signaling lymphocyte activation molecule (SLAM), and nectin-4. Unlike nectin-4, which is highly conserved across species, the SLAM/CD150 molecule is more divergent and specific to different species of hosts. The structure of SLAM is based around 35 residues that allow it to bind to the viral H protein. The 35 residues of interest are conserved between pinnipeds and are highly similar to the residues of the suborder *Caniformia* (dogs, otters, mink), suggesting a potentially large community for which both canine distemper virus and phocine distemper virus may infect. The set of 35 residues is more divergent in cetaceans, potentially suggesting an encoded H protein with broader binding specificity to host receptors in these populations.[8]

There is evidence that this virus is capable of cross-species transmission, with the Dolphin morbillivirus (DMV) strain's ability to use both dolphin and seal SLAM/CD150 as host cell receptors. Using Bayesian phylogeographic analysis, CeMV is estimated to have a mutational rate of 2.34 x 10-^{4} nucleotide substitutions/site/year, regardless of host or geographic region.[7] The rinderpest virus (or of closely related cattle origin) is believed to have crossed the bovine-human barrier 1000-5000 years ago, leading to an offshoot that became the measles virus. A common ancestor between rinderpest virus and cetacean morbillivirus has been hypothesized, though it may be equally likely that CeMV originated directly from rinderpest virus. The ability for these viruses to cross species increases the likelihood that new morbillivirus species will continue to evolve and affect animals, both on land and sea.[3]

Knowledge gaps still exist in our understanding of this virus and disease. While some receptor interactions are relatively well characterized, characterization of cell receptors in the central nervous system may help define its neurotropism. We also do not yet know the magnitude or contribution from environmental contaminants on the host's manifestation of disease. Toxins like polychlorinated biphenyls, methylmercury, dioxins, and others, accumulate in long lived marine animals as a process of bioaccumulation (also termed biomagnification). We do not yet know how predominantly Th1 response differs from predominately Th2 response in the host. Different characterization of host immune response is critical for the development of many known disease, such as AIDS in humans. Similarly, CeMV has been identified in newborns and fetuses. The host immune response is often operating at reduced efficiency during pregnancy, and we do not yet understand what effect that may have on the development of disease.[2]

During the case discussion, a pathologist raised the possibility that this striped dolphin had a co-infection that worsened the meningoencephalitis. A recent study of viral, bacterial, protozoal, and metazoan causes of cetacean meningoencephalitis, viral etiologies (CeMV, Herpesvirus) were the most common cause, but generally only resulted in minimal or mild non-suppurative meningitis. However, the animals that presented with more severe cases of meningitis had a coinfection with *Brucella* sp. Unfortunately, a culture was not performed in this case.[10]

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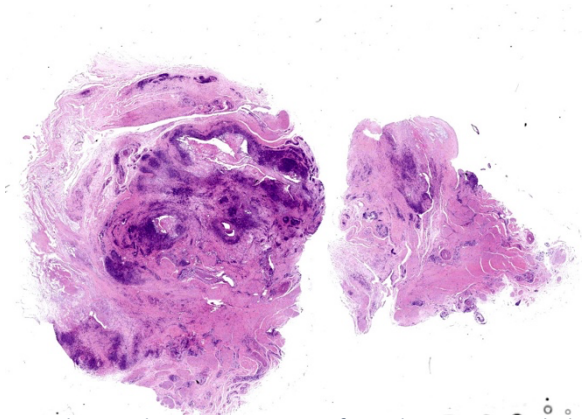
CASE 4: C008 (4153941-00)

Signalment:
6-year-old, female, Rasa Aragonesa, Ovine (*Ovis orientalis aries*)

History:
The animal was part of an experimental study that aimed to characterize the evolution of arthritis in sheep naturally infected by small ruminant lentiviruses (SRLV). At clinical examination, the animal showed bilateral asymmetric carpal arthritis and mild diffuse increase of respiratory sounds.



Carpal joint, sheep. Two sections from the joint, to include the thickened joint capsule and synovium are submitted for examination. (HE, 5X)

Gross Pathology:
At necropsy, the sheep showed body score of 2/5. Carpal joints were swollen and showed marked synovitis and moderate cartilage erosion. Lungs showed rubbery consistence and were diffusely increased in size.

Laboratory results:
The animal was seropositive to small ruminant lentivirus (SRLV, Elitest MVV/CAEV, Hyphen Biomed). Carpal joint qPCR (EXOone Maedi Visna – CAEV, Exopol) were performed on carpal swaps and the results were Ct: 35,07 and Ct: 32,47, respectively for each carpi (considered positive if Cq≤38). qPCR against *Mycoplasma agalactiae, Chlamydia abortus, Streptococcus dysgalactiae, Erysipelothrix rhusiopathiae* were negative.

Microscopic description:
Joint capsule, synovia. Diffusely expanding the stroma there is an inflammatory process associated with moderate synovial membrane villar proliferation. Stroma in thickened (up to 1 cm) by abundant lymphocytes, plasma cells and macrophages occasionally arranged in lymphoid-like structures and rare neutrophils admixed with increased numbers of plump fibroblast and matures collagen fibers (fibrosis) and proliferation of neovessels. Additionally, some macrophages contain abundant brown-golden intracytoplasmic pigment (hemosiderin) and

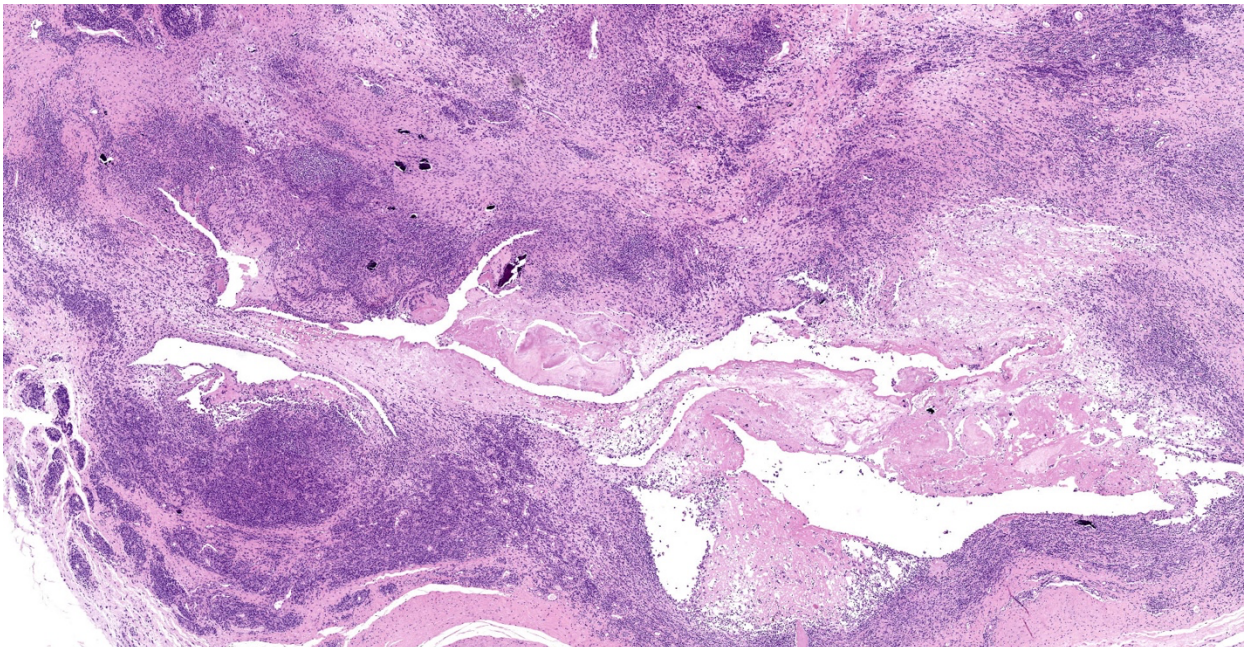
there are multifocal areas of basophilic mineral deposition (calcification). Stromal arteries show marked thickening of the tunica media with smooth muscle hypertrophy and reactive endothelium and lymphatics are dilated. Diffusely, synovial membrane is thickened (up to 3 times) and show fingerprint projections towards the lumen lined by swollen synoviocytes with finely vacuolated cytoplasm (hypertrophy) that occasionally piles up (hyperplasia). Additionally, there are multifocal areas of synoviocyte loss (erosions and ulcers) and replacement by moderate amounts of fibrillary eosinophilic polymerized material (fibrin), occasional cellular debris and rare neutrophils.

Contributor's morphologic diagnosis:
Joint capsule, synovia. Lymphoplasmacytic synovitis. Diffuse. Severe. Chronic. With synovial hypertrophy and hyperplasia and stromal fibrosis.

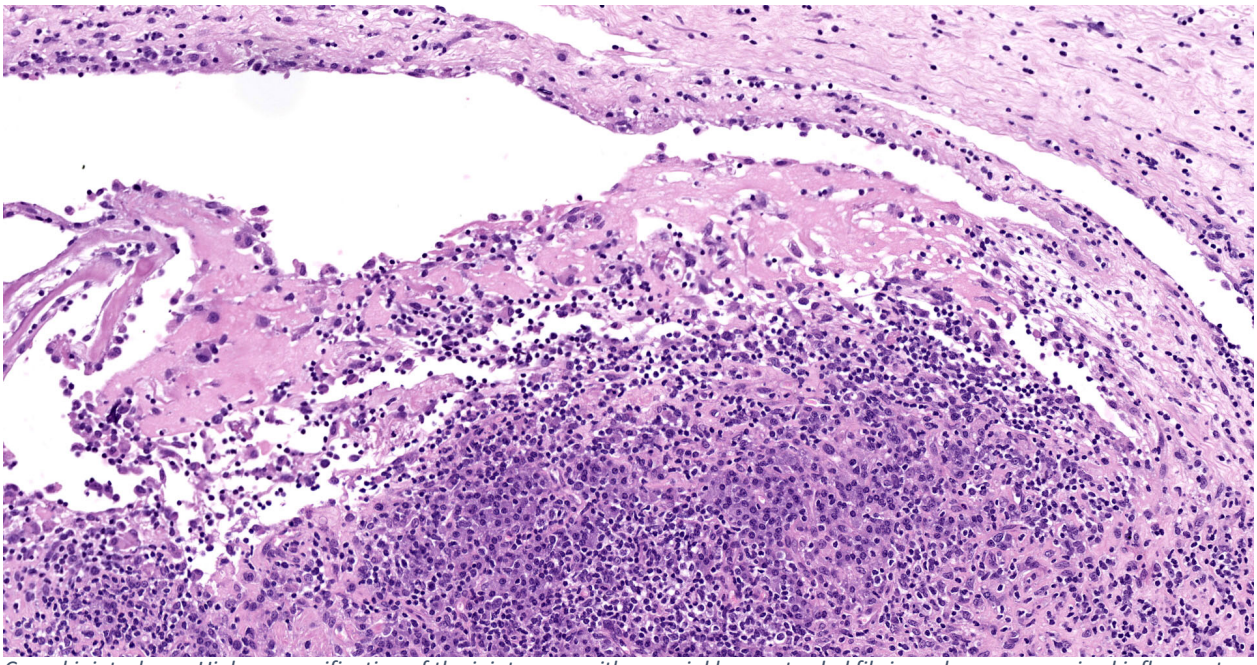
Contributor's comment:
This is the articular form of small ruminant lentiviruses (SRLV), a highly prevalent chronic infection of sheep and goats linked to important deleterious effects in animal production and animal trade restrictions.[4]

The SRLVs are a group of non-oncogenic exogenous retroviruses with marked tropism for the mononuclear-phagocyte system. SRLV are single stranded RNA viruses with high mutational potential.[13] Indeed, four main genotypes (A, B, C, and E) and more than 35 subgroups with significant antigenic heterogeneity have been already characterized.[8] These viruses induce chronic and persistent inflammation that usually remains subclinical. When it becomes clinical, the disease can express mainly in four different locations: lung, joints (mainly carpus), central nervous system, and mammary gland.[9] The expression of each form as well as the severity of the lesion depends on the host immune response and the viral factors.[5,11]

The main routes of vertical and horizontal transmission are via colostrum and via inhalation of nasal secretions, respectively.[9] The virus infects a variety of cell types (mammary epithelium, fibroblast, endothelial cells, monocytes, choroid plexus) but its replication mainly occurs in mature macrophages.[13] Lentiviral infected macrophages produce cytokines that recruit and activate other leukocytes but also enhance lentiviral replication.



Carpal joint, sheep. The joint capsule contains fibrin and necrotic debris, and there is marked inflammation within the synovial lining. (HE, 64X)



Carpal joint, sheep. Higher magnification of the joint space with synovial loss, extruded fibrin and numerous mixed inflammatory cells within the joint space and underlying synovium. (HE, 128X)

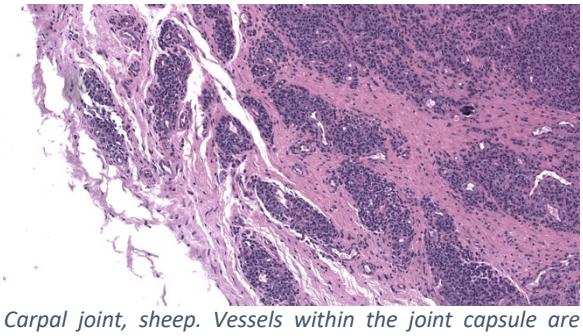
Indeed, clinical signs and histopathological lesions are the result of the inflammatory process instead of direct viral damage to the organ. There is neither treatment nor commercial vaccines for the disease, what makes challenging the immunization of the ovine population against the virus.[14]

The articular form, characterized by arthritis, is mainly caused by genotype B2 strains.[7,10] It mainly targets carpal joint although tarsal joints may also become affected. Typical gross findings include, serosanguinous joint effusion, thickened joint capsule and tendon sheaths, fibrin attached to synovium, and hygromas (flattened, cystic, subcutaneous distensions over the anterior carpus filled with serosanguinous fluid containing fibrinous or gelatinous masses). In most severe cases, fibrillation and erosion of cartilage can be present. Histologically, lentiviral arthritis is characterized by villous synovial hyperplasia, subsynovial lymphoplasmacytic inflammation with occasional lymphoid follicle formation and stromal fibrosis.

The respiratory form is the most common presentation of the disease in sheep and consists of progressive pneumonia with marked dyspnea and weight loss.[2] Lungs are characterized by a rubbery firm texture and lack of collapse when the thorax is opened. Indeed, this animal presented with a diffuse interstitial pneumonia with BALT hyperplasia.

The neurologic form is characterized by demyelinating leukoencephalomyelitis that leads to ataxia and profound weight loss.[12] This form progresses faster than the respiratory one and usually occurs in adult sheep and goat kids (2-4 months).

The mammary form is difficult to detect and mainly consists of mammary gland induration, progressive bilateral atrophy, and milk



Carpal joint, sheep. Vessels within the joint capsule are surrounded by large numbers of lymphocytes and plasma cells. (HE, 200X)

production loss that leads to deficient lamb growth.[6,9] Both mammary glands are usually symmetrically affected. Microscopical lesions are characterized by abundant lymphocytes and plasma cells that infiltrate the interstitial and periductal connective tissue and even form lymphoid nodules. In advanced lesions degeneration and loss of acinar and ductal epithelium may happen as a consequence of inflammation rather than direct virus effect.

The main differential diagnosis for this kind of arthritis in the Mediterranean countries are *Mycoplasma agalactiae, Chlamydia abortus, Streptococcus dysgalactiae, Erysipelothrix rhusiopathiae*.

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Departamento de Patología Animal
https://patologiaanimal.unizar.es

JPC diagnosis:
Joint capsule: Synovitis, proliferative and lymphoplasmacytic, chronic, diffuse, marked, with fibrin, synovial ulceration, granulation tissue, and mineralization.

JPC comment:
Numerous countries have active eradication programs for small ruminant lentivirus (SRLV), including Belgium. Recent phylogenetic analysis of the *gag-pol* region of samples from sheep and goats of various provinces in Belgium showed that 13 of 14 sheep SRLV strains belonged to subtype A1, with 7 of 7 goat SRLV strains belonging to subtype B1. One sheep sample was found to be of the B1 subtype, illustrating the ability of these viruses to cross species and cause disease. Interestingly, analysis of the *pol* region of sheep all clustered in subtype A1, but three goats clustered in subtype B1, two clustered in subtype A1, and two samples clustered in a new separate cluster within genotype B. The new subtype has been designated subtype B5.[8]

A robust monitoring and eradication program was tested in Switzerland when a flock of seropositive goats was imported. ELISA and western blot results were consistent and indicated a high prevalence of infection in the new animals. Isolation of the virus was most successful from the spleen, confirmed B1 subtype, and PCR analysis of the *gag-pol* and *env* regions revealed a high degree of heterogeneity within viral sequences, indicating multiple infections within single animals, and within the flock. Excellent infrastructure and communication allowed this program to succeed, in large part.[3]

Numerous aspects of the host immune response have previously been investigated, including Toll-like receptors, antiviral proteins (APOBEC family, tetherin, TRI-M5alpha), and various cytokines, with recent research focused on miRNAs. A total of 212 miRNAs were identified, with 46 highly conserved across species. However, 12 novel miRNAs were identified in infected sheep. Differences were found in expression of these miRNAs between uninfected sheep versus seropositive sheep. However, no differences in miRNA expression were found between asymptomatic seropositive animals and those with clinical disease. While some differential expression of miRNAs has been found, additional research may be warranted.[1]

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