Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2024-2025

Conference #15 28 March 2025

CASE I:

Signalment:
Table egg layers (leghorn chickens), 27-weeks old.

History:
Flock of 15000 birds. 900 new birds were added to the flock on Feb 5, 2024. A sharp drop in egg production (14%) was reported on February 15, 2024. Mortality begins on February 17 with 3+ birds dead per day until March 12, 2024. The cumulative mortality in the flock was 127 birds. The clinical signs included swollen face, eyelids, wattles, lacrimation, and mucoid nasal discharge.

Gross Pathology:
Two culled birds were submitted. The carcasses were fresh. The body condition was fair with apparent fat stores. The face, eyelids and wattles of the chickens were markedly swollen and red. The eyes were closed, and mucoid exudate expressed from the nasal sinuses. On reflecting skin, the subcutaneous tissue was markedly edematous. The cut surface of the wattles was dark red and soft in the center and surrounded by yellow-white tissue at the periphery. The thoracic and abdominal air sacs were yellowish and cloudy. There were multifocal, few, 1 mm, pale white foci in the liver parenchyma. The ovarian follicles were very small or had a few enlarging follicles (not in production). No significant gross findings in other organ systems.

Figure 1-1. Head, chicken. The face, eyelids and wattles of the chickens were markedly swollen and red. (*Photo courtesy of* Diagnostic Services Unit, Faculty of Veterinary Medicine, University of Calgary)

Laboratory Results:

Avibacterium Paragallinarum cultured from wattle and sinus swab.
Infectious Bronchitis Virus (IBV) and Mycoplasma spp. generic PCR- Low positive for IBV (CT= 32), negative for Mycoplasma spp.

Microscopic Description:
Wattle: Diffusely the center of the wattle was necrotic and hemorrhagic which is characterized by eosinophilic, cellular, and heterophilic debris mixed with pale eosinophilic fibrin, edema fluid, and hemorrhage. At the periphery was a layer of giant cells. The adjoining tissue was variably necrotic and congested and there are multifocal perivascular

Figure 1-2. Head, chicken. On reflecting the skin, the subcutaneous tissue was markedly edematous (top). The cut surface of the wattles was dark red and soft in the center and surrounded by yellow-white tissue at the periphery (bottom). (*Photo courtesy of* Diagnostic Services Unit, Faculty of Veterinary Medicine, University of Calgary)

and lymphocyte infiltrates. The infraorbital sinus was filled with pale blue homogenous mucous mixed with cellular debris, pale eosinophilic fibrin, and heterophils. The lining epithelium was multifocally sloughed and the lamina propria was multifocally congested, edematous, and infiltrated by lymphocytes, plasma cells, macrophages, and heterophils. A similar lesion is present in the lining epithelium of the nasal cavity. Lacrimal and harderian glands are moderately infiltrated by lymphocytes, plasma cells, and heterophils. The air spaces in the calvarium are filled with eosinophilic fluid, mixed with fibrin, a small number of heterophils, and sloughed lining epithelium. Other lesions included multifocal, hepatic necrosis and mild focal airsculitis, diffuse, lymphocytic and heterophilic, conjunctivitis, necro-hemorrhagic cellulitis.

Contributor's Morphologic Diagnosis:

1. Wattles: dermatitis, fibrino necrotizing and hemorrhagic, diffuse, marked, acute.
2. Infraorbital sinus and nasal cavity: Sinusitis and rhinitis, lymphoplasmacytic and heterophilic, mucoid, marked, diffuse, acute.
3. Cranial osteomyelitis, necrotizing, severe, multifocal, acute.

Contributor's Comment:

Infectious coryza (IC) is an economically important disease of intensively raised commercial chickens around the world. IC is an acute, sometimes chronic, contagious, upper respiratory tract disease that results in airsacculitis and condemnation in the broiler chickens in the processing plant and reduced egg production (up to 10-40%) in the breeders and layers.[2] The disease is spread by close contact and droplets, fomites, or the introduction of carrier chickens into a closed flock. The incubation period is about 24 hours. The affected birds in uncomplicated cases recover within 2 weeks.[1] IC is caused by *A. paragallinarum*, which is a gram-negative, fastidious organism bacterium (previously, *Hemophilus paragallinarum*). It requires factor nicotinamide dinucleotide (NAD) for invitro growth, which is often provided by striking a satellite

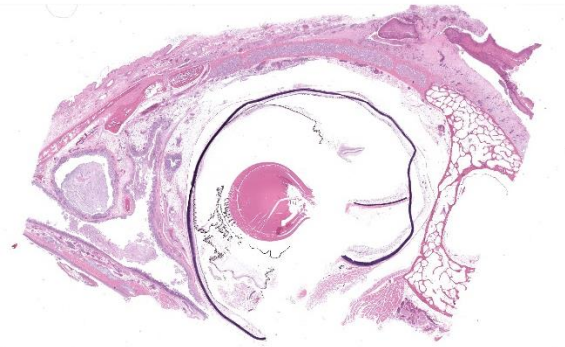

Figure 1-3. Head, chicken. A cross section of the head is submitted for examination. There is abundant exudate in the infraorbital sinus and nasal cavity at left, and a heterophilic core to the wattle at top right. (HE, 5X)

Staphylococcus aureus nurse colony on the plate. NAD- independent bacterial strains exist which sometimes pose a diagnostic challenge. In field conditions, it is often difficult to recover this bacterium, hence sending whole heads for bacterial culture is preferred if the veterinarians prefer to do necropsy in the field. *A. paragallinarum* can be categorized into 3 serovars or sub-serovars by hemagglutination inhibition tests [3,4] and/ or recently genotyping is used to categorize the bacteria with a high correlation with serovars, but the technique needs further studies.[2] The important virulence factors of *A. paragallinarum* include hemagglutinins (HAs), capsule, and RTX cytotoxin. HA is a 210 kDa protein coded by the *HMTp210* gene. The HA protein confer hemagglutination, cell adhesion, and biofilm formation. The capsule protects the bacteria from bactericidal activity of immune cells.

In this case, two whole bodies were submitted with swollen face, eyelids, wattles, lacrimation, and mucoid nasal discharge. Given the history of a sharp decline in egg production and upper respiratory signs, *Avibacterium paragallinarum, Mycoplasma gallisepticum,* and *Mycoplasma synoviae* were top differentials. An underlying Infectious bronchitis virus (IBV) was also speculated based on the history of bronchitis in the source flock from where the birds were sourced. Hemorrhagic wattles and nasal swabs were sent for bacterial culture and sensitivity, and trachea was sent for the detection of generic *Mycoplasma* and IBV PCR. The clinical signs were non-specific and can be observed with several other disease agents that can act as a primary pathogen or coexist concurrently with IC. The list includes *Mycoplasma gallisepticum, M. synoviae, Ornithobacterium rhinotracheale, Gallibacterium anatis, Pasteurella multocida,* and viral pathogens such *as* Avian Metapneumovirus virus (aMPV), IBV and infectious laryngotracheitis

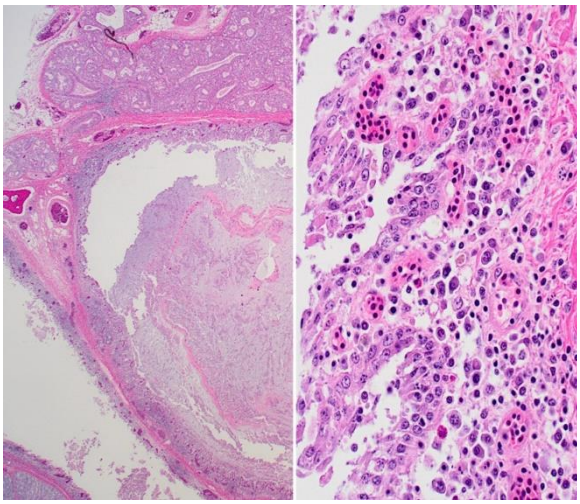

Figure 1-4. Head, chicken. The infraorbital sinus contains a mucocellular exudate within the lumen (left) and marked hyperplasia and profound mixed inflammation of the mucosal lining (right). A similar lesion is present in the nasal cavity (not pictured). (HE, 40X and 400X) (*Photo courtesy of* Diagnostic Services Unit, Faculty of Veterinary Medicine, University of Calgary)

virus (ILTV). In addition, poor management and ventilation can increase the severity of the disease.[2] No other bacteria were cultured other than *A. paragallinarum.* Low level of IBV was detected in the tracheal samples and *Mycoplasma* spp. were negative on PCR. Owing to the low level of the virus, genotyping was not possible in this case. ILT was excluded based on histopathology and the aMPV was not tested in this case as it was not in Canada at the time of the current case. The birds were not laying in this case and were not treated and culled. In general, if laying birds get infected, they can be treated, though they can become carriers following treatment and recurrence is possible. It is recommended to cull the flock as soon as it is close to the cycle completion due to biosecurity risk to commercial poultry. The affected flocks can be depopulated if the disease is not reported in the region to avoid future outbreaks. Inactivated bacterins are available to

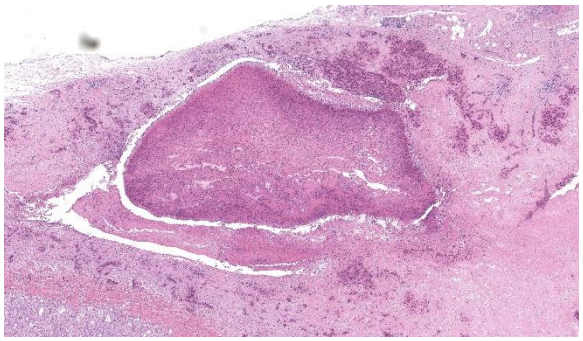

Figure 1-5. Head, chicken. The wattle is expanded by a necrotic core composed of abundant heterophilic debris and lined by a layer of epithelioid macrophages. The surrounding dermis and subcutis is effaced by a thick layer of vascularized fibrous connective tissue.

use in endemic areas to minimize the occurrence. Enhancing biosecurity and avoiding adding replacement birds to closed flocks are some of the strategies to reduce the occurrence of the disease.[1] Prior testing before movement can be done. In this case, replacement birds were introduced into the current flock without any testing which resulted in the disease outbreak. On further investigation, the source flock had a similar disease outbreak in the past. This highlights the importance of strict biosecurity, and quarantining of newly introduced birds when not raised on the same site. While the disease is very prevalent in the US and other countries, there is not much information on the occurrence of IC in Canada.

Contributing Institution:

Pathology & Diagnostic Services | Diagnostic Services Unit (DSU) | Faculty of Veterinary Medicine | University of Calgary (ucalgary.ca).
Faculty of Veterinary Medicine | University of Calgary (ucalgary.ca)

JPC Diagnosis:

1. Nasal cavity and infraorbital sinuses: Rhinitis and sinusitis, heterophilic and granulomatous, chronic, diffuse, severe, with luminal bacilli.
2. Comb: Dermatitis, necrotizing and heterophilic, chronic, diffuse, severe.
3. Harderian and conjunctival lacrimal glands: Dacryoadenitis, lymphoplasmacytic, chronic, diffuse, mild to moderate.

JPC Comment:

This conference's moderator was Dr. Tom Cecere of Virginia-Maryland CVM who led conference participants through a broad cross-section of infectious agents and production animal species. We enjoyed reviewing this first slide given that it is a microcosm of the gross pathology that the contributor nicely demonstrates, though orientation required consideration of the glands, sinuses, and unaffected epithelium accordingly. Our Gram stain confirmed gram-negative coccobacilli present within the infraorbital sinus (consistent with *Avibacterium*) along with fewer gram-positive bacilli and cocci. We interpreted the large region of necrosis and cellulitis as arising within the comb of this bird; we did not see any bacteria within this region on H&E or Gram stains. We differed from the contributor on osteomyelitis as bony changes within our section were mild (perhaps reflecting variation in submitted slides) though we did capture additional inflammation within adjacent glands as a secondary change in this case.
The contributor lays out a good differential diagnosis list for this case. Participants noted multinucleated giant cells (especially within the overlying skin) that prompted a discussion of viral syncytia (e.g. ILTV) versus granulomatous inflammation and macrophage activation. Given the likely polymicrobial infection at play in these birds, consider-

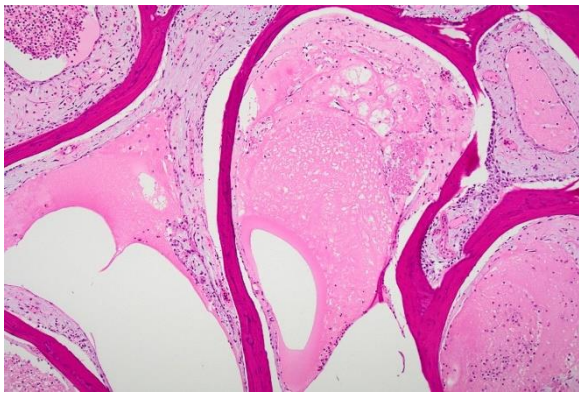

Figure 1-6. Head, chicken. The lining of the air spaces in the calvarium are markedly edematous and filled with proteinaceous fluid. (HE, 200X) (*Photo courtesy of* Diagnostic Services Unit, Faculty of Veterinary Medicine, University of Calgary)

ation of relevant histologic features and ancillary diagnostics such as culture and PCR is prudent for the supporting pathologist. Infectious coryza has also been reported on poultry farms and among smaller hobby flocks within the United States.[5,6] In contrast to this case, more acute/fulminant disease may have more fibrinous exudate and infection can extend to the abdominal air sacs and/or the pericardial sac and cause pericarditis and/or periphepatitis.[6] Complicated (polymicrobial) infections often lead to septicemia and marked flock mortality.[5,6] Flock susceptibility to IC can reflect vaccine failure (poor preparation, timing/delivery failure) as well as lack of cross-protection between different circulating serovars; use of vaccines in laying birds, meat birds, and breeder flocks varies by facilty.[6] Farm-farm transmission between poultry workers is a likely control factor as wild birds and insects have been shown to be ineffective vectors for IC.[6]

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CASE II:

Signalment:
4-week- suckling piglet, male neutered, Swiss Large White, *sus scrofa domesticus*, porcine.

History:
In March 2023, an increase in spinal deformities was detected on a Swiss breeding farm, characterized by thoracic lordosis and lumbar

kyphosis ("humpy-back syndrome") and suckling as well as weaning piglets showed focal thickening of multiple ribs and facial oedema in an otherwise unremarkable clinical condition.

Gross Pathology:
Seven adjacent ribs show a marked focal callus formation. The longitudinal sections reveal rib fractures.

Laboratory Results:
qPCR for PCV-3 revealed high viral loads in kidney (Ct 21), mesenteric lymph node (Ct 12), rib (Ct 26) and brain (Ct 23).

Immunohistochemistry for detection of PCV-2 yielded a negative result.

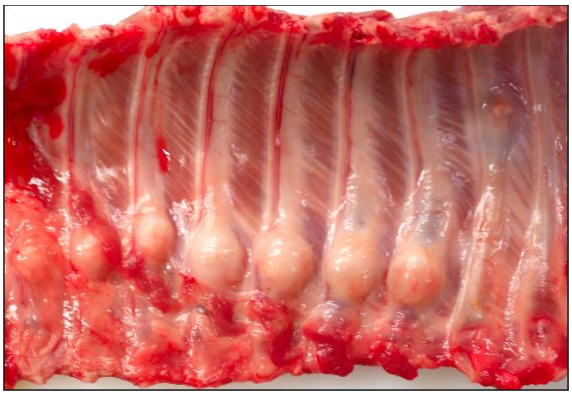

Figure 2-1. Rib, piglet. Left thoracic rib cage of a 4-week- suckling piglet showing seven adjacent rib fractures with marked callus formation. (*Photo courtesy of* Institute of Veterinary Pathology, Vetsuisse-Faculty, University of Zurich, https://www.vetpathology.uzh.ch/de.html)

Microscopic Description:
Bone, rib: In the calcification zone of the chondrocostal junction, a particularly high number of osteoblasts and osteoclasts are found (bone remodeling). Primary trabeculae vary in thickness, are fragmented and show cross-connections. The bone marrow contains all three hematopoietic cell lines with a slight increase in fibroblasts (myelofibrosis).

At the level of the macroscopically described bone distension, a blunt discontinuity of the osseous tissue is visible (fracture). The edges of the woven bone and multiple bone fragments within the fracture gap are lined by numerous multinucleated cells (osteoclasts) adjacent to scalloped, irregularly shaped bone (Howship's lacunae). Within the fracture gap, the tissue is focally extensive replaced by irregular trabeculae of woven bone at varying stages of maturation along with granulation tissue. The immature woven bone, oriented perpendicular to the periosteum, is composed of densely organized collagen fibers and is often surrounded by numerous plump, large-nucleated mesenchymal cells (reactive osteoblasts) lying in a single layer. The granulation tissue extends into the adjacent bone marrow and is multifocally infiltrated by a moderate number of macrophages, lymphocytes, plasma cells, scattered neutrophils and few erythrocytes (acute hemorrhage).

In addition to multiple bone fragments, there are multifocal to coalescing areas of fibrous connective tissue and chondrocytes producing cartilage fragments (soft callus formation).

Furthermore, multifocal osteoid formations surrounded by osteoblasts are present. Neutrophils, fibrin and eosinophilic cellular and karyorrhectic debris (necrosis) as well as numerous multinucleated giant cells are found in the fracture gap.

Focally extending from the cortical surface and markedly elevating the periosteum, there is a periosteal proliferation of reactive, woven bone with trabeculae aligned perpendicular to the cortex (exostosis). The periosteum is diffusely highly expanded by collagenous connective tissue (periosteal fibrosis) and includes small fragments of

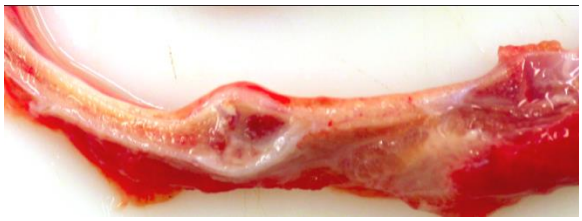

Figure 2-2. Rib, piglet. Longitudinal section of a rib with focal fracture and extensive callus formation (*Photo courtesy of* Institute of Veterinary Pathology, Vetsuisse-Faculty, University of Zurich, https://www.vetpathology.uzh.ch/de.html)

osseous tissue and abundant extravascular erythrocytes (acute hemorrhage).

The arteries in the periosteal tissue and those in the adjacent intercostal muscles and adipose tissue show infiltration of the media and the adventitia by a moderate number of small lymphocytes, plasma cells and sporadic macrophages. Similar infiltrates are found periarterially. The venous and lymphatic vessels, as well as the nerves, do not exhibit any alterations.

ISH detected abundant PCV-3 RNA in the rib (periosteal arterial walls, osteocytes and osteoblasts).

Contributor's Morphologic Diagnosis:
Bone, rib: Fracture with bone remodeling, focally extensive, marked with reactive woven bone formation (fracture callus), myelofibrosis and hemorrhage, multifocal, acute, mild to moderate as well as exostosis, periosteal, circumferential, moderate.

Arteritis and periarteritis, lymphoplasmacytic and histiocytic, multifocal, moderate to severe.

Contributor's Comment:
Circoviruses are single-stranded DNA viruses with a circular genome and one main capsid protein. They are prevalent across a variety of animals, including mammals, fish, birds, and insects. In pigs, four different porcine circoviruses (PCV) have been identified up to now: PCV-1, PCV-2, PCV-3 and PCV-4. PCVs are ubiquitous in global pig populations. While PCV-1 is accepted as non-pathogenic, PCV-2 is considered as an economically challenging pathogen on a global scale. Similarly to PCV-2, PCV-3 is widespread and detected in both healthy and diseased pigs, often in mummified and stillborn fetuses, indicating vertical and horizontal transmission of the viruses. PCV-4 has only recently been discovered and further information on this virus is required to understand its potential impact.[1]

PCV-3 infections are associated with various clinical and pathological manifestations, especially reproductive disorders, PDNS and multisystemic inflammatory diseases.[2,4] Furthermore, viral DNA has been detected in asymptomatic pigs and coinfections comprised of PCV3 with other swine pathogens have been frequently reported. This suggests that PCV-3 may act as a cofactor for some pathogens or may need other cofactors to cause clinical signs of disease.[3]

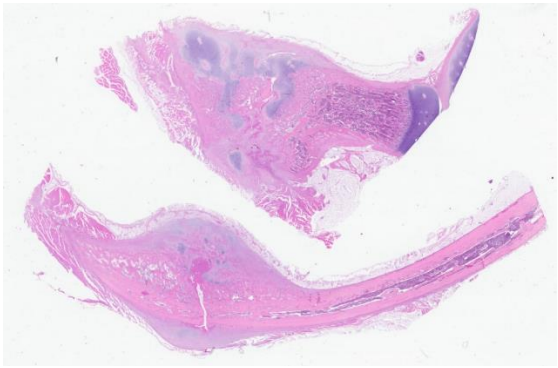

Figure 2-3. Rib, piglet. Two sections of rib with a diaphyseal fracture are submitted for examination. The physis is present in the section at top. (HE, 7X)

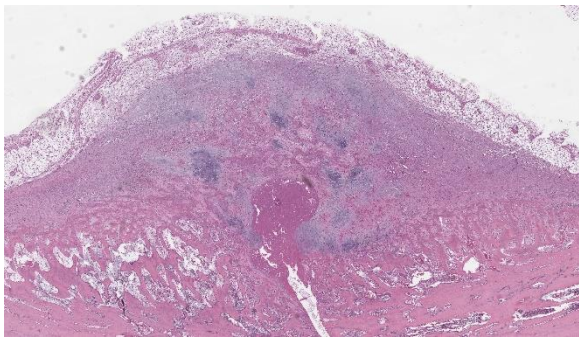

Figure 2-4. Rib, piglet. There is a large mature callus with anastomosing trabeculae of woven bone and large islands of cartilage over the fracture site (bottom center). (HE, 60X)

In situ hybridization (ISH) is used for the detection of PCV-3 in lesions. Histopathologic expertise is crucial as associated lesions may be subtle, requiring a degree of confidence to recognize these conditions. To diagnose PCV-3, a histopathologic specimen including heart, lung, spleen and lymph nodes should be submitted.[3] However, the clinical findings associated with PCV-3 are non-specific and require consideration of different pathogens. The "humpy-back-syndrome" is earliest observed in pigs of 3 weeks but most often detected at an age of 8 to 16 weeks.[4] It can arise as a secondary condition linked to multiple primary lesions within the vertebrae.[5] Primary lesions include osteomyelitis, fractures, neoplasms and metabolic diseases.[6] In addition, factors such as painful diseases of the legs and back[7], Musculo-mechanical stress on the lumbar spine[8], early onset of puberty in male pigs[9] and intrauterine viral infections[10] are contributing factors. A hereditary influence on the development of porcine kyphosis is suggested as well.[10]
The histological lesions in this case match the descriptions of PCV-3 systemic disease, but for the first time the virus has been detected by qPCR and ISH in bone lesions. In the last decade, authors reported cases of "humpy-back" pigs exhibiting histologically inflammatory vascular lesions comparable to those reported here.[4,11] Therefore, pathomorphological investigations and possible detection of PCV-3 is recommended in pigs displaying bone lesions and "humpy-back" posture.

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JPC Diagnosis:
Bone, rib: Diaphyseal fracture with maturing callus.

JPC Comment:
This was a case that generated a lively discussion. As Dr. Cecere did his PhD on circoviruses, he guided the group through assessing the changes present in this slide which were descriptively rewarding (and quite detailed). Ultimately, we summarized this case in a simple way and chose to focus on the H&E features before considering the ISH (and question of causation) presented in this case.

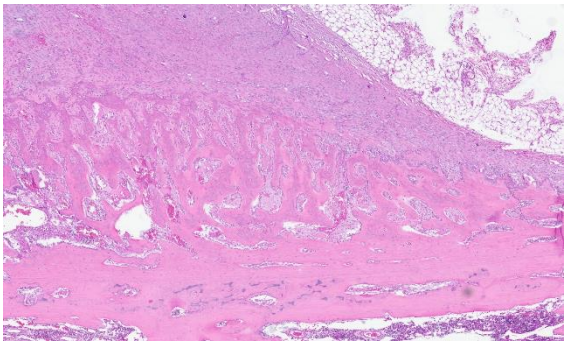

Figure 2-5. Rib, piglet. At the periphery of the callus, there is marked woven bone proliferation beneath the hypercellular periosteum. (HE, 75X)

We differed from the contributor in several

key aspects. Foremost, the section that we reviewed lacked a significant vascular component, though there is mild lymphocytic perivasculitis and lymphocytic infiltration within peripheral nerves present. There is also a lack of overt necrosis. These features make it harder to assess the role of viral-induced insult as the cause of the weakening and eventual fracture of these ribs (i.e. through disruption of blood flow to the developing bone) as there is no evidence to support this interpretation. Likewise, we reviewed the ISH image supplied by the contributor (Figure 2-6) and agree that the localization of nucleic acid within the vessel wall comports with the expected behavior of PCV-3, though the signal also extends beyond osteocytes/osteoblasts to include numerous cells within the adjacent fibrous tissue and callus. Another potential interpretation is that the non-vascular signal represents transfer of circoviral nucleic acid sto antigen-presenting cells (e.g. infiltrating histiocytes). As such, this result raises the question of "correlation or causation" i.e., changes *because of* PCV-3 or *in addition to* PCV-3. Although not included with this submission, additional information about the spleen, kidney, liver, and/or peripheral lymph nodes to confirm the presence of circoviral-associated disease (especially with corroborating ISH) was suggested by Dr. Cecere to develop this hypothesis further in future cases. Conference participants were also skeptical of myelofibrosis in this piglet and interpreted mesenchymal cells as part of the developing callus to stabilize the fracture – by convention, myelofibrosis implies a primary defect which seems less likely in this case.

It is worth briefly discussing several of the slide features in weighing differential diagnoses for this case. As this was a very young piglet, it is important to know that the

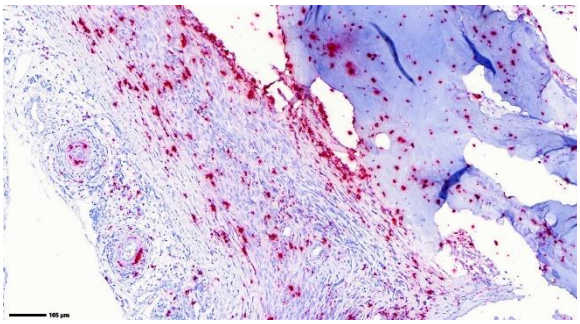

Figure 2-6. Rib, piglet. ISH detected abundant PCV-3 RNA in the rib (periosteal arterial walls, osteocytes and osteoblasts). (*Photo courtesy of* Institute of Veterinary Pathology, Vetsuisse-Faculty, University of Zurich, https://www.vetpathology.uzh.ch/de.html)

width of cortical bone closer to the physis/metaphysis (i.e., the "cut-back zone") is typically thinner for physiological reasons (this is where elongation of the bone takes place) which does not reflect osteoporosis or osteopetrosis. This animal had not yet developed a secondary center of ossification as well. Similarly, the degree of cartilage present within the primary spongiosa is appropriate for the age of this animal. Comparing the hue of physeal cartilage to cartilage within the developing callus is helpful to determine if both growth plates are in section (they are not in the slides submitted to the conference). Conference participants also discussed the 'rosary' gross appearance (Figure 2-1) which resembles a rachitic (rickets) . In the submitted sections, the physis at the costochondral junction in this animal is relatively normal histologically.

References:

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Figure 3-1. Presentation, pig. The pig was presented in poor body condition, with prominent tuber ischia (pin bones) and ribs. (*Photo courtesy of*: State Veterinary Diagnostic Laboratory, Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australiahttps://www.dpi.nsw.gov.au/about-us/services/laboratory-services/veterinary)

CASE III:

Signalment:
6-12 week old, female, hybrid, *Sus scrofa domesticus*, pig.

History:
Multiple weaners on the property with poor body condition and wasting, leading to death.

Gross Pathology:
The pig was presented in poor body condition, with prominent tuber ischia (pin bones) and ribs. There was marked faecal staining around the anus. The large intestine, predominantly colon and caecum, was diffusely dilated with diffuse dark grey to red-brown discoloration of the serosa. Multifocally, the mucosa had white fibrinous lesions admixed with dark necrotic areas and areas of haemorrhage.

Laboratory Results:
Porcine circovirus type 2 real-time PCR: Positive

Salmonella enrichment culture: Positive.

Identification of *Salmonella* by serotyping: *Salmonella enterica* serovar infantis.

Brachyspira hyodysenteriae and *B. pilosicoli* multiplex PCR: Negative

Microscopic Description:
Colon or caecum (depending on the slide), H&E: Multifocally to coalescing, affecting the epithelium, lamina propria and submucosa, is marked loss of mucosa, with replacement by abundant mats of fibrillar eosinophilic material (fibrin), cellular and karyorrhectic debris, degenerate neutrophils, lymphocytes and macrophages, and colonies of basophilic bacteria with rod morphology. Small vessels in affected areas have disrupted endothelium and are often filled with thrombi. In less affected areas, crypts are markedly elongated, ectatic, with attenuated epithelium and contain amphophilic to pale basophilic fibrillar material (mucus) or filled with degenerate neutrophils and necrotic debris (crypt abscess). Multifocally, there is mild goblet cell hyperplasia. Multifocally, the lamina propria and submucosa are expanded by lymphocytes, macrophages, and plasma cells; with occasional small vessels containing thrombi. Multifocally, numerous ciliated protozoa (*Balantidium coli*) are free in the lumen or admixed with necrotic material and buried deep in the mucosa. The serosa is focally markedly expanded by neutrophils and fibroblastic proliferation, underlying a thin mat of fibrin, clear areas, and a florid perivascular infiltrate of lymphocytes and plasma cells.

Colon or caecum (depending on the slide), Warthin-Starry: Multifocally, abundant colonies of bacteria with rod morphology are highlighted within the areas of ulceration, necrosis and inflammation, previously described in H&E.

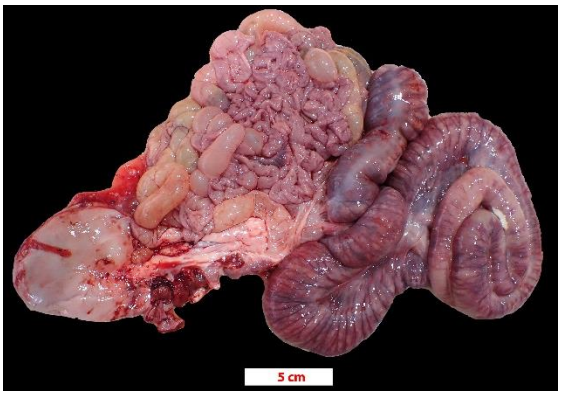

Figure 3-2. Abdominal viscera, pig. The colon and caecum are diffusely dilated with diffuse dark grey to red-brown discoloration of the serosa. (*Photo courtesy of*: State Veterinary Diagnostic Laboratory, Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australiahttps://www.dpi.nsw.gov.au/about-us/services/laboratory-services/veterinary)

Contributor's Morphologic Diagnosis:
Colon or caecum: Colitis or typhlitis, fibrinonecrotizing, multifocal to coalescing, severe, with diphtheritic membranes, goblet cell hyperplasia, thrombosis, abundant intralesional rod-shaped bacteria, and numerous intralesional protozoan ciliates, hybrid breed, *Sus scrofa*.

Contributor's Comment:
Salmonellosis is a common bacterial infection of mammals and birds, resulting in various syndromes such as septicaemia, enterocolitis, abortion and pneumonia. There are two recognized species of Salmonella*: S. enterica* and *S. bongori*, of which there are a vast number of serovars and serotypes.[5] Additionally, there are three broad groups of Salmonella serovars to consider: those that are host adapted to humans and higher primates, those that are host adapted to certain animal species and those that are not host adapted.[1] Several *Salmonella enterica* serovars, most commonly Typhimurium, Derby and Cholerasuis, have been identified

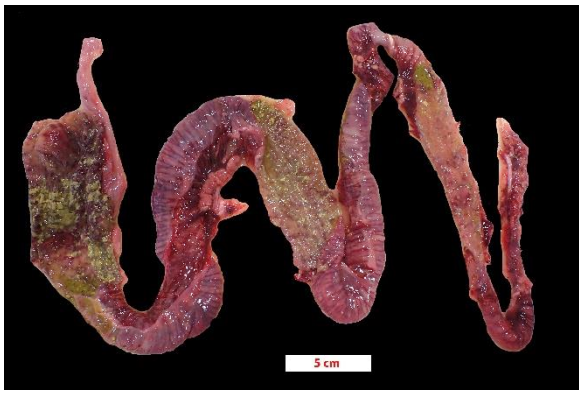

Figure 3-3. Colon, pig. Multifocally, the mucosa had white fibrinous lesions admixed with dark necrotic areas and areas of haemorrhage. (*Photo courtesy of*: State Veterinary Diagnostic Laboratory, Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australiahttps://www.dpi.nsw.gov.au/ about-us/services/laboratory-services/v eterinary)

to cause enterocolitis and septicaemia in swine;[13] Cholerasuis is host adapted to swine, while Typhimurium and Derby also commonly cause disease in ruminants and poultry.

The *Salmonella enterica* serovar isolated in this case, *Salmonella* Infantis, has been identified from both clinically normal and ill pigs, as well as in the environment and carcasses of pigs at slaughterhouses.[7] While this serotype is less commonly identified compared to Typhimurium, Cholerasuis, and Derby, prevalence of this serotype is increasing. In a number of studies across Canada, the United States and Brazil, *Salmonella* Infantis was consistently the 3rd to 4th most identified serotype in farms, slaughterhouses and in diagnostic samples at veterinary laboratories, with evidence for increasing prevalence over time.[6-8,12,15] This serotype is also of public health concern due to its zoonotic potential and antibiotic resistance.[4,7,9] *Salmonella* Infantis has been isolated in a number of food safety trials, involving both contaminated pork and poultry meat, presenting increasing risk to human health.[4,10,11] Interestingly, poultry are asymptomatic carriers of *Salmonella* Infantis.[10,14]

There are no published reports of the gross or histological changes observed with *Salmonella* Infantis infection in swine, as far as the authors are aware. Comparatively, the gross and histological changes seen in this case are most similar to infection with *Salmonella* Typhimurium in swine, where diphtheritic enterocolitis is also generally confined to the large intestine and rectum, with minimal involvement of the distal ileum.[13] Other bacterial agents that were considered in this case given the gross and histological findings included *Brachyspira hyodysenteriae* and *B. pilosicoli* as well as *Clostridium perfringens* types A and C. Both *B. hyodysenteriae* and *pilosicoli* cause fibrinonecrotic and erosive lesions limited to the colon and caecum. Diphtheritic membranes are also a common gross feature of *B. pilosicoli* infection. *Clostridium perfringens* types A and C both cause necrohaemorrhagic enterocolitis in neonatal piglets. While lesions generally involve the small intestine, severe cases also involve the large intestine.

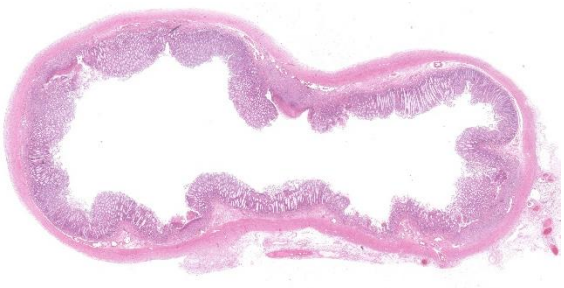

Figure 3-4. Colon, pig. One section of colon is presented for examination. There are multifocal full-thickness mucosal ulcers. (HE, 11X)

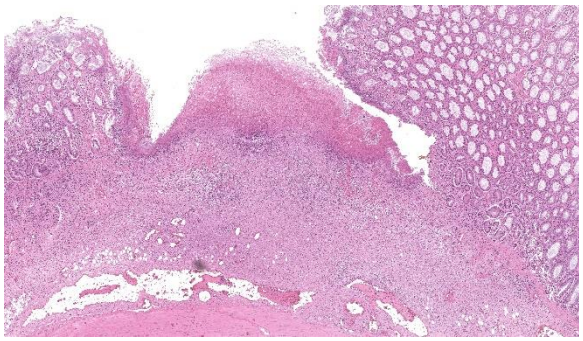

Figure 3-5. Colon, pig. Ulcers are covered by a fibrinocellular membrane and consist of abundant cellular debris admixed with infiltrating viable and necrotic neutrophils and macrophages. Inflammation and necrosis extends downward into the underlying submucosa and peripherally into the lamina propria of the adjacent mucosa. (HE, 68X)

The pathogenicity of *Salmonella* relies on a number of *Salmonella* pathogenicity islands (SPIs) and other virulence genes within the genome. The SPI-1 proteins are mainly associated with invasion of the cell, while SPI-2 proteins are involved with survival and replication within host cells; a number of virulence markers contained within SP-1 and SP-2 are present in *Salmonella* Infantis isolates, indicating the pathogenicity of this serotype.[2] Virulence associated plasmids are also vitally important for the survival and growth of *Salmonella* within macrophages.[2] Observable histological changes (reduction in villi length) can be present in the small intestine from one day post-infection with *Salmonella* Typhimurium, with severe changes such as villous atrophy and epithelial damage seen as early as two days post-infection.[3]

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JPC Diagnosis:
Colon: Colitis, ulcerative, subacute, multifocal, marked, with vasculitis, thrombosis, and diffuse lymphoid depletion.

JPC Comment:
We thank the contributor for sharing this slide with us as the ancillary diagnostics opened up a great conference discussion. Our Gram stain demonstrated gram-negative bacilli adjacent to ulcerated areas of the colon, consistent with the presence of *Salmonella* Infantis outlined above and likely the primary process in this animal though this feature was not apparent to us on H&E. Conference participants offered a number of

other possible etiologies (and/or coinfections) which had varying levels of support, including *Lawsonia* (here lacking a marked proliferative component and the degree of hemorrhage in this case is not severe), *Clostridium (Clostridioides difficile* - a good differential in a newborn animal), and African or Classical Swine fever (better supported by marked hemorrhage in additional organs). We debated the relevance of *Brachyspira* in this case despite the PCR result as we noted many spirochetes present

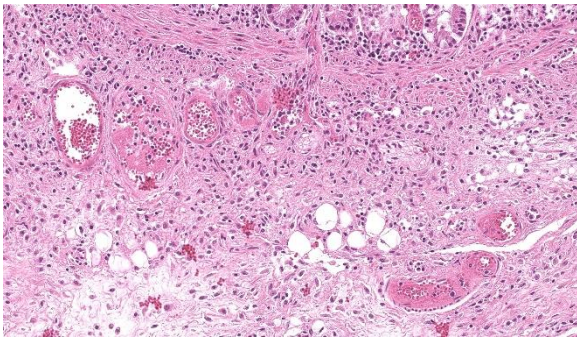

Figure 3-6. Colon, pig. Submucosal vessels often contain fibrin thrombi. (HE, 758X)

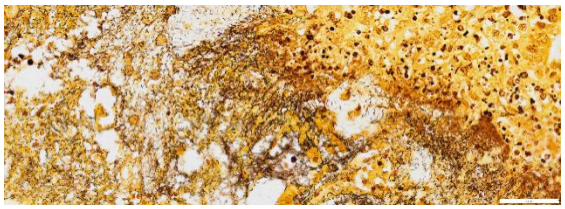

Figure 3-7. Colon, pig. A silver stain demonstrates large numbers of bacterial rods within areas of ulceration. (Warthin-Starry 3.2, 400X) (*Photo courtesy of*: State Veterinary Diagnostic Laboratory, Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australiahttps://www.dpi.nsw.gov.au /about-us/services/laboratory-services/ veterinary)

within colonic crypts on our silver stain as well (similar to Figure 3-7), though it is possible that this could reflect a normal amount to a slight overgrowth secondary to ulceration induced by *Salmonella* – the degree of mucus present on this section is not overwhelming as well. There is also a moderate number of ciliated trophozoites present within the lumen of the colon (consistent with *Balantidium coli*, a normal colonic commensal) which we did not assign any pathologic significance to.

One subtle feature of this case that should not be overlooked is the depletion in lymphoid cells within the colon. Simply put, there isn't lymphoid tissue present at all in this section which underscores the need to review tissues systemically to detect severe changes. That this animal was PCR-positive for PCV-2 is a potential explanation, though there is no corroborating evidence such as cytoplasmic botryoid viral inclusions despite the vasculitis present. *Salmonella* is another major cause of lymphoid depletion, and often accomplishes this 'task' shortly after entering aggregated lymphoid nodules (GALT; Peyer's patches) via overlying M cells. Conference participants extrapolated that the ulcers in the colon of this pig ('button-like') probably reflected this pathogenesis with accompanying infarction of the adjacent submucosal blood vessels.

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CASE IV:

Signalment:
16 weeks, male, domestic white turkey (*Meleagris gallopavo*).

History:
A flock of 7,500 domestic white male turkeys was experiencing elevated mortality as a result of both aortic rupture and culling of lame, recumbent birds. Recumbent birds had swollen intertarsal (hock) joints with bruising of the nonfeathered skin of the hock. Five affected legs were removed at the coxofemoral joint from culled carcasses and shipped to the Minnesota Veterinary Diagnostic Laboratory.

Gross Pathology:
The submitted turkey legs had skin bruising of the caudal aspect of the hock. The hocks were swollen as a result of periarticular subcutaneous edema (serosanguinous fluid) and increased volume of synovial fluid within the hock joints and sheaths of the gastrocnemius tendon and digital flexor sheaths. In two legs, the gastrocnemius tendon was partially rup-

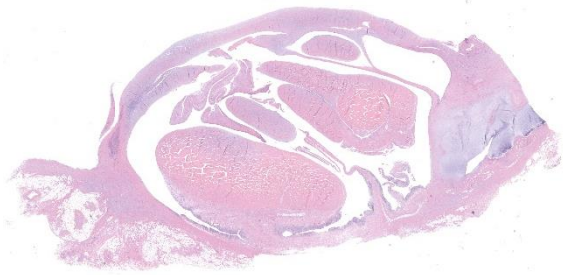

Figure 4-1. Tendons and tendon sheath, turkey. A section through the gastrocnemius and digital flexor tendons is submitted for examination. There is an adhesion between the gastrocnemius tendon and the adjacent tendon sheath (center, bottom). (HE, 10X)

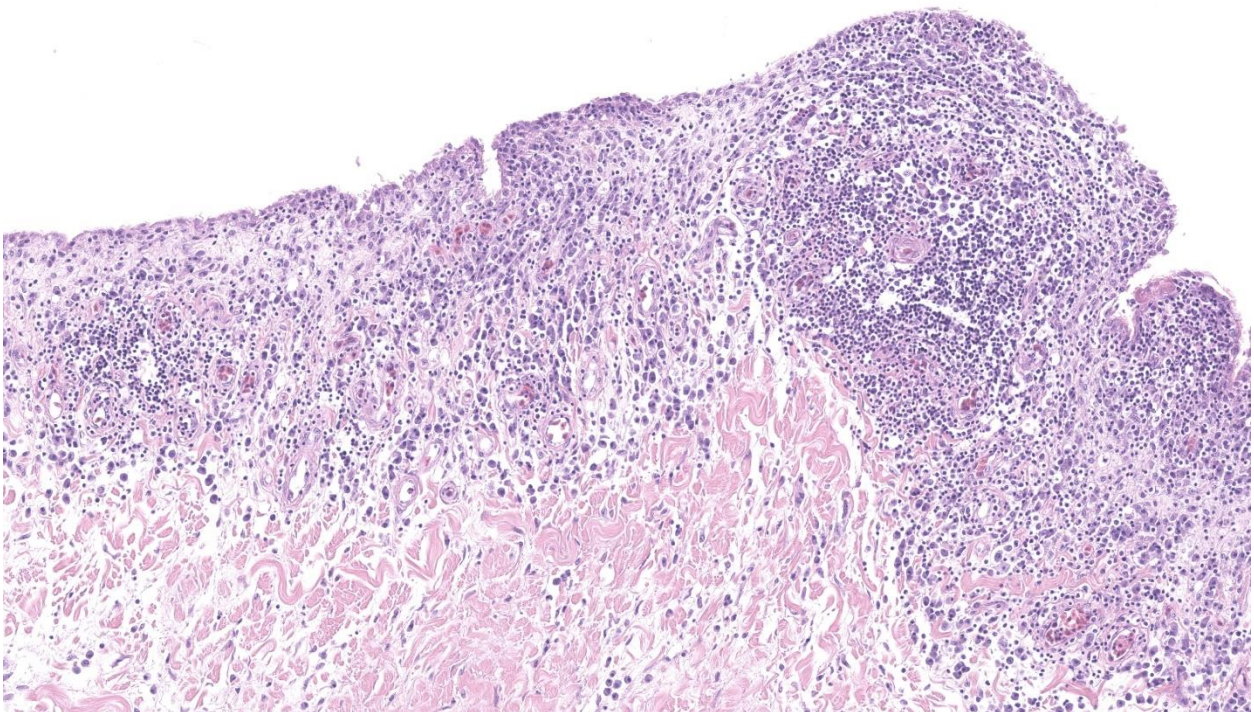

Figure 4-2. Tendon sheath, turkey. There is marked chronic inflammation of the edematous tendon sheath, with numerous lymphocytes and plasma cells, occasionally in aggregates, beneath the synovial lining. (HE, 268X)

tured proximal to the hock joint. Longitudinal sections of the femur, tibiotarsus and tarsometatarsus showed no overt lesions (no evidence of osteomyelitis or chondrodysplasia) in bone or growth plate.

Laboratory Results:
-Aerobic culture: Hock joint fluid- no significant growth

-Molecular diagnostics: Gastrocnemius tendon was positive (CT 32.5) for avian reovirus by universal avian reovirus PCR

Synovial fluid was negative for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by PCR

-Virology: Reovirus was isolated after one pass on embryonated eggs (yolk sac inoculation) and after two passes on QT-35 (quail fibroblast) cell line

Microscopic Description:
The tissue is a cross-section of gastrocnemius tendon complex, composed of the tendon and sheaths of the primary gastrocnemius tendon and multiple smaller digital flexor tendons and sheaths. Inflammation largely affects the tendon sheaths and consists of multifocal, moderate to marked infiltrates of lymphocytes and plasma cells either scattered or arranged in perivascular fashion within the edematous subsynovium. Rare heterophils are also observed. Adjacent synoviocytes are hypertrophic. No microorganisms are observed.

Contributor's Morphologic Diagnosis:
Gastrocnemius and digital flexor tendons: tenosynovitis, lymphoplasmacytic with subsynovial edema and synoviocyte hypertrophy

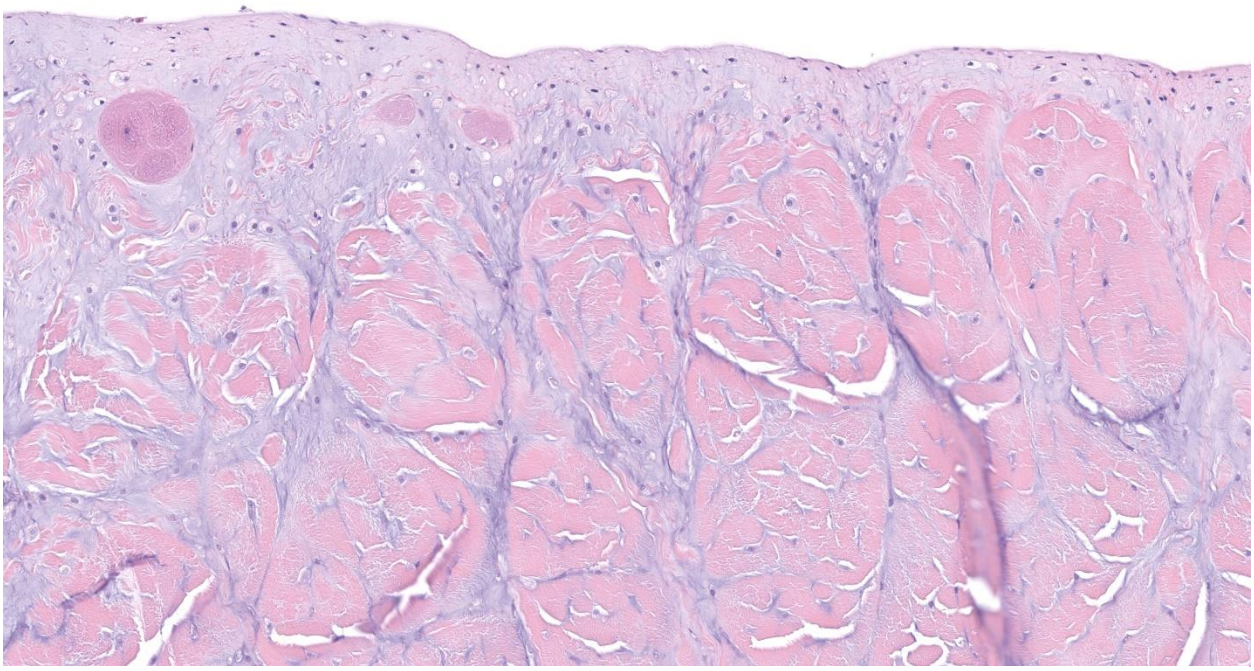

Figure 4-3. Tendon sheath, turkey. There are degenerative changes in the gastrocnemius tendon with abundant ground substance within the interstitium between collagen fibers, which is undergoing cartilaginous metaplasia. Similar, but less severe changes are seen in other tendons as well as the tendon sheath. (HE 323X)

Contributor's Comment:
Turkey arthritis reovirus (TARV) causes lameness in domestic turkeys, including both turkey breeders and meat-type turkeys, and affecting both sexes by 12-17 weeks of age. Males generally show clinical signs more often than females, likely because of the greater male body weight. In the 1980s, there were two reports of reovirus isolated from the gastrocnemius tendons of domestic turkeys affected with arthritis/tenosynovitis, but this condition was not experimentally reproduced at the time and was not observed again for nearly 25 years when it was reported in Minnesota.[2,4,5] Thereafter, there were multiple reports of TARV-associated outbreaks of lameness in market age turkeys,[3,10] resulting in substantial economic losses in the form of increased culling, mortality, poor feed efficiency, low rates of weight gain, aortic rup tures and increased condemnations at the processing plant.[3,6,10] The disease has been experimentally reproduced to confirm the involvement of reovirus and the infection has consistently been associated with uni- or bilateral lameness due to swelling of the intertarsal (hock joints), periarticular fibrosis, tenosynovitis, occasional erosion of articular cartilage of the hock joints and rupture of the gastrocnemius tendon or digital flexor tendons.[4,6,7,8] More recently, reoviruses that are genetically identical to turkey arthritis reovirus have been shown to cause or have been associated with hepatitis and meningoencephalomyelitis in turkey poults.[1] Avian reoviruses of turkeys are members of the genus *Orthoreovirus* in the family *Reoviridae* containing a double-stranded, segmented RNA genome in a double-shelled capsid. The ten genome segments are classified as L class (L1-L3), M class (M1-M3) and S class (S1-

S4) based on their electrophoretic mobility.[9] A similar condition of reoviral tenosynovitis in chickens ("viral arthritis") has been recognized for many years; however, gene segments of the chicken arthritis reoviruses bear only 80-85 % homology with the turkey arthritis reoviruses. It is likely that the turkey reoviruses represent a more recent mutation of the chicken reoviruses.

Contributing Institution:
University of Minnesota Veterinary Diagnostic Laboratory

https://vdl.umn.edu/

JPC Diagnosis:
Gastrocnemius and digital flexor tendons (presumptive): Tenosynovitis, lymphoplasmacytic, chronic, multifocal to coalescing, moderate.

JPC Comment:
The final case of this conference is a cross-section of multiple large tendons. From the H&E alone, we speculated that this likely represented the gastrocnemius and digital flexor tendons (given the approximate size/width presented) and adjusted our morphologic diagnosis accordingly. The section is nicely presented, with the distribution of lymphoplasmacytic inflammation being readily apparent even from low magnification. Conference participants also considered *Enterococcus* and *Mycoplasma synoviae* as less likely differential diagnoses for this case.

An interesting finding in this slide is the large amount of amphophilic to basophilic substance present in the affected tendon reflects notable deposition of ground substance (including proteoglycans, glycosaminoglycans;) and even cartilaginous metaplasia (Fig 4-3). We debated its origin carefully, weighing the natural progression of 'turkeydom' and rapid weight gains effect on the tendons of the legs versus virally-induced changes. The bulk of participants felt that the deposition of anastomosing ground substance was most attributable to the rapid weight gain (and increased shear and concussive force in this area.) Though it is possible that reoviral changes could weaken collagen and augment rupture of tendons, we did not have clear evidence of inflammatory cells associated with this material (i.e. tendonitis). As such, we probably could (should?) have two morphologic diagnoses for this case, but we could not agree on what to call this process with some participants unsatisfied with the vagueness of 'tendinopathy' or 'tendinosis' or if (in true WSC fashion) an expected lesion in a heavy meat bird merited an entire paragraph to explain it (it appears it does)! It is some consolation perhaps to consider a comparison to human sports-induced tendinopathies,[11] which have analogous gross and histologic appearance – something worth considering for those that enjoy running like the JPC residents do. Careful description of disorganized collagen, ground substance, the presence or absence of inflammatory cells, and changes in supporting stroma are helpful in categorizing these conditions.[11]

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