Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2016-2017

C o n f e r e n c e 5

28 September 2016

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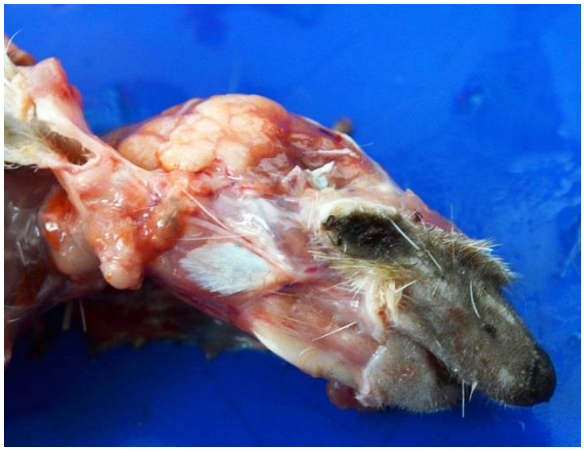
CASE I: Case 1 (JPC 4084648).

Signalment: Three-year-old, female, African hedgehog (*Atelerix albiventris*).

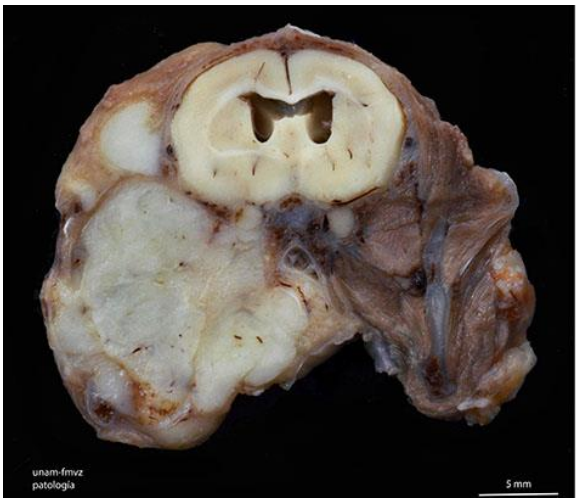
History: The animal presented for bloody vulvar discharge, right-sided facial paralysis, and multiple skin masses. Elective ovario-hysterectomy was performed and multiple skin masses were removed. A firm multi-nodular mass was observed arising from the internal ear canal. Due to the worsening of clinical signs, the animal was humanely euthanized and submitted for postmortem examination.

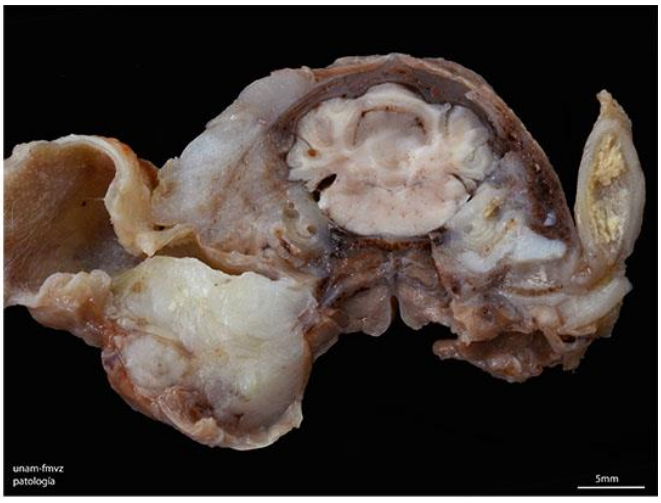
Gross Pathology: On necropsy, the animal was in good body condition. Gross examination revealed a multilobulated, white and tan mass measuring 2.5 cm arising from the right parietal bone and extending toward the right occipital lobe, surrounding the external auditory meatus and protruding through the internal ear canal, as well as rostrally to the right frontal lobe involving the right turbinates. The cut surface was yellow, white, and tan, with few white areas of mineralization. In transverse sections, the mass focally compressed the cerebral hemi-spheres, cranial nerves and partially



Cranium, hedgehog. A 2.5cm white-tan multilobular mass arises from the right parietal bone and extends to the right occipital bone, surrounding the external auditory meatus. (Photo courtesy of: Departamento de Patología (Pathology Department). Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México. Mexico city, Mexico.Web site: http://fmvz.unam.mx/fmvz/departamentos/patologia/acerca.html

obliterating the ear meatus. Both lateral ventricles were moderately dilated.



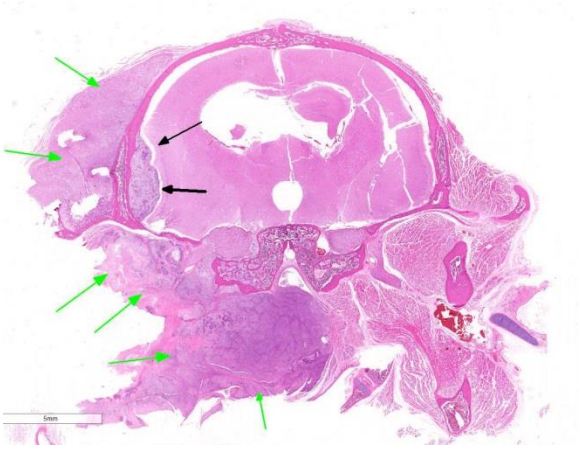


Transverse sections at the level of midbrain and hindbrain, hedgehog: In transverse sections, the mass compresses the cerebral hemispheres, cranial nerves and partially obliterates the ear meatus. Lateral ventricles were moderately dilated. (Photo courtesy of: Departamento de Patología (Pathology Department). Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México. Mexico city, Mexico.Web site: http://fmvz.unam.mx/fmvz/departamentos/patologia/acerca.html)

Laboratory results: N/A

Histopathologic Description: Microscopic examination revealed that this mass arises from the skull, protrudes both internally and externally, and compresses the brain. The mass is composed of multiple well-demarcated nodules, rimmed by a thick layer of fibrous connective tissue arranged in sheets of polyhedral to spindle-shaped cells with large interspersed areas of coagulative necrosis. Numerous islands and lakes of osteoid are present throughout the tumor. The neoplastic cells have abundant eosinophilic cytoplasm and large, round to ovoid nuclei with finely stippled chromatin and often prominent nucleoli. There is marked anisocytosis and anisokaryosis. The mitotic rate is low, averaging two per ten 400x high powered fields. In some sections, cranial nerves are markedly compressed by the neoplasm, with multifocal axonal swelling and spheroid formation (Wallerian degeneration). Neurons at this level are shrunken and hypereosinophilic (necrosis).

Contributor's Morphologic Diagnosis: Skull: Osteoblastic osteosarcoma, with cranial nerve compression, Wallerian degeneration, and neuronal necrosis.



Transverse section of head at the level of hippocampus. The neoplasm is present external to the cranium (green arrows) as well as traversing the cranium and compressing the lateral cerebral hemisphere (black arrow). (HE, 5X)

Contributor's Comment: Neoplasms in African hedgehogs are common and represent one of the main causes of disease in this species. In retrospective studies, the prevalence of neoplasia has ranged from 29% to 51%. Of these, 85% are malignant, portending an overall poor prognosis.[3] Osteosarcomas in this species have been reported in skeletal and extraskeletal locations. Skeletal locations include: mandible,[3] ribs,[1,6] and vertebra.[8] Reported extraskeletal sites include the subcutis over the scapula[2] and flank.[7] One documented case of osteosarcoma is theorized to be associated with retroviral infection.[6] Multiple concurrent neoplasms are present in a small percentage of hedgehogs with neoplasia.[3] In this case, multiple trichoepitheliomas and an endometrial stromal sarcoma were also present.

Although osteosarcoma accounts for up to 85% of malignant bone tumors in dogs and 70% in cats,[2] the occurrence in African hedgehogs is unusual. In dogs, osteosarcomas can be subclassified according to the predominant histologic pattern into six different categories: poorly differentiated, osteoblastic, chondroblastic, fibroblastic, teleangiectatic and giant-cell rich osteosarcoma. This classification scheme is an adaptation of a system developed for use in human medicine. Osteoblastic osteosarcoma is the most common subtype, and it can be further sub-classified into non-productive and productive, based on the tumor bone produced. According to this classification, this osteosarcoma would be classified as a productive osteoblastic osteosarcoma.[2]

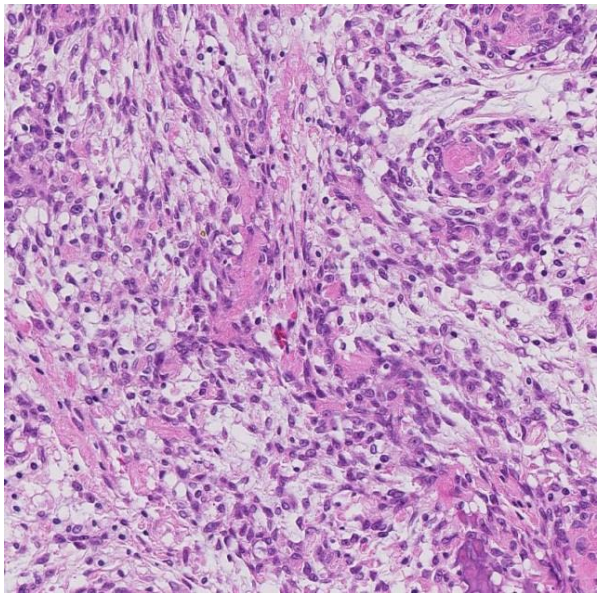
Additionally, intracranial, extracranial, and skull base neoplasms often compress the facial nerve and may result in facial paralysis.[5] In this case, cranial nerve involvement can be seen in some slides. Nerves are clearly compressed and surrounded by the neoplastic tissue, leading to Wallerian degeneration. Wallerian degeneration is a process that occurs when a nerve fiber is cut or crushed, leading to axonal separation from the neuronal cell body which degenerates distal to the injury.[9]

JPC Diagnosis: Bone, cranium: Osteosarcoma, African hedgehog, *Atelerix albiventris*.

Conference Comment: Transverse histologic sections of the cranium from this case spanned from the level of the rostral diencephalon to the caudal metencephalon. As a result, conference participants noted marked slide variation based on the anatomic location of this neoplasm and its effects on the adjacent tissue. Due to extensive variation in tissue sections submitted, histologic lesions noted by participants that may not be present on every slide include: Wallerian degeneration of the cerebrum and peripheral nerves; compression of the cerebrum and peripheral nerves with adjacent neuronal necrosis; hydrocephalus of the lateral, third, and fourth ventricles; osteosarcoma (OSA)

tumor emboli within adjacent vessels; skeletal muscle degeneration, regeneration, and necrosis; and hyperkeratosis of the external ear canal.

As mentioned by the contributor, neoplasia is an extremely common antemortem and postmortem finding in the African hedgehog. However, mesenchymal neoplasms, such as in this case, are relatively uncommon with an incidence of only about 4% at necropsy.[1,3,8] Epithelial neoplasms of the integument are the most common type, followed by round cell tumors.



Cranium, hedgehog. The neoplasm is composed of short streams of spindled cells which produce moderate amounts of osteoid. (144X)

Osteosarcoma is the most common mesenchymal neoplasm reported in African hedgehogs.[3]

In dogs and cats, osteosarcoma is characterized by rapid progression and early metastasis to the lungs, resulting in short survival times and high mortality rate.[2] This aggressive biologic behavior is similar in reported cases of osteosarcoma in African hedgehogs.[1,3,8] The contributor describes six different categories of osteosarcoma in dogs based on the predominant histologic pattern. These include:

1. **Poorly differentiated**:
    - Variation in cell size from small primitive mesenchymal to large and pleomorphic undifferentiated cells
    - Identification depends on presence of unequivocal tumor osteoid
    - Highly aggressive and associated with pathological fractures.
2. **Osteoblastic**:
    - Anaplastic osteoblasts; variable amounts of basophilic cytoplasm, and hyperchromatic, eccentric nuclei
    - Further subclassified as nonproductive or productive based on presence or absence of tumor bone production
    - Productive osteoblastic OSA is the most common subtype in dogs
3. **Chondroblastic**:
    - Neoplastic cells produce both chondroid and osteoid matrices
4. **Fibroblastic**:
    - Interlacing bundles of spindle cells resembling fibrosarcoma that produce osteoid or bone
5. **Telangiectatic**:
    - Solid areas and large blood-filled spaces lined by malignant osteoblasts that occasionally form spicules of osteoid
    - Resembles hemangiosarcoma, but is

immunonegative for factor VIII and CD31
    - Most malignant classification with least favorable prognosis.
6. **Giant cell-rich**:
    - Tumor giant cells predominate with nuclear atypia and a high mitotic rate
    - Must be differentiated from the benign giant cell tumor of bone[2]

The conference moderator cautioned participants that histologic classification of osteosarcoma is often complicated by their heterogeneous nature, as several microscopic patterns are often evident within a single neoplasm. Regardless of classification, the prognosis for all subtypes of canine central osteosarcoma is considered poor.[2] The conference moderator also mentioned different grading systems for osteosarcoma based on nuclear pleomorphism, mitotic index, necrosis, multinucleated cells, the amount of tumor matrix, and vascular invasion. Unfortunately, no histologic grading system has gained widespread acceptance by veterinary pathologists due to marked variation in histomorphology of the neoplastic cells within different regions of the same tumor.[2]

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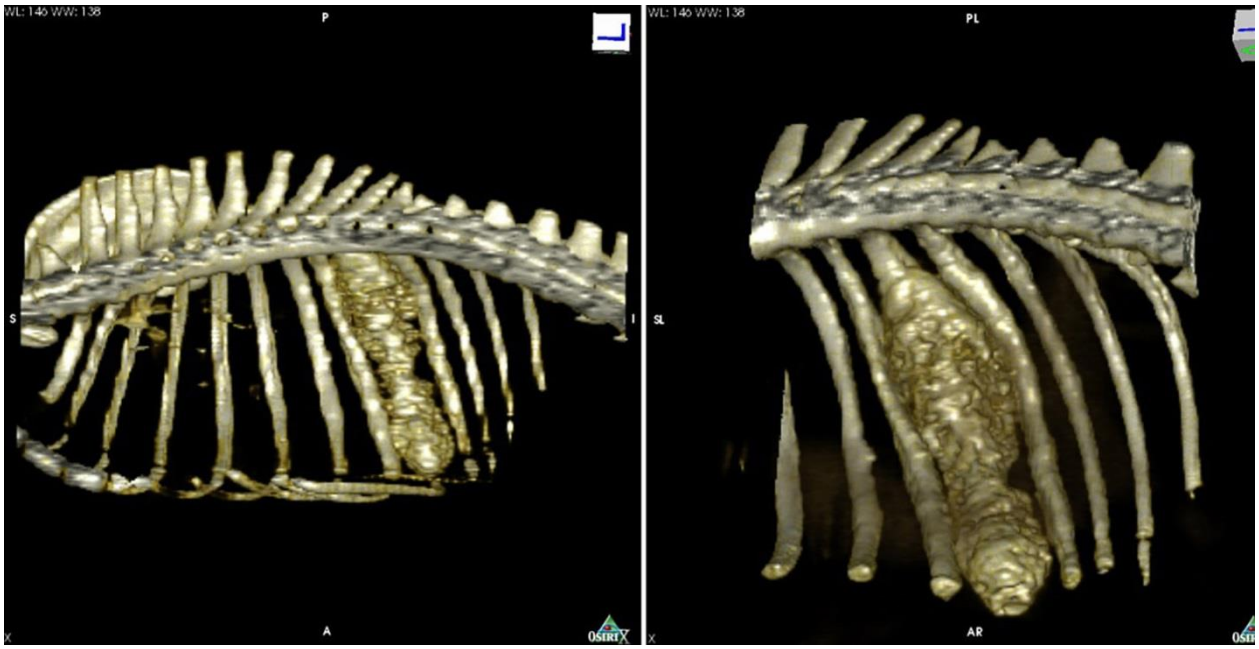
Disease. 5th ed. p.p. 959 St. Louis, MO: Elsevier; 2012.

CASE II: V254/10 (JPC 4003264).

Signalment: Four-year-old, castrated male, Labrador retriever (*Canis familiaris*).



Rib, dog. Three-dimensional CT reconstruction of a rib mass. (Photo courtesy of: Dep. Vet Resources, Weizmann Institute, Rehovot 76100, Israel http://www.weizmann.ac.il/vet/)

History: This sample is from a rib mass. The animal had an initial history of moderate thrombocytopenia, mild leukopenia and mild anemia detected during a routine CBC. Ehrlichiosis was suspected and the dog was started on a course of doxycycline. CBC values did not normalize in response to the antibiotics and the dog was referred to a specialty practice, where pronounced thrombocytopenia (40,000/microL), mild anemia and mild leucopenia were documented. PCR for *Ehrlichia canis* could not be performed while the dog was on antibiotic therapy; however, the main differential diagnosis remained chronic ehrlichiosis. Cytology of the marrow showed general hypocellularity and a mild increase in plasma cells. The dog was clinically healthy and no other abnormalities were noted. As marrow destruction in ehrlichiosis has been shown to be immune-mediated, a course of prednisone in an immunosuppressive dose was added to the doxycycline. Subsequent CBC showed improvement in all 3 blood cell lines but whenever steroids were tapered, the dog relapsed. Treatment was therefore continued and eventually the dog became cushinoid. Another immunosuppressive drug (mycophenolate) was added to the regimen. CBC improved but the dog developed ulcerated subcutaneous abscesses on the limbs. These were treated conservatively and resolved but additional abscesses developed and there were several episodes of fever. Culture of one abscess yielded *Nocardia* spp. The culture results of the remaining abscesses are unknown.

Immunosuppressant therapy was discontinued, but later resumed because of severe thrombocytopenia. A few weeks later, the dog presented with a 15cm diameter swelling on the side of the chest. The swelling appeared to be due to a subcutaneous abscess. The abscess was drained but recurred, at which point surgical debridement was performed. During this procedure, a connection to the rib was identified. The mass was evaluated by CT and resected. Material was submitted for bacterial culture and *Corynebacterium* spp and *Nocardia farcinica* were isolated.
Eventually, the dog recovered, his CBC normalized, and he is now reportedly in good health.

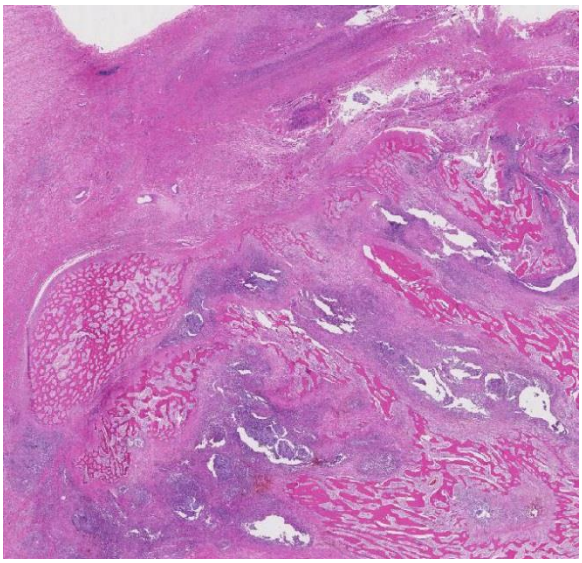
Gross Pathology: There is a 15 cm diameter firm mass attached to a rib.

Laboratory results: N/A

Histopathologic Description: Mass on rib: There are multiple large bacterial colonies surrounded by abundant accumulation of cellular debris, neutrophils, histiocytes and rare multinucleated cells. In the surrounding tissue, there is widespread fibrosis and proliferation of woven bone. The fibrous tissue is of variable maturity and within it there is multifocal hemorrhage and severe multifocal to coalescing infiltration of neutrophils, histiocytes with lesser plasma cells and lymphocytes. The woven bone is arranged in a lattice. Bone trabeculae are commonly invested with a single layer of active osteoblasts and fibrovascular tissue of low cellularity occupies the intertrabecular spaces (reactive bone). The bacterial colonies (sulfur granules) are amphophilic to basophilic, have irregular circular, undulant and vermiform shapes and are composed of dense mats of rods and filamentous bacteria. The outer perimeter of the colonies is covered by a thin eosinophilic rim.

On special stains, the bacteria are gram positive and strongly stain with a modified acid fast stain, Fite-Faraco, and inconsistently with Ziehl Neelsen (ZN). In the modified ZN stain, long filamentous and beaded organisms are easily seen.

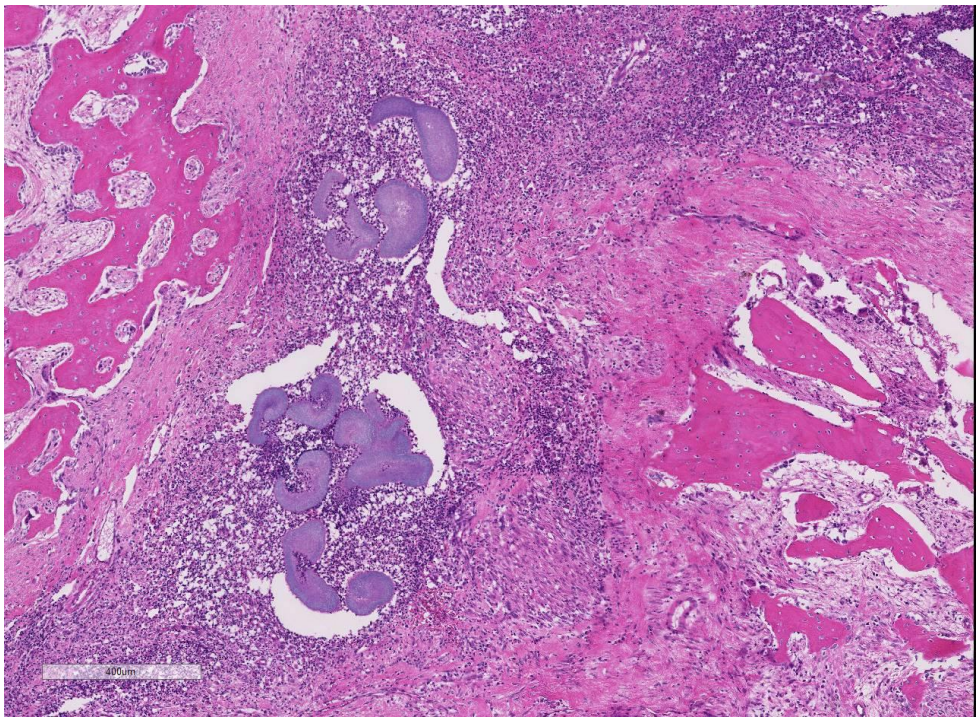
Contributor's Morphologic Diagnosis: Rib: Severe pyogranulomatous osteomyelitis with bacterial colonies consistent with *Nocardia* spp.



Rib, dog. There is loss of cortical architecture, and a dense inflammatory infiltrate traverses among trabeculae of proliferating woven bone. (HE, 5X)

Contributor's Comment: The morphologic features and staining characteristics of the bacterial colonies are consistent with *Nocardia* spp. which was supported by culture. In dogs, distemper and other causes of immunosupression may predispose to nocardiosis.[3] There are two recent reports of nocardial infection in dogs treated with immunosuppressive agents.[6] In the current case, immunosuppressive treatment was used because the clinicians thought the pancytopenia with preferential involvement of the platelets was due to subacute to chronic ehrlichiosis. In their experience,

dogs often recover from acute and subclinical ehrlichiosis but go on to develop subclinical and progressive bone marrow suppression, which is typically detected too late in the course of the disease. In this case, the ongoing myelosuppression was detected fortuitously but long-term immuno-suppression (to which putative *E. canis*-induced myelosuppression may have been a contributing factor) led to opportunistic bacterial infection.



Rib, dog. The suppurative and histiocytic infiltrate is centered on large colonies of filamentous bacilli enmeshed in a pink matrix of Splendore-Hoeppli material. (HE, 47X)

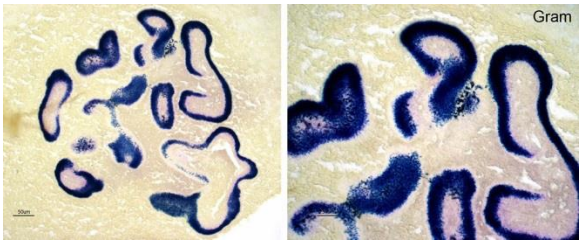
Actinobacteria are gram-positive, terrestrial, or aquatic bacteria which constitute one of the dominant bacterial phyla. Most *Actino-bacteria* of medical significance belong to the order *Actinomycetales*. Due to their filamentous appearance, these organisms were thought to be fungi for many years but were later shown to be higher bacteria.[3,7] *Actinomyces* and *Nocardia* are the most common *Actinomycetes* which cause disease.[7] Organisms in the genus *Nocardia* are aerobic, gram-positive and partially acid fast saprophytes with a worldwide distribution. They commonly occur in soil and decaying organic matter and may cause opportunistic infection. *N. asteroides* is the species most commonly implicated in disease and has been recovered from lesions in humans, dogs, cats, cattle, goats, horses, pigs, marine mammals and fish.[6] Cattle and dogs are the most commonly affected animals, but these infections are sporadic.[3] Infection is not contagious and affected animals do not constitute a public health hazard.[7] Failure to distinguish properly between *Nocardia* and *Actinomyces* in earlier reports has caused confusion in the literature regarding nocardiosis in animals.[3]

Typically, nocardial infection originates from organisms introduced into skin wounds or aspirated into the respiratory tract, leading to superficial skin lesions, and necrotizing pneumonia, respectively.[3,7] Infections may remain localized at the site of introduction but there is a tendency for the organisms to spread either by direct extension or vascular invasion and hematogenous dissemination.[4,7] There is evidence that strains within the same species vary markedly in their virulence.[4] Cell-mediated immunity and neutrophils appear to be of critical importance in defense against these bacteria.[6,7] In dogs, infections are more

common in the young (<1-year-old), which may be due to increased exposure or to reduced resistance.[7]

The typical gross cutaneous lesions caused by infection with filamentous bacteria (*Actinomyces* and *Nocardia*) consist of abscesses, cellulitis, draining fistulous tracts, and dense fibrous masses.[7] Lesions progress slowly by local extension. The exudate is variable and can contain white, yellow, tan or gray "sulfur granules."[4,7] Histologically, pyogranulomatous inflammation is commonly seen.[4,7] The sulfur granules consist of masses of organisms, which may be bordered by clubbed corona of brightly eosinophilic Splendore-Hoeppli material.[4,7] It has been reported that the shape of this material is characteristic for the agent involved.[1] *Nocardia* spp. have a limited tendency to clump together, thus they typically do not form granules.[4,7] However, in some cases, nocardial lesions are morphologically indistinguishable from those induced by *Actinomyces*[7], as appears to be the case here. In gram-stained sections, the bacteria are seen as branched and beaded filaments up to 1µm wide. Fragmentation of the filaments produces coccobacillary forms. The beaded appearance is due to alternating gram-positive and gram-negative regions in the filament and is more evident in *Nocardia* spp. *Actinomyces* are acid-fast negative with most acid-fast stains. Many, but not all, *Nocardia* spp. stains strongly with modified Ziehl-Neelsen. Some acid-fast negative *Nocardia spp.* cannot be differentiated from *Actinomyces* in sections, and culture is required.[7]

The cutaneous and subcutaneous nodules are progressive and may extend to involve underlying bone, as appears to have occurred in this case. The lesion is similar to "lumpy jaw" in cattle, a classic example of actinomycotic mycetoma. In this condition, traumatic implantation of *Actinomyces bovis* in the mandibular mucosa progresses to involve the mandibular bone.[7] In cattle, *N. asteroides* can also cause granulomatous mastitis when contaminated drugs for the treatment or prevention of mastitis are introduced through the teat canal. This condition may also occur in outbreaks.[2] As noted above, a second relatively common site of infection with filamentous bacteria (*Nocardia*, *Actinomyces* and *Bacteroides*) in dogs and cats is the thoracic cavity, causing pyogranulomatous pleuritis with intra-thoracic accumulation of blood-stained pus and reactive mesothelial cells, so-called "tomato soup." This is no longer considered pathognomonic of nocardial infection.

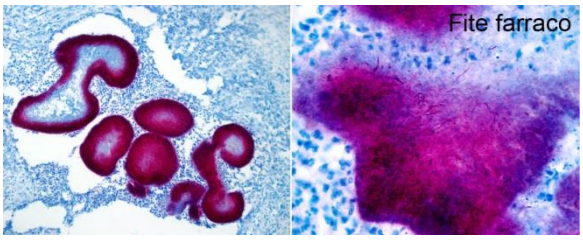


Rib, dog. A tissue Gram stain demonstrates that the bacilli are gram-positive. (Gram, 200X) (Photo courtesy of: Dep. Vet Resources, Weizmann Institute, Rehovot 76100, Israel http://www.weizmann.ac.il/vet/)

JPC Diagnosis: Bone: Osteomyelitis, pyogranulomatous and sclerosing, with new bone formation and colonies of filamentous bacteria, Labrador retriever, *Canis familiaris*.

Conference Comment: This case nicely demonstrates the histopathologic appearance of sulfur granules in tissue section. Sulfur granules, which are usually located within neutrophil abscesses or pyogranulomas, are distinct masses of bacteria bordered by eosinophilic radiating projections of Splendore-Hoeppli material characteristic for actinomycetes bacteria.[2,7] Although, formation of granules is a more common feature of actinomycosis, *Nocardia* spp may

form sulfur granules which are indistinguishable from *Actinomyces* spp. These structures can be seen grossly as small yellow granular material present within the exudate.[2,7]



Rib, dog. The filamentous bacilli are also acid-fast. (Fite-Furaco, 200X) (Photo courtesy of: Dep. Vet Resources, Weizmann Institute, Rehovot 76100, Israel http://www.weizmann.ac.il/vet/)

Conference participants briefly discussed the pathogenesis of the Splendore-Hoeppli phenomenon. As mentioned above, Splendore-Hoeppli reaction is typically the brightly eosinophilic, radiating, club-shaped, material around bacterial colonies in histologic sections.[2,5,7] This material is composed of antigen-antibody complexes, tissue debris, and fibrin. Although the exact nature of this reaction is unknown, it is thought to be a localized immune response to an antigen-antibody deposition related to fungi, parasites, bacteria or inert materials. The characteristic formation of the Splendore-Hoeppli reaction around infectious agents or biologically inert materials is likely the body's attempt to contain the injurious agent on the part of the host. However, it also likely prevents phagocytosis and intracellular killing of the agent leading to prolonged damage or infection.[5]

Identification of the tissue as costal bone in this section was difficult for all conference participants. As a result, participants discussed effective strategies for differentiating reactive new bone formation from neoplastic bone disease, given the lack of normal tissue architecture. The conference moderator instructed that first one must look for evidence of the parent bone structural elements such as osteons within complex mature lamellar bone to differentiate new bone formation from osseous metaplasia. Next, to differentiate reactive bone from neoplastic bone, one must look at the characteristics and orientation of the osteoblasts. Given that osteoblasts are terminally differentiated products of mesenchymal stem cells, there should be no mitotic activity within this cell population.[8] Additionally, in reactive bone formation, osteoblasts form highly organized groups connected by gap junctions allowing the cells to function in a well-regulated manner. In tumor bone, neoplastic mesenchymal cells are haphazardly arranged and produce an osteoid matrix without the regularity and regimentation present in reactive bone osteoblasts.

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http://www.weizmann.ac.il/vet/

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CASE III: HV6474 (JPC 4085013).

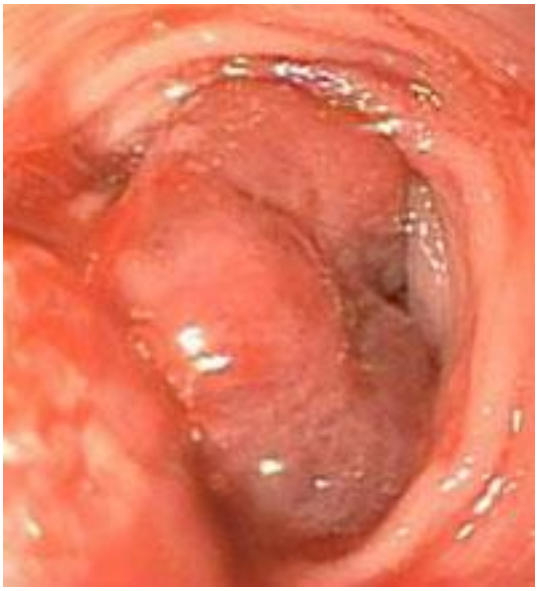
Signalment: Ten-year-old, ovario-hysterectomized female, miniature dachshund (*Canis familiaris*).

History: The animal had a one-month history of hematochezia. A rectal mass was found and surgical excision of the mass was performed by the rectal pull-through technique.

Gross Pathology: There was a polypoid mass (5×3×1 cm) on the rectal mucosa.

Laboratory results: N/A

Histopathologic Description: The large polyp showed severe cellular infiltration, granulation tissue, epithelial cell proliferation, interstitial mucus accumulation, hemorrhage, and osteoid formation. At the base of the polyp, goblet cells proliferated without cellular atypia and crypts were dilated due to abundant mucinous material accumulation. Mucous inflammation consisted of predominant neutrophil infiltration and less frequent macrophage, plasma cell, and lymphocyte infiltration. There are similar small lesions in the adjacent polyps.



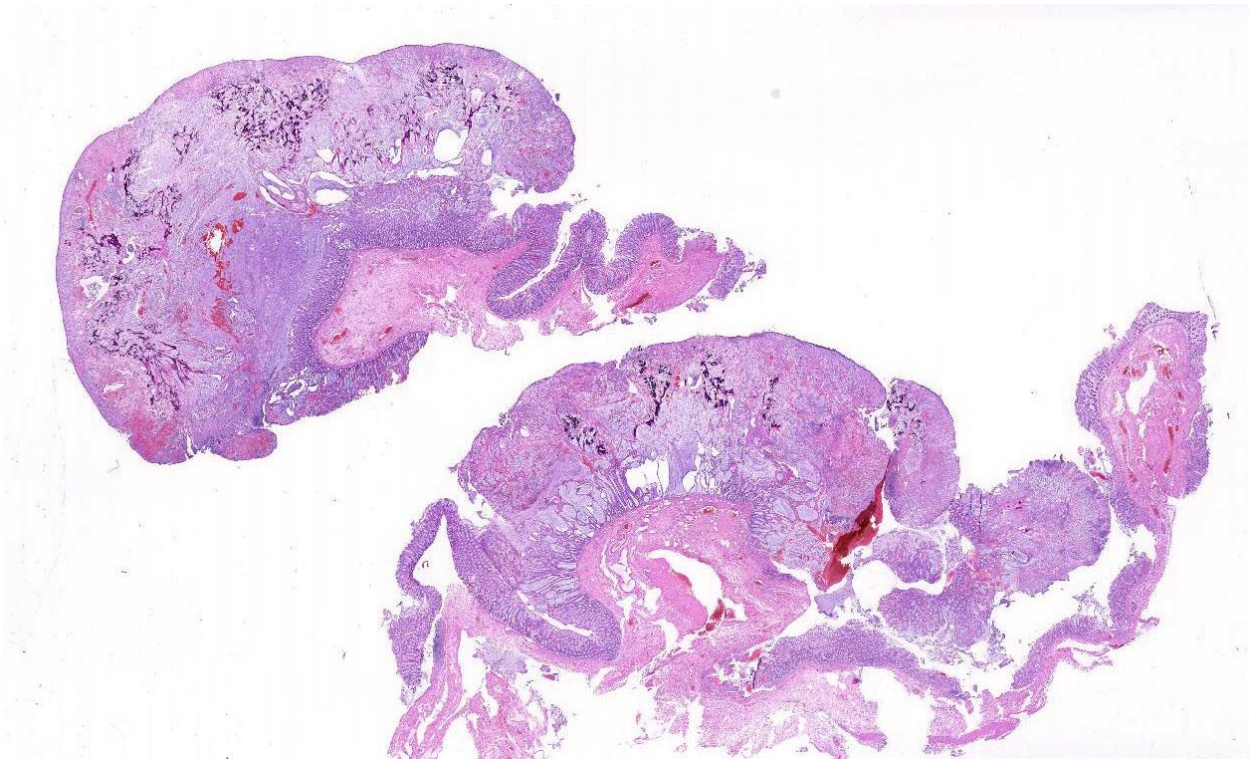
Rectum, dog. A polypoid mass measuring 5×3×1 cm arises from the rectal mucosa (Photo courtesy of: Laboratory of Comparative Pathology, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University.)

Contributor's Morphologic Diagnosis: Rectum: Polyp, with severe inflammation, epithelial cell proliferation, mucus accumulation in crypts (Inflammatory polyps of miniature dachshunds).

Contributor's Comment: Colorectal polyps are relatively common in dogs. These polyps include non-neoplastic (hyperplastic or inflammatory) polyps and neoplastic polyps such as adenoma and adenocarcinoma. In Japan, Miniature Dachshunds show a significantly higher tendency to develop multiple polyps than other breeds.[5] Recently, it was proposed to refer to these polyps as inflammatory polyps of miniature dachshunds.[7] These polyps are non-neoplastic single or multiple polypoid lesions arising from the rectum and distal colon, and are characterized by mucosal proliferation without cellular atypia and significant inflammatory cell infiltration.[5] Microscopically, the inflammatory polyps show inflammatory cell infiltration, hyperplastic goblet cells with dilated crypts, mucus accumulation in crypts, granulation tissue, and occasional osteoid formation.[1,8]

In comparisons based on epithelial composition, the polyps displayed a thickened mucosa containing increased goblet cells, dilated crypts filled with a large amount of mucus, and mild inflammatory infiltration (mainly lymphocytes and macrophages) in the early stage.[8] In later stages, leukocyte infiltration was more severe and consisted of mostly neutrophils and macrophages. Additionally, interstitial mucus accumulation, granulation tissue, and occasional osteoid formation were seen.[8] In this case, predominant neutrophil infiltration, interstitial mucus accumulation, granulation tissue and osteoid formation were observed in the polyp; therefore, the lesion is comparable to that in late stage.

Although the pathogenesis remains unclear, some studies suggested that the arachidonic acid cascade, increased IL-8 production in macrophages, dysregulation of toll-like receptors and proinflammatory cytokines, and IL-17A upregulation in T cells are associated with the lesion formation.[2,6,7] Additionally, 80% of cases responded to immunosuppressive therapy with prednisolone and cyclosporine.[5] These findings indicate that inflammatory polyp of miniature dachshunds is an immune-



Rectum, dog. The submitted material contains two sections of a dome-shaped polyp arising from the rectal mucosa. (HE, 5X)

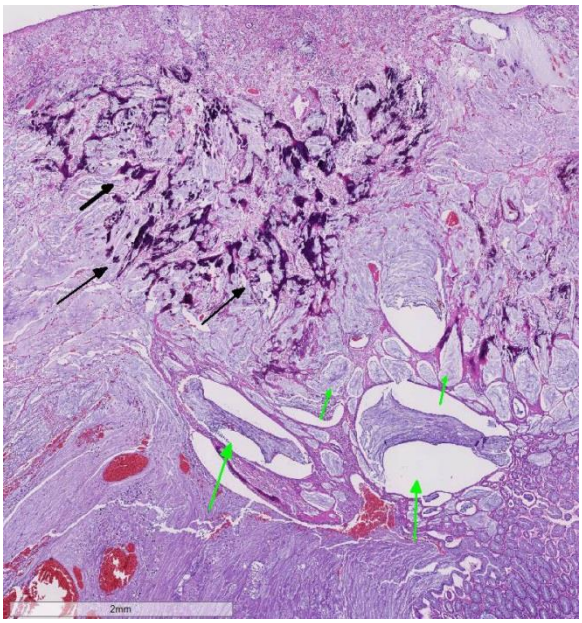
mediated disease. However, Igarashi et al reported that two cases developed adenomas secondary to inflammatory colorectal polyps during long-term medical therapy.[4] Further investigations are required to elucidate the pathogenesis, oncogenesis, and prognosis in inflammatory polyps of miniature dachshunds.

JPC Diagnosis: Rectum: Inflammatory polyp, miniature dachshund, *Canis familiaris*.

Conference Comment: The contributor provides a compelling example and concise summary of colorectal inflammatory polyps of miniature dachshunds (IPMD). It is unknown why inflammatory polyps occur much more commonly in miniature dachshunds than any other breed of dog in Japan.[8] Colorectal polyps are commonly present as single or multiple friable, lobulated, sessile, or pedunculated lesions on the mucosa.[1-8] Among the polypoid masses present in the intestinal tract of dogs, the major histopathologic subtypes include nonneoplastic and hyperplastic polyps, papillary adenomas, and papillary adenocarcinomas.[8] Most conference participants agreed that the late-stage inflammatory polyp, in this case, was difficult to differentiate from a neoplastic papillary adenoma or mucinous papillary adenocarcinoma.

In a recent study by Uchida et al. published in *Veterinary Pathology*, they classify IPMD into early (stage 1) and late (stages 2 and 3) lesions based on the histopathology of the mucosal epithelial components and inflammatory cells present.[8] In addition, they use immunohistochemistry to distinguish inflammatory polyps from neoplastic cells of adenomas and adenocarcinomas using cytokeratin 20 (CK20) and beta-catenin. Normally, intestinal goblet cells and enterocytes are strongly immunopositive for CK20. The markedly hyperplastic goblet cells, which is a key histologic feature of IPMD, are also strongly immunopositive for CK20, while neoplastic cells of adenomas and adenocarcinomas were immunonegative. This indicates that inflammatory polyps are a proliferation of well-differentiated epithelial tissue and non-neoplastic. The authors also demonstrate that IPMD expressed only intracytoplasmic beta-catenin, while neoplastic cells of adenomas and adenocarcinomas expressed both intracytoplasmic and intranuclear beta-catenin.[8]

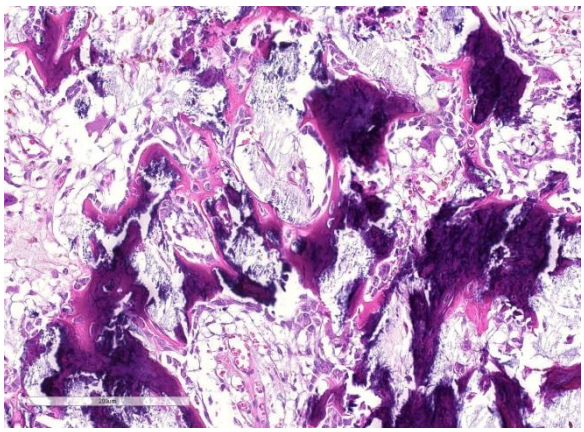
There have been reports of dogs with later stage (stage 3) IPMD developing rectal adenomas secondary to neoplastic transformation.[5] Chronic inflammation and the production of pro-inflammatory cyto-kines can induce mutations in oncogene and tumor suppressor genes. In humans, the connection between chronic inflammatory



Rectum, dog. The polyp contains numerous dilated glandular structures filled with mucin at the interpace with the mucosa (green arrows), and numerous trabeculae of mineralized woven bone (black arrows). (HE, 5X)

bowel disease and development of both inflammatory polyps and colorectal carcinoma is well established.[5] Aberrant activation of the Wnt/beta-catenin pathway occurs in the vast majority of colorectal cancers in humans and dogs and likely plays a role in neoplastic transformation of late stage IPMD.[5,8]

Inflammatory colorectal polyps are typically treated with immunosuppressive therapy and endoscope guided polypectomy.[1,5,8] Unfortunately, recurrence after polypectomy is common. Recent research suggests that dysbiosis of the gastrointestinal tract is



Rectum, dog. Higher magnification of the traveculae of woven bone. Bony spicules are lined by osteoblasts and rare osteoclasts, and interstices contain fat and mucin. (HE, 144X)

associated with IPMD and may be a potential future therapeutic target.[3]

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CASE IV: D15-000804 (JPC 4066248).

Signalment: Six-month-old female mixed breed calf (*Bos taurus*).

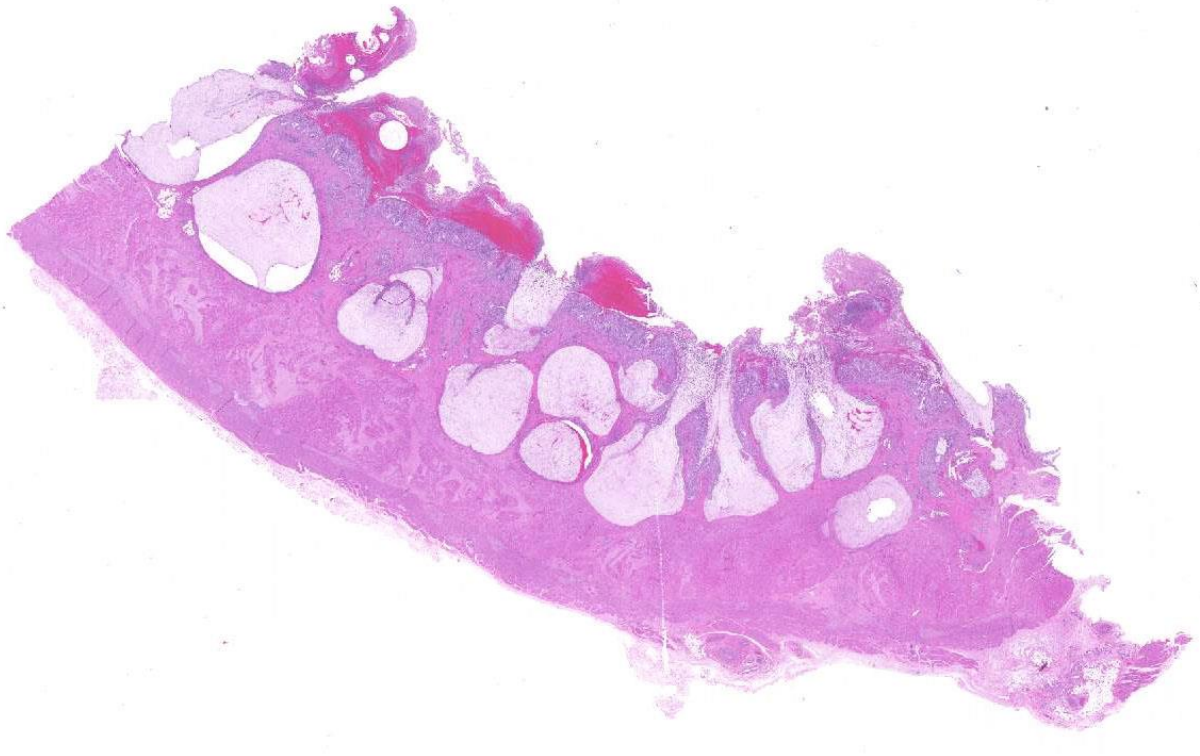
History: This heifer was running a fever of 103.6 F and was suspected of a respiratory disease. She was treated with Baytril® and oxytetracycline, but died a week later. The animal was submitted for necropsy to the Kansas State Veterinary Diagnostic Laboratory.

Gross Pathology: The mucosa of the distal part of the small intestine was diffusely dark red and the lumen contained dark brown, mucoid, sludgy material. The wall of spiral colon and cecum was thick, edematous and the lumen contained thick clots of blood. The colonic and cecal mucosae were diffusely dark red.

Laboratory results: Immunohistochemistry (IHC) revealed strong positive immuno-reactivity for bovine viral diarrhea virus (BVDV) in the crypt epithelial cells. IHC was negative for bovine corona virus.

Histopathologic Description: Diffusely, there is marked necrosis of the epithelial cells lining the crypts. Multifocally, the crypts are markedly ectatic and filled with abundant mucus admixed with karyorrhectic cellular debris and low to moderate numbers of degenerate leukocytes. Remaining crypts are lined with attenuated epithelium that is occasionally hyperplastic. The crypts contain moderate numbers of degenerate neutrophils, admixed with necrotic debris (crypt abscesses). The adjacent lamina propria is moderately expanded by large aggregates of lymphocytes, plasma cells, fewer neutrophils, and moderate amounts of fibrin.

The overlying mucosa is diffusely eroded



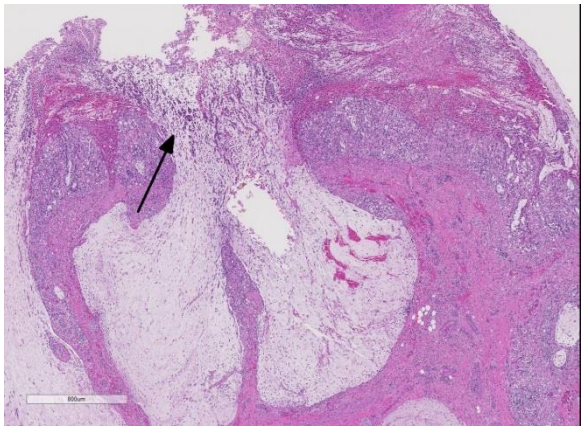
Colon, ox: At low magnification, colonic crypts are markedly ectatic and invade into the underlying muscular tunics. The mucosa is decreased in thickness and covered by a layer of hemorrhage and necrotic debris. (HE, 4X)

and covered by a thick layer of necrotic cellular debris admixed with numerous degenerate neutrophils, abundant fibrin, large numbers of extravasated erythrocytes, and rare bacterial colonies. The tunica muscularis is expanded by moderate amounts of edema and fibrin.

Contributor's Morphologic Diagnosis: Colon: Colitis, necrotizing, diffuse, severe, with multifocal crypt abscessation.

Contributor's Comment: The clinical history, gross and microscopic findings are consistent with mucosal disease caused by persistent infection with bovine viral diarrhea virus. Bovine viral diarrhea is an economically important disease of cattle and other even-toed ungulates that are caused by bovine viral diarrhea virus (BVDV). BVDV belongs to the *Pestivirus* genus of the family *Flaviviridae*. It has two recognized genotypes, (BVDV-1 and BVDV-2) based on antigenic and genetic differences. These genotypes are further classified into cytopathic (cp) and non-cytopathic (ncp) biotypes based on *in vitro* cell culture characteristics. Ncp BVDV strains do not induce apoptosis in cultured cells, are ubiquitous in nature and frequently isolated from bovine tissues while cp BVDV strains are infrequently isolated unless accompanied by ncp BVDV and induce apoptosis in susceptible cell lines. The molecular basis for cytopathogenicity was reported to involve mutations that lead to increased expression of NS3 protease in cp strains versus little to no expression in ncp strains.[5] However, it is important to note that *in vitro* cytopathogenicity does not correlate with virulence or the clinical severity of the disease.

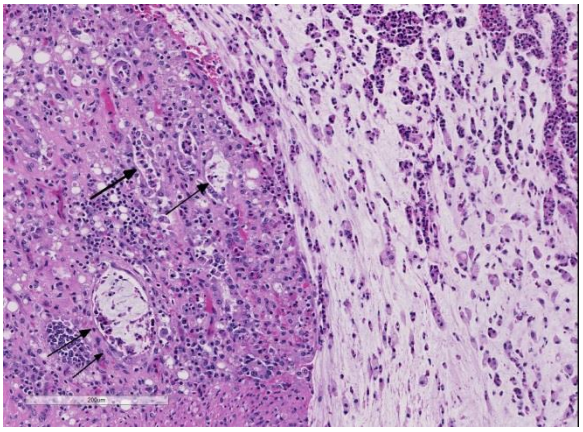
The pathogenesis and the clinical manifestations of BVDV infection in cattle are dependent on the age, immune status as well as the biological properties of the infecting strain.[3] Previously described cp and ncp biotypes are closely associated with two fundamentally different forms of infection in cattle; either transient or persistent. In areas where BVDV infection is endemic, acute infection with ncp BVDV results in transient viremia with mild clinical signs that include low-grade fever, diarrhea and coughing. The infection is cleared within a few days and the seropositive animals are protected from re-infection for life. In contrast, fetal infection within second to fourth months of gestation results in immunotolerance and lifelong persistent infection. Persistently infected (PI) calves develop severe clinical disease referred to as mucosal disease that is characterized by widespread necrosis of the alimentary mucosa and lymphoid tissues. Mucosal disease only develops in PI cattle and is the result of persistent infection with a ncp strain followed by a subsequent post-natal infection with a cp strain that arise from mutation/s in already persisting ncp strain. The ability of the ncp strain to inhibit the induction of type-I interferon (IFN) in the fetus is generally believed to enable the virus to establish persistent infection. Research in last decade has identified two



Colon, ox: In the submitted section, the necrotic mucosa is interrupted by markedly dilated crypts which infiltrate down into the underlying muscularis. Such crypts often invade underlying Peyer's patches, but there is an almost total loss of lymphoid tissue in this section. (HE, 25X)

candidate genes that interfere with host cell's IFN induction namely E^{rns}, a structural glycoprotein with RNase activity and N^{pro}, an N-terminal autoprotease.[2]

The PI calves are a major concern because they shed large amounts of virus and are an important source of infection for the uninfected animals in the herd. Control programs are specifically designed to detect and eliminate PI animals. There are a wide range of diagnostic tools available to detect virus and virus-specific immune response.[4] Multiple ELISA kits are commercially available for detection of BVDV-specific antibodies in various biological samples including serum and milk. These kits are cost effective and generally used for screening large herds or conducting seroprevalence studies. It is important to

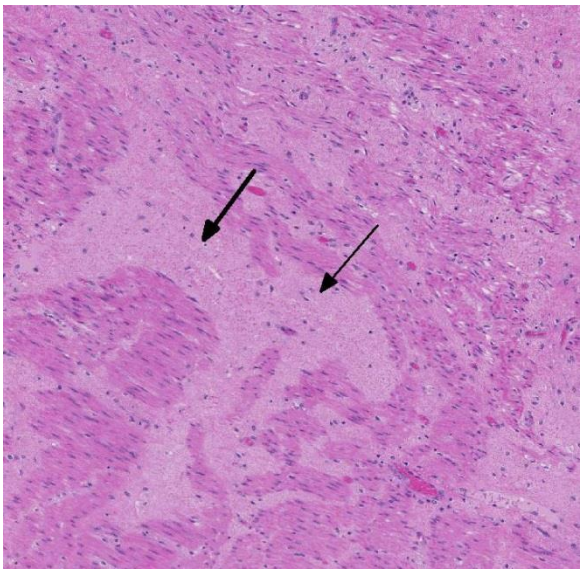


Colon, ox: Higher magnification of the colonic mucosa at the edge of the necrotic crypt. Glands are dilated and contain a combination of sloughed epithelial cells as well as neutrophils and are only segmentally lined by attenuated regenerating epithelium (crypt abscesses) (arrows). The massively dilated crypt contains aggregates of neutrophils, individualized muciphages, and abundant mucin. (HE, 100X)

note that PI infected animals are seronegative. RT-PCR is now widely accepted as a standard for BVDV diagnosis while immunohistochemistry remains one of the popular methods for antigen detection from the ear notch tissue samples.

JPC Diagnosis: Colon: Colitis, necrotizing diffuse, severe with pseudomembrane formation, marked crypt abscessation, crypt herniation, and lymphoid depletion, mixed breed calf, *Bos taurus*.

Conference Comment: The contributor provides an excellent summary of the epidemiology, pathogenesis, and diagnostic testing for persistent infection and mucosal disease in animals infected with bovine pestivirus. Other members of the *Pestivirus* genus of veterinary importance are classical swine fever virus and border disease virus.[6] Emerging pestiviruses include: Bungowannah virus, isolated from swine in an Australian piggery; atypical (HoBi-like) pestiviruses identified in Europe, Asia, and

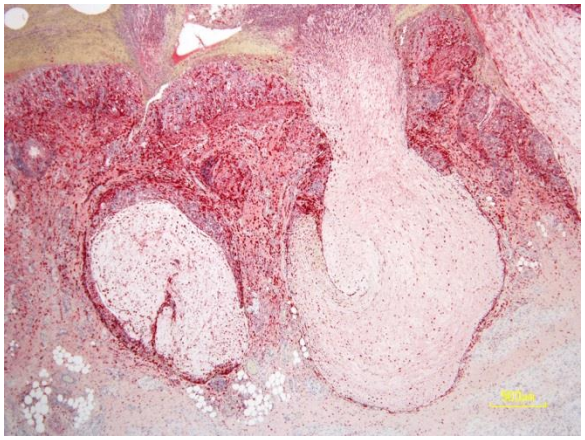


Colon, ox: Smooth muscle of the muscular tunics contains large lakes of edema fluid (arrows) (HE, 48X)

South America, discussed below; the pronghorn antelope pestivirus strains; and the giraffe strain of pestivirus (PG2).[5]

There are basically two forms of clinically severe BVDV in cattle. The first is mucosal disease in persistently infected cattle

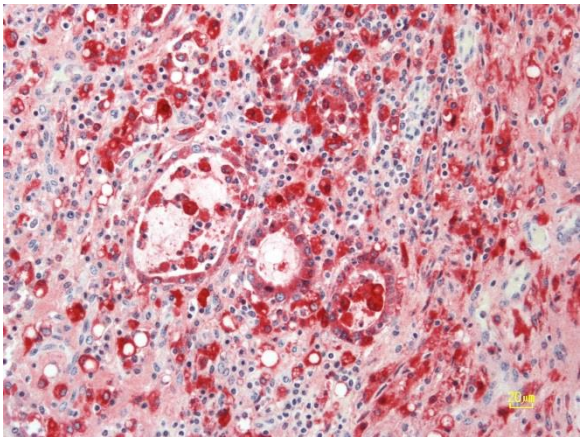
described by the contributor. The second is the more recently recognized severe acute BVD caused by highly virulent strains of bovine pestivirus. Viruses from both BVDV-1 and BVDV-2 species can induce severe acute BVD, however, this form is usually caused by BVDV-2.[2, 6] Severe acute BVD is characterized by high morbidity and high mortality in all age groups of susceptible animals and has a peracute to acute course, with fever, sudden death, diarrhea, or pneumonia. It can also be associated with thrombocytopenic syndrome



Colon, ox. Immunohistochemistry reveals strong positive immunoreactivity for bovine viral diarrhea virus throughout the mucosa, and submucosa. (Photo courtesy of: Department of Diagnostic Medicine and Pathobiology, Kansas State Veterinary College of Veterinary Medicine, 1800 Denison Avenue, Manhattan, KS 66506, http://www.vet.k-state.edu/depts/dmp/index.htm) (anti-BVDV, 100X)

characterized by severe epistaxis, hyphema, mucosal hemorrhage, and bloody diarrhea. This syndrome is typically associated with infection by non-cytopathic high-virulence BVDV-2 species.[2,6] Severe acute disease resembles both mucosal disease, malignant catarrhal fever, and rinderpest virus infection grossly. Histologically, rinderpest (*Paramyxoviridae*, morbillivirus) is distinguished from BVDV by the presence of intranuclear and intracytoplasmic inclusion bodies as well as syncytial cells. Malignant catarrhal fever (*Herpesviridae*, gammaherpesvirus) can be distinguished from BVDV by the appearance of the lymphoid organs; involution of lymphoid tissue occurs in BVD and lymphoproliferation occurs in MCF.[6] Regardless, antigenic or molecular characterization of the virus is required for definitive diagnosis. In this case, the contributor used immunohistochemistry, which is a highly specific modality to test for BVDV antigen in affected tissue.

In addition to persistent infection, early embryonic death, and abortion, in utero infection with bovine pestivirus can induce teratogenic lesions if a fetus is infected between 90-120 days of gestation. These lesions include: microcephaly, cerebellar



Colon, ox. Immunohistochemistry reveals strong positive immunoreactivity for bovine viral diarrhea virus within crypt epithelium. (Photo courtesy of: Department of Diagnostic Medicine and Pathobiology, Kansas State Veterinary College of Veterinary Medicine, 1800 Denison Avenue, Manhattan, KS 66506, http://www.vet.k-state.edu/depts/dmp/index.htm) (anti-BVDV, 400X)

hypoplasia and dysgenesis, hydranencephaly, hydrocephalus, and defective myelination of the spinal cord, microphthalmia. Other reported lesions include congenital cataracts, retinal degeneration, and optic neuritis. By day 135, the fetus is immunocompetent and will produce antibodies against the virus.[6]

Conference participants briefly discussed the emerging atypical pestivirus known as BVDV-3 or HoBi-like virus. This strain was recently recognized in Europe in fetal bovine serum imported from Brazil, where the disease is likely endemic. The clinical disease caused by this virus closely resembles BVDV infection, including growth retardation, reduced milk production, respiratory disease, reduced reproductive performance, and increased mortality among young animals.[1,6] In addition, commercially available BVDV diagnostic tests may not be able to detect HoBi-like viruses or to differentiate between BVDV and HoBi-like viruses. Current vaccines against BDVD have very little cross-protection against HoBi-like viruses.[1,6] Emergence of this pathogen could have negative implications for control and eradication programs for BVDV worldwide.

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