



## WEDNESDAY SLIDE CONFERENCE 2016-2017

# Conference 4

14 September 2016

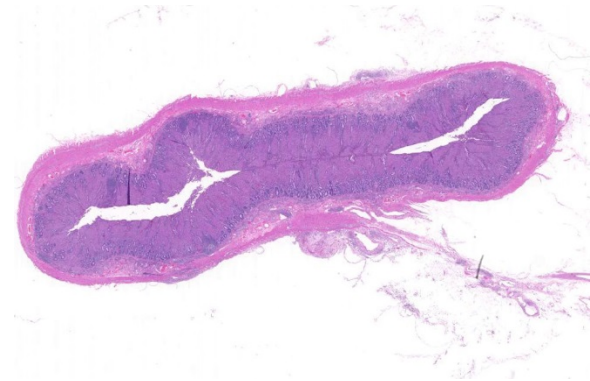
### CASE I: D16-02 (JPC 4083954).

**Signalment:** One-year-old, female, Hampshire sheep (*Ovis aries*).

**History:** A one-year-old, female, Hampshire sheep was received for necropsy to the Kansas State Veterinary Diagnostic Laboratory at Kansas State University (KSU). She was presented earlier to the KSU teaching hospital with a clinical history of being down and unable to get up. She was being treated with Baytril® for two days, but her condition worsened over time. She was eventually euthanized. The sheep belonged to a herd with a history of chronic malnutrition. The owner has lost a steady number of sheep over the years. The flock was being treated for coccidiosis at the time of submission.

**Gross Pathology:** The sheep was thin with prominent ribs and scant amounts of body fat. There were no significant gross lesions in any organ, including the gastrointestinal tract.

**Laboratory results:** Real-time PCR from paraffin block shavings was strongly



*Intestine, sheep. The intestinal mucosa is moderately thickened by an eosinophilic cellular infiltrates that separates and replaces crypts. Villi are markedly blunted. (HE, 4X)*

positive for *Mycobacterium avium paratuberculosis* (MAP).

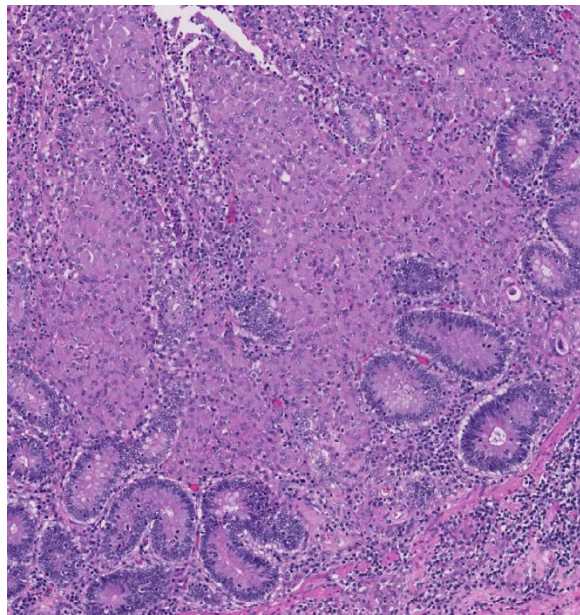
**Histopathologic Description:** Small intestine (ileum): There is diffuse blunting, shortening and fusion of the intestinal villi. The lamina propria is markedly expanded and crypts at the base of mucosa are widely separated by a diffuse sheet of epithelioid macrophages admixed with rare multinucleated giant cells, and small numbers of lymphocytes and plasma cells. These macrophages have abundant, finely-granular to foamy, eosinophilic cytoplasm. The submucosa is diffusely expanded by infiltrating

macrophages admixed with small to moderate numbers of lymphocytes, plasma cells, and increased clear space and ectatic lymphatics (edema). The serosa is diffusely expanded by mild edema, dilated lymphatics that contain intraluminal plugs of macrophages, and small to moderate numbers of macrophages, lymphocytes and plasma cells that predominantly surround the lymphatics. There are rare intraepithelial coccidian parasites within the crypts. The macrophages in the lamina propria of the intestine contain numerous intracytoplasmic acid-fast bacilli.

**Contributor’s Morphologic Diagnosis:**

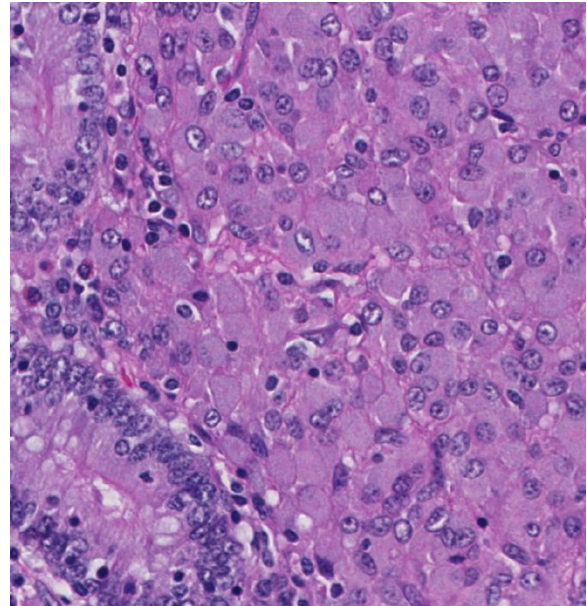
Small intestine (ileum): Granulomatous enteritis with villus atrophy, lymphangitis, diffuse, severe, chronic with numerous intrahistiocytic acid-fast bacilli.

**Contributor’s Comment:** The microscopic findings are diagnostic for ovine paratuberculosis. Paratuberculosis, or Johne’s disease, is an infectious and chronically



*Intestine, sheep. Higher magnification of the infiltrate of macrophage with fewer lymphocytes and neutrophils which markedly expands the lamina propria and effaces intestinal crypts. (HE, 63X)*

progressive granulomatous disease which affects domestic and wild ruminants worldwide. The causative agent, *Mycobacterium avium paratuberculosis* (MAP), is a slow growing mycobactin-dependent acid-fast bacillus that has been linked to Crohn’s disease in humans. The bacteria may persist in the environment for extended



*Intestine, sheep. Still higher magnification of the mucosal infiltrate, demonstrating large plump macrophages with gray, granular cytoplasm (which corresponds to the large number of intracytoplasmic bacilli.) (HE, 300X)*

periods of time, a continuing concern while implementing control programs.<sup>7</sup>

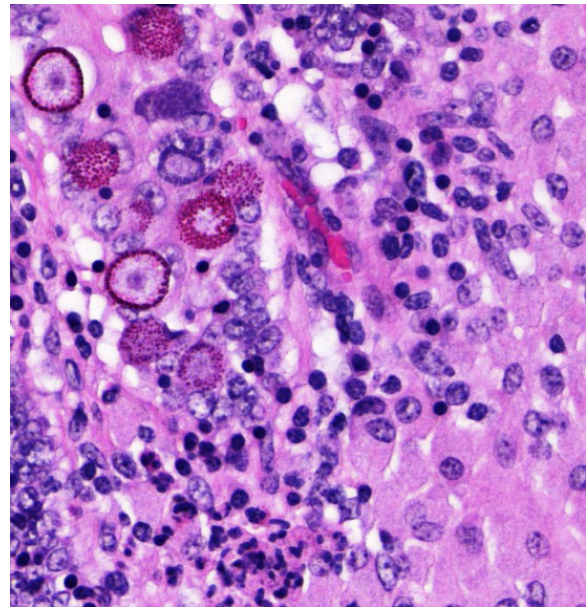
Sheep are usually infected early in life via the fecal-oral route, although infection can also be acquired through consumption of contaminated colostrum or *in utero* during advanced stages of the disease. A recent study characterized the pathology following experimental infection of MAP in adult sheep and found focal lesions restricted to the intestinal lymphoid tissue.<sup>2</sup> The M cells, specialized non-villous epithelial cells located in the Peyer’s patches of small intestine, act as a main portal of entry and facilitate translocation of MAP across the intestinal epithelium where they are

phagocytized by macrophages. Macrophage recognition of MAP is mediated by host pathogen recognition receptors (PRRs), including Toll-like receptors (TLRs) and intracellular NOD-like receptors (NLRs). The MAP bacterium has a unique ability to replicate within macrophages by blocking or modulating antimicrobial activities that allow long-term survival.<sup>5</sup>

Broadly, two distinct phenotypes have been recognized based on the histologic features and the pathogen load: the lepromatous (organism-rich or multibacillary) form and the tuberculoid (paucibacillary) form. It is generally accepted that animals with paucibacillary lesions have a dominant Th1 type (IFN- $\gamma$ ) immune response, while animals with multibacillary lesions tend to have predominant Th2 type (antibody) response.<sup>8</sup> The progression in the severity of disease and degree of intestinal bacterial load parallels a switch from Th1 to a Th2 response.<sup>3</sup> In the present case, the ileum showed diffuse multibacillary feature (Type 3b) according to well-established histological criteria for classifying lesions associated with natural paratuberculosis in sheep.<sup>6</sup>

Although the pathogenesis of Johne's disease in small ruminants is generally accepted to be similar to that in cattle, the clinical manifestations and gross pathology tend to be more insidious. Affected sheep show poor quality fleece, chronic wasting, and submandibular edema secondary to hypoalbuminemia, but overt diarrhea is not a common feature unlike in cattle. Enteric gross lesions are mild with little obvious thickening and no transverse ridges. Mineralized tubercle-like lesions in the mucosa, submucosa, serosa, and lymphatics of the intestine or lymph nodes have also been reported in goats and occasionally in sheep.<sup>9</sup>

The diagnosis of Johne's disease is difficult because of the organism's fastidious growth requirement and the lack of a specific diagnostic test that is sensitive enough to detect subclinical animals. Traditionally, fecal culture for MAP is considered the gold standard for diagnosis but is time-consuming and has poor reliability. As the humoral immune response is elicited late during infection, serological tests such as enzyme-linked immunosorbent assay (ELISA) are even less sensitive than fecal culture. The use of PCR for detection of MAP by amplifying the IS900 gene



*Intestine, sheep. Some sections contained coccidial gametocytes within the intestinal epithelium. (HE, 400X)* (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>)

sequence in fecal samples has vastly improved the detection of low shedders.<sup>10</sup> Vaccination can be useful, but current vaccines have significant drawbacks that prevent their widespread use.

**JPC Diagnosis:** 1. Ileum: Enteritis, granulomatous, diffuse, severe, with villar

blunting, crypt loss, moderate serosal granulomatous lymphangitis and numerous acid-fast intrahistiocytic bacilli, Hampshire sheep, *Ovis aries*.

2. Ileum, crypts: Rare coccidian gamonts, schizonts, and oocysts.

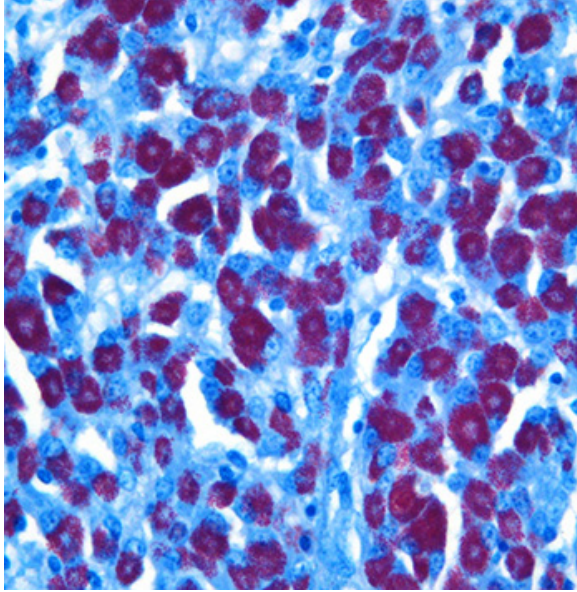
**Conference Comment:** The contributor provides an excellent example, description, and thorough characterization of *Mycobacterium avium* subsp *paratuberculosis* (MAP) infection in sheep. Conference participants discussed different types of lesions associated with MAP infection, as well as virulence factors that allow the organism to evade immune destruction. The type of lesion produced is associated with the stage of disease. The major lesions of MAP are typically confined to the ileum, large intestine, and draining lymph nodes.<sup>9</sup> Occasionally, aortic and endocardial subintimal fibrosis and metastatic mineralization occurs due to overproduction of a vitamin D analog produced by macrophages during the granulomatous inflammatory disease.<sup>1,9</sup>

The **focal** form of MAP infection is typified by small, well-demarcated, granulomas in the lymphatic tissue of the intestine and lymph nodes; these are characteristic for the initial and latent stages of infection in adult animals. **Multifocal** forms are present in subclinical infections, and animals manifest with small granulomas in lymphoid tissue and in the lamina propria of the intestine; however, the normal histological architecture of the intestine is not drastically changed. This multifocal form is often subclinical and affected animals can still shed the organism and be infectious to herd

mates resulting in the “iceberg effect,” because in an infected herd, only a small percentage of the animals demonstrate clinical signs. Sheep and goats are generally thought to be more susceptible to clinical disease and have a shorter incubation period.<sup>2,3,4,9,11</sup>

**Diffuse** lesions are characterized by generalized granulomatous enteritis that affects both lymphoid tissue and the lamina propria of the intestine. This form, unlike the multifocal form, causes characteristic widespread cerebriform thickening of the intestinal wall. The diffuse form is divided into two categories based on the cell types and numbers of acid-fast bacteria (AFB) present: 1) the multibacillary/histiocytic form is composed of sheets of epithelioid macrophages with large numbers of intracytoplasmic AFB; and 2) the paucibacillary/lymphocytic form is typified by numerous lymphocytes within the lamina propria surrounding scattered granulomas with macrophages containing few or no AFB.

The focal, multifocal, or diffuse paucibacillary lesions are associated with the tuberculoid form mentioned by the contributor, and are a consequence of host bias toward a cell-mediated immunity response, with predominance of M1 or classically activated macrophages. Classically activated macrophages are more effective at killing intracellular bacteria. The diffuse multibacillary lepromatous form, present in this case, is associated with the less effective humoral response and M2, or alternatively activated, macrophages.<sup>4,9,11</sup>



*Intestine, sheep. An acid-fast stain on the mucosal infiltrate demonstrates large numbers of intracytoplasmic acid-fast bacilli. (AF, 400X) (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>)*

The virulence factors expressed by mycobacterial agents play a major role in the lesion type and pathogenicity of the organism. A recent study identified the macrophage subsets within granulomatous lesions in bovine paratuberculosis,<sup>4</sup> and as mentioned by the contributor, the pathogenesis in sheep is likely similar to that of cattle. The MAP organism, like other mycobacterial agents, does not secrete toxins; instead, its virulence is based on properties of its cell wall. Mannose and CD14 receptors expressed on macrophages stimulate phagocytosis of the organism. After phagocytosis, the mycobacterial cell wall receptor lipoarabinomannan (LAM) inhibits macrophage phagosome maturation by inducing expression of cytokines IL-10 and TGF- $\beta$  through activation of TLR2 in the phagosome.<sup>4,9,11</sup> The bacterium can also inhibit acidification of the phagosome and phagosome-lysosome fusion. The cytokine

IL-10 suppresses M1 macrophage activation, and thus the cell-mediated response, and enhances the Th2 humoral response. Further recruitment of alternatively activated M2 macrophages leads to widespread infection and progressive inflammation.<sup>4,9,11</sup> As the severity of the inflammation increases, MAP secretes exochelins, iron reductases, and siderophores to acquire iron from ferritin stored in macrophages, and inhibits iron-dependent conversion of H<sub>2</sub>O<sub>2</sub> into hydroxyl radicals via the Fenton reaction.<sup>11</sup>

Several conference participants also noted rare intraepithelial coccidian microgamonts, macrogamonts, schizonts, and oocysts within the intestinal crypts. In sheep, the likely causes of coccidiosis in the small intestine are *Eimeria christensenii*, *E. ahsata*, *E. brakuensis*, and *E. crandallis*. In the cecum and colon of sheep, the most likely cause is *E. ovinoidalis*.<sup>9</sup>

#### **Contributing Institution:**

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>

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**CASE II: RUSVM Case 2 (JPC 4085381).**

**Signalment:** Ten-year-old female mixed breed dog (*Canis familiaris*).

**History:** This dog presented to the RUSVM Spay Clinic for preoperative evaluation for elective ovariohysterectomy. The owner reported no significant medical history. Physical exam abnormalities included severe tick infestation; mildly but uniformly enlarged peripheral lymph nodes, and multiple mammary masses ranging in size from 1cm to 1.5cm diameter. Masses were present throughout the mammary chain but were concentrated in the caudal glands. Hematologic and chemistry findings included a moderate thrombocytopenia (65 x 10<sup>9</sup>/l, range 200-500); severe lymphocytosis (16.2 x 10<sup>9</sup>/l, range 1-4.8); and significantly elevated plasma protein (10.1 g/dl, range 6-7.5). The patient was prescribed doxycycline 100mg (8.5mg/kg) q24h for 21 days on the presumptive diagnosis of rickettsial disease. Further diagnostics were strongly recommended but declined. Surgical sterilization was postponed until further workup could be pursued.

Nine months later the patient represented to the RUSVM Emergency Service in respiratory distress. The owner indicated the patient's condition had been deteriorating and elected euthanasia, which was performed with 3ml of pentobarbital (200mg/ml).

**Gross Pathology:** At necropsy, a 12 x 9 x 8 cm, spheroid, hard mass was within the



*Presentation, dog. The patient presented at autopsy with multiple mammary masses. (Photo courtesy of: Ross University School of Veterinary Medicine, 485 US Highway 1, South Building B, 4th floor, Iselin, NJ 08830 <http://www.rossu.edu/veterinary-school/>)*

subcutis of the ventrocaudal abdomen, apparently replacing the left inguinal mammary gland. On cut surface, the mass was mottled pale pink and grey, gritty, and had a peripheral 3 cm cavitated area containing soft gelatinous opaque material. Six smaller firm nodular masses were present in other mammary glands, ranging in size from 0.5 to 2 cm. Similar 0.5-1.5 cm discrete, grey-white, nodular to sessile masses were heavily disseminated throughout the lungs, replacing nearly 90% of the parenchyma. The kidneys, spleen, and liver had a few similar masses, and a similar solitary 0.7cm mass spanned the epicardium and superficial myocardium of the right ventricular free wall.

**Laboratory results:** N/A

**Histopathologic Description:** Histologically, the large mammary mass and the kidney, spleen, liver, and lung masses consisted of embryonal rhabdomyosarcoma. A section of lung with multiple metastases is submitted for examination. The tumors were composed of large elongate tube-like to fusiform cells admixed with fewer round myoblastic cells and organized into interwoven bundles and streams of within myxomatous stroma. The elongate cells

resembled myotubes, often having multiple tandemly aligned oval nuclei and poorly discernable sarcoplasmic cross striations (“strap cells”). Sarcoplasmic cross-striations were accentuated with phosphotungstic acid hematoxylin (PTAH) stain and enhanced by polarized light on preparations stained with Picrosirius red stain.

Tumor cells express moderate to marked and diffuse vimentin protein, moderate to marked and segmental desmin protein, but were negative for myoglobin, and smooth muscle actin.

When evaluated with electron microscopy, tumor cells varied from round, immature myoblast cells with variable amounts of cytoplasm to multinucleated myotubular developing skeletal muscle cells. The cytoplasm contained moderate amounts of mitochondria, glycogen, and ribosomes. Immature myoblast cells have eccentrically located nuclei with heterochromatin and one or two large nucleoli. The multinuclear tumor cells had indented nuclei with heterochromatin and abundant mitochondria and glycogen. Tumor cells contained myofilaments with Z-band like structures.



*Lung, dog. Much of the pulmonary parenchyma was replaced by numerous 0.5-1.5cm whitish nodules. (Photo courtesy of: Ross University School of Veterinary Medicine, 485 US Highway 1, South Building B, 4th floor, Iselin, NJ 08830 <http://www.rossu.edu/veterinary-school/>)*

Unequivocal cell junctions were present in the tumor cells.

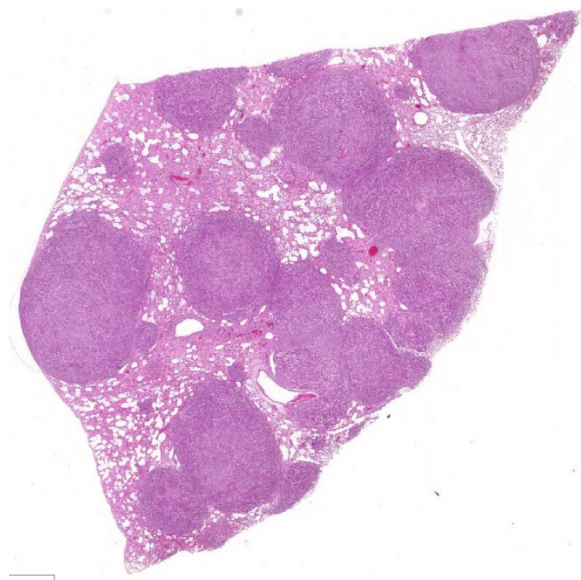
**Contributor's Morphologic Diagnosis:**

Lung: Metastatic rhabdomyosarcoma, embryonal

**Contributor's Comment:**

Canine rhabdomyosarcomas are histologically classified as embryonal, botryoid, alveolar, and pleomorphic.<sup>1</sup> The mixture of round immature myoblastic cells and multinucleated myotubular cells resembling developing skeletal muscle are consistent with the embryonal subtype of rhabdomyosarcoma. In this case, the predominance of the myotubular cells is further indicative of the myotubular variant of embryonal rhabdomyosarcoma.

In humans, embryonal rhabdomyosarcoma is the most common type, and predominantly affects children.<sup>7</sup> Similarly, embryonal rhabdomyosarcoma is the most common type diagnosed in dogs, and usually involves dogs under two years of age. However, when excluding the botryoid



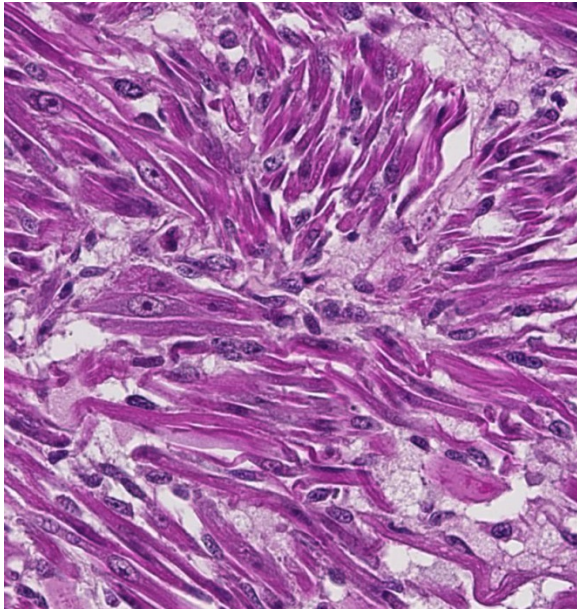
*Lung, dog. Subgross examination of the submitted section reveals that neoplastic nodules replace approximately 80% of the tissue. (HE, 4X)*

type of embryonal rhabdomyosarcoma of the urinary bladder, approximately half of the reported cases of canine embryonal rhabdomyosarcoma occur in adult dogs, as observed in this case.<sup>1</sup> In humans, embryonal rhabdomyosarcoma is considered an intermediately aggressive variant of rhabdomyosarcoma. Canine rhabdomyosarcoma, in general, is considered an aggressive malignancy having a metastatic rate which appears to approximate that of grade three soft tissue sarcomas. However, there is too little data to make conclusions regarding the prognostic significance of histological subtypes in dogs. The present case would appear to comprise the first report of histologically-confirmed metastasis of embryonal rhabdomyosarcoma in an adult dog (>2 years), but appears to be of intermediate malignancy since the dog survived over nine months following clinical diagnosis of the mammary mass.

Rhabdomyosarcoma has occasionally been reported in dogs to emerge from organs lacking striated muscle, including skull,<sup>4</sup> meninges,<sup>6</sup> greater omentum,<sup>8</sup> gingiva,<sup>9</sup> spleen, perirenal, and mammary gland.<sup>2</sup> In such instances, the neoplasm is thought to arise from stem cells capable of myogenic differentiation. In the present case, the large mammary mass is presumed to be the primary tumor because it was the largest of all masses and was first recognized clinically. Mammary rhabdomyosarcoma is very rare in humans and usually affects adolescent girls, is metastatic rather than primary breast tumor, and is alveolar subtype.<sup>5</sup> Similar to the present case, the other reported case of canine mammary rhabdomyosarcoma involved an adult female, and was also of the embryonal subtype.<sup>2</sup>

**JPC Diagnosis:** Lung: Rhabdomyosarcoma, metastatic, mixed breed dog, *Canis familiaris*.





*Lung, dog. Higher magnification reveals that neoplastic cells are spindled to strap-like with abundant cytoplasm, and one or more nuclei which line up in the center of the cell. (HE, 400X)*

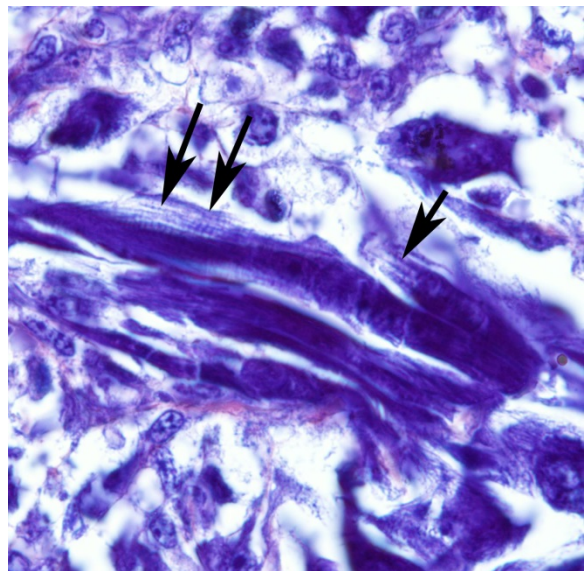
**Conference Comment:** The contributor provides an excellent example of a metastatic rhabdomyosarcoma (RMS) in the dog. RMS is a rare, malignant, neoplasm arising from pluripotent mesenchymal stem cells that differentiate toward skeletal muscle.<sup>1,3</sup> As a result of their pluripotent origin, RMS has a highly variable age of onset, location, gross, and histological appearance.<sup>3</sup>

As mentioned by the contributor, skeletal muscle neoplasms often arise in areas where skeletal muscle is not normally present. Common locations in the canine are the larynx and urinary bladder.<sup>3</sup> Laryngeal rhabdomyomas are formally known as laryngeal oncocytomas due to their characteristic eosinophilic, granular, PAS-positive cytoplasm. The name was changed to rhabdomyoma due to the presence of myofilaments with Z-bands on transmission electron microscopy (TEM), and positive immune-reactivity for muscle markers.<sup>3</sup> In

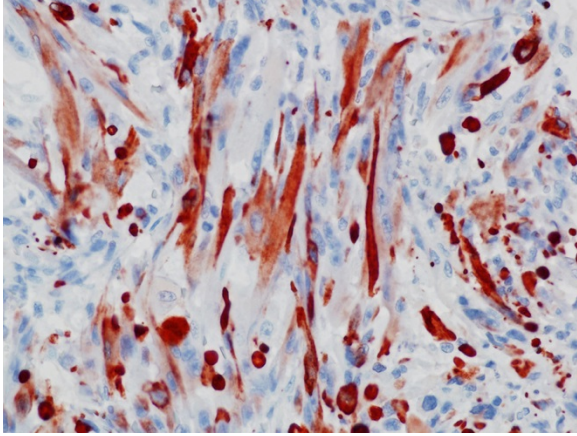
the urinary bladder, RMS is also known as botryoid rhabdomyosarcoma. This most commonly occurs in young (<2 years), large breed bitches, with Saint Bernard dogs over-represented. Grossly, these botryoid neoplasms occur at the trigone and cause urinary obstruction.<sup>3</sup> Interestingly, they have also been associated with hypertrophic osteopathy in dogs.<sup>3</sup> In pigs, cardiac rhabdomyomas are benign incidental findings and are thought to be Purkinje cell origin due to expression of protein gene product (PGP) 9.5, a Purkinje fiber marker.<sup>3</sup>

Almost all RMSs are typically aggressive with local infiltration and distant metastasis to other muscles, liver, spleen, and the lung.<sup>3</sup> In this case, in addition to the lung and adjacent mammary tissue, there is metastasis to the kidneys, spleen, liver, epicardium, and myocardium.

RMS in humans and veterinary species has been classified into different categories



*Lung, dog. Cross-striations are demonstrable on a phosphotungstic acid-hematoxylin stain (arrows). (PTAH, 400X) (Photo courtesy of: Ross University School of Veterinary Medicine, 485 US Highway 1, South Building B, 4th floor, Iselin, NJ 08830 <http://www.rossu.edu/veterinary-school/>)*

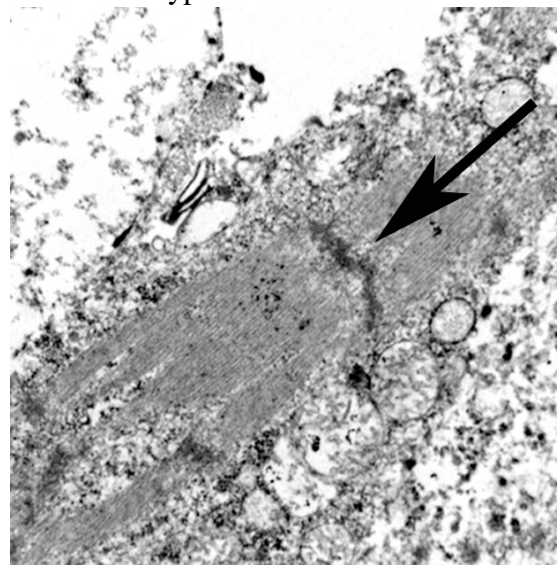


*Lung, dog. Neoplastic cells exhibit strong cytoplasmic immunopositivity for desmin. (anti-desmin, 400X)*  
 (Photo courtesy of: Ross University School of Veterinary Medicine, 485 US Highway 1, South Building B, 4th floor, Iselin, NJ 08830 <http://www.rossu.edu/veterinary-school/>)

depending on the degree of differentiation towards skeletal muscle:

- **Embryonal:** Divided based on cell morphology: myotubular, myoblastic, spindle cell, and botryoid. Embryonal RMS is typically encountered in animals <2 years old, and occur on the face, skull, masticatory muscle, oropharynx, trachea, axilla, scapula, perirenal, tongue, flank, leg, mammary gland, and hard palate. Botryoid RMS occurs in the urinary bladder and uterus.<sup>1,2,3</sup> The mammary gland is theorized to be the primary tumor location in this case.
  - Myotubular: Multinucleated strap cells and racquet cells with cross-striations.
  - Myoblastic: Most common form, and is composed of small round cells with abundant eosinophilic cytoplasm.

- Spindle cell: Low cellularity and arranged in a storiform pattern.
- Botryoid: Submucosal location with mixed round and myotubular cells. The term botryoid is due to grape-like appearance grossly.<sup>1,3</sup>
- **Alveolar:** Sheets of small, undifferentiated round cells on a fibrous framework arranged in “alveolar-like” structures due to lack of cohesiveness in the center of neoplastic nests. These are usually found in the hip, maxilla, omentum, and uterus.<sup>1,3</sup>
- **Pleomorphic:** Extremely rare and only reserved for RMS that do not display any features of embryonal or alveolar RMS. They are characterized by haphazardly arranged plump spindle cells with marked anisocytosis and anisokaryosis and bizarre mitotic figures. This type is more common in



*Lung, dog. Ultrastructurally, neoplastic cells demonstrate well-formed myofibrils and Z-bands (arrow).* (Photo courtesy of: Ross University School of Veterinary Medicine, 485 US Highway 1, South Building B, 4th floor, Iselin, NJ 08830 <http://www.rossu.edu/veterinary-school/>)

adults.<sup>1,3</sup>

The use of immunohistochemistry (IHC) to identify vimentin, desmin,  $\alpha$ -actins, myoglobin, myogenin, and MyoD1 in conjunction with TEM to identify of sarcomeric structures, Z-bands, and large mitochondria, can also be useful in the diagnosis RMS.<sup>1,2,3</sup>

#### **Contributing Institution:**

Ross University School of Veterinary Medicine

485 US Highway 1

South Building 1, 4<sup>th</sup> Floor

Iselin, NJ 08830

<http://www.rossu.edu/veterinary-school/>

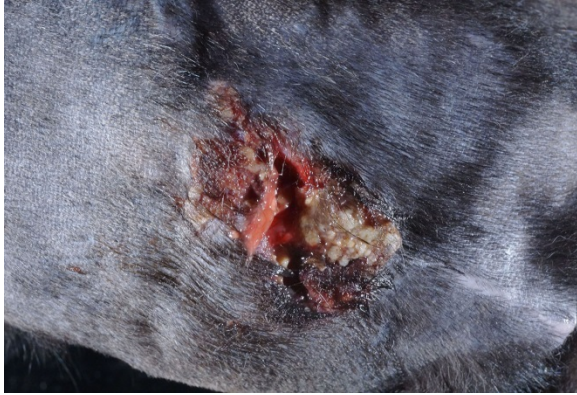
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#### **CASE III: 15183 (JPC 4082892).**

**Signalment:** Three-year-old ovario-hysterectomized female domestic shorthair cat (*Felis catus*).

**History:** This cat had a history of tail chewing, hair loss and recurring dermatitis over the tail and caudodorsum, occurring



*Haired skin, cat. The skin of the caudodorsum had a large area of ulceration and crusting in proximity to a previous caudectomy site. (Photo courtesy of: University of Minnesota, Veterinary Diagnostic Laboratory, <http://www.vdl.umn.edu>)*

since this cat was acquired as a kitten from Florida. Affected areas of skin were alopecic with erythema, crusted papules, plaques, and nodules. There was limited to no clinical response to treatment with fluoxetine, topical antibiotics and regular flea prevention and the cat deteriorated following injections of depomedrol and cefovecin. Following biopsy and initial diagnosis, the cat underwent tail amputation; however, the cat had recurring dermatitis over the caudodorsum 3 weeks postoperatively and was euthanized and submitted for necropsy.

**Gross Pathology:** At necropsy, there were two reddened, ulcerated areas over the caudodorsum with variable brown crusts and dried red-brown exudate. The caudal, larger area of ulceration and crusting was overlying a healing scar within the skin (interpreted as part of the surgical site from the previous tail amputation). The cranial, smaller area of ulceration was not associated with the previous surgical wound. On cut section, these areas extended into and expanded the subcutis, with poorly demarcated, tan to pink, firm, multifocal to coalescing nodules. There was no gross involvement of the underlying vertebrae and

no gross evidence of spread to lymph nodes or visceral organs.

**Laboratory results:** Previous biopsy and culture of the affected areas yielded scant mold; further identified as *Lagenidium* sp. by 18S/ITS (internal transcribed spacer) nucleic acid amplification and sequence analysis. Culture of necropsy samples was unable to repeat isolation of *Lagenidium* spp.

**Histopathologic Description:** The section examined was taken from the cranial, smaller area of skin ulceration not associated with the previous surgical wound. Underlying a locally extensive area of ulcerated epidermis and expanding the dermis and subcutis, there is a poorly demarcated, non-encapsulated infiltration of large numbers of eosinophils, moderate lymphoplasmacytic and histiocytic infiltrates and fewer neutrophils, with extensive, multifocal areas of eosinophilic necrotic and karyorrhectic cellular debris. Within areas of necrosis, there are small to moderate numbers of predominantly negatively stained, extracellular, 10-15  $\mu\text{m}$  diameter hyphae with variably prominent round or bulbous dilatations and intermittent right angle branching. Areas of necrotic cellular debris and inflammatory cellular infiltrate surround



*Haired skin, cat. On cut section, the dermis is expanded by numerous white-tan nodules. (Photo courtesy of: University of Minnesota, Veterinary Diagnostic Laboratory, <http://www.vdl.umn.edu>)*

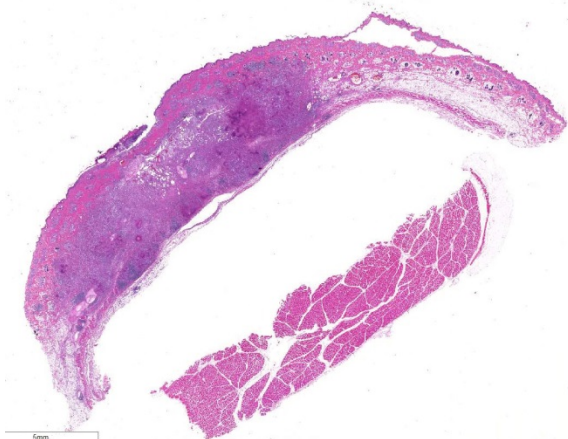
and isolate small aggregates of irregular, eosinophilic collagen fibers (collagenolysis).

**Special Stains:** Staining with Grocott's methenamine silver (GMS) yielded moderate numbers of broad, thick-walled hyphae of varying diameters that are occasionally septate. Staining with Periodic-Acid-Schiff (PAS) highlights hyphae with similar morphology, albeit less prominently.

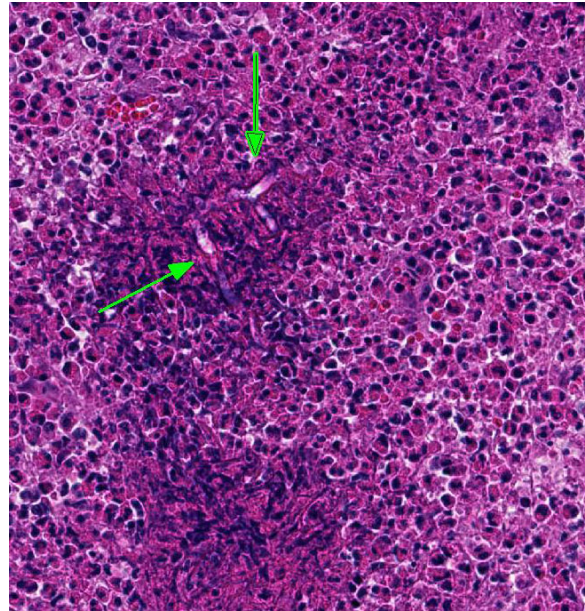
**Contributor's Morphologic Diagnosis:**

1. Skin, caudodorsum: Dermatitis, panniculitis, steatitis and myositis, eosinophilic and histiocytic, with lymphoplasmacytic infiltrates, marked, locally extensive, chronic with multifocal necrosis and hyphae
2. Skin, caudodorsum: Ulceration, multifocal, moderate to marked, chronic

**Contributor's Comment:** The fungal hyphae present in this case are consistent with a recurrence of dermatitis associated with *Lagenidium* sp., previously confirmed in this cat by 18S/ITS nucleic acid amplification and sequence analysis. Members of the genus *Lagenidium* sp. are a group of the *Oomycetes*, closely related to *Pythium* sp. and often referred to as 'water molds'. *Oomycetes* are frequently pathogens of plants, nematodes, and insect larvae;



*Haired skin, cat. Subgross examination reveals diffuse cellular infiltration by a profound cellular infiltrate with multifocal areas of necrosis. (HE, 5X)*



*Haired skin, cat. Large sheets of eosinophils surround areas of necrosis, which contain outlines of fungal hyphae. (HE, 400X)*

however, they are occasionally associated with disease in mammals.<sup>2</sup> The most widely known *Lagenidium* species is *Lagenidium giganteum*, a pathogen of mosquito larvae that has previously been implemented as a biologic control agent.<sup>2</sup> Although stages of oomycetes may be morphologically similar to fungal hyphae, oomycetes are phylogenetically distinct from fungi.<sup>7,8</sup> In contrast to fungi, the cell wall of oomycetes contains cellulose and  $\beta$ -glucan rather than chitin<sup>8</sup>, and the cell membranes lack ergosterol.<sup>7</sup> Oomycetes also differ from fungi with respect to life stages produced, including the production of sporangia and biflagellate zoospores.<sup>7</sup>

Most case reports of lagenidiosis involve dogs; however, there have been several recent reports in cats.<sup>7</sup> Infection with *Lagenidium* sp. in dogs typically occurs in young to middle-aged dogs and most frequently occurs in southeastern United States.<sup>2,3,7</sup> Exposure to water bodies such as lakes and ponds is frequently, but not always, reported.<sup>3</sup> Recent molecular work has led to the description of two closely

related pathogens in dogs; *Lagenidium giganteum* forma *caninum* and *Paralagenidium karlingii*.<sup>5</sup> *Lagenidium giganteum* forma *caninum* causes cutaneous or subcutaneous disease with frequent widespread dissemination, involving visceral organs, lymph nodes and/or great vessels.<sup>3</sup> *Paralagenidium karlingii* infection in dogs results in chronic ulcerative and/or nodular dermatitis that does not typically disseminate.<sup>5</sup> Recommended treatment for lagenidiosis is wide surgical resection where possible.<sup>1,3,7</sup> Prognosis is poor for disseminated disease, with lagenidiosis poorly responsive to medical therapy.<sup>2,9</sup>

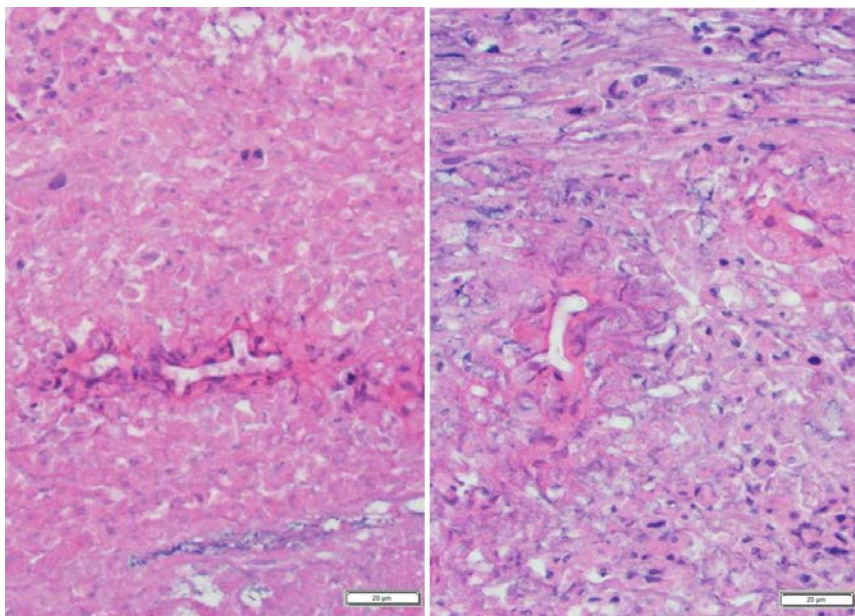
Differential diagnoses for lagenidiosis on clinical presentation and histopathology may include pythiosis, resulting from infection with the oomycete, *Pythium insidiosum*; and zygomycosis, involving infection with fungal organisms such as *Basidiobolus ranarum* and *Conidiobolus* spp..<sup>1,2,5</sup> All may result in granulomatous and/or eosinophilic inflammation and morphologically appear as

broad, irregular branching hyphae that are rarely to occasionally septate.<sup>1</sup> Differentiation is clinically important due to differing treatment and prognosis<sup>7,8</sup>.

Diagnosis of lagenidiosis can be challenging. Cytology and histopathology yield hyphae with similar morphology to pythiosis and zygomycosis.<sup>4</sup> Although there may be subtle differences in size and/or morphology of hyphae, histopathology cannot be used for definitive differentiation.<sup>3</sup> Fungal culture is possible but can be difficult due to the fastidious nature of *Lagenidium* sp. (particularly the sexual stages). Definitive diagnosis requires confirmation through molecular assays on tissue or cultured isolates.<sup>3,7,9</sup> *Lagenidium* sp. should be considered a differential in cats with granulomatous to eosinophilic, nodular to ulcerative dermatitis.

**JPC Diagnosis:** Haired skin and subcutis: Dermatitis, panniculitis and myositis, eosinophilic and gran-ulomatous, and eosinophilic, focally extensive, marked, with multifocal necrosis, ulceration, and fungal hyphae, domestic shorthair, *Felis catus*.

**Conference Comment:** The contributor provides an excellent example and overview of the pathogenic Oomycete water mold, *Lagenidium* sp. As mentioned above, *Lagenidium* sp. is strikingly similar in geographic distribution, clinical, and histologic appearance to the more commonly diagnosed Oomycete, *Pythium insidiosum*.<sup>6</sup> As a result, the

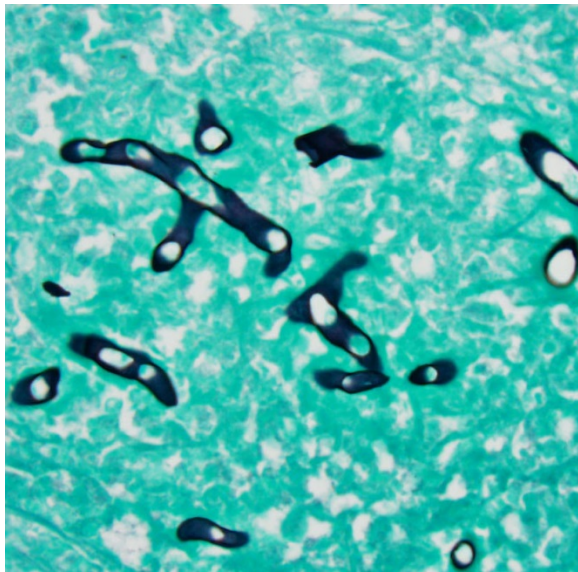


Haired skin, cat. Fungal hyphae measure 10-15 um with right angle branching. (Periodic acid-Schiff, 600X)(Photo courtesy of: University of Minnesota, Veterinary Diagnostic Laboratory, <http://www.vdl.umn.edu>)

majority of conference participants had pythiosis as their top differential for this lesion.

Infection with both *Pythium insidiosum* and *Lagenidium* spp. typically, but not always, occurs when the host has prolonged contact with standing or stagnant water containing the motile aquatic flagellate zoo-spores.<sup>2,3,6</sup> This infectious form of the organism is attracted by animal fur, damaged skin, and intestinal mucosa. As a result of contact with standing water, infections in domestic animals are most commonly reported in the limbs, ventral thorax, and abdomen. When a mammalian host with a skin injury enters a contaminated pond, the oomycete zoospores will encyst upon contact with the injured skin and mechanically penetrate the tissue, causing clinical disease.<sup>6</sup>

Like pythiosis, this disease is typically highly aggressive and lesions in the great



*Haired skin, cat. Fungal hyphae are thick-walled with non-parallel walls and occasional septa. (Gomori methenamine silver, 600X)(Photo courtesy of: University of Minnesota, Veterinary Diagnostic Laboratory, <http://www.vdl.umn.edu>)*

vessels, mediastinum, lungs, and esophagus have been reported in dogs. However, unlike

pythiosis, gastrointestinal disease has not been reported in *Lagenidium* spp.<sup>6</sup> Both entities are associated with a poor to grave prognosis even with wide surgical excision of cutaneous masses because the majority of animals infected with this pathogen have occult, non-resectable, disease in regional lymph nodes or distant sites when initially diagnosed.<sup>2,3</sup> In dogs infected with the less aggressive species, *Paralagenidium karlingii* mentioned by the contributor, surgery that achieves three cm margins is often curative.<sup>7</sup> Medical therapy for lagenidiosis is typically ineffective because ergosterol, the target for most antifungal drugs, is lacking in the Oomycete cell membrane.<sup>6,7</sup>

Conference participants discussed this lesion as a great example of chronic-active inflammation. Chronic-active inflammation occurs when the inciting inflammatory stimulus has not been removed from the chronic inflammatory process and continues to elicit an acute inflammatory response.<sup>1</sup> In this case, surrounding fungal hyphae are numerous eosinophils, neutrophils, and fibrin admixed with brightly eosinophilic plasma protein that is reminiscent of Splendore-Hoeppli material.

#### **Contributing Institution:**

University of Minnesota  
Veterinary Diagnostic Laboratory  
1333 Gortner Ave.  
St. Paul, MN 55108  
<http://www.vdl.umn.edu>

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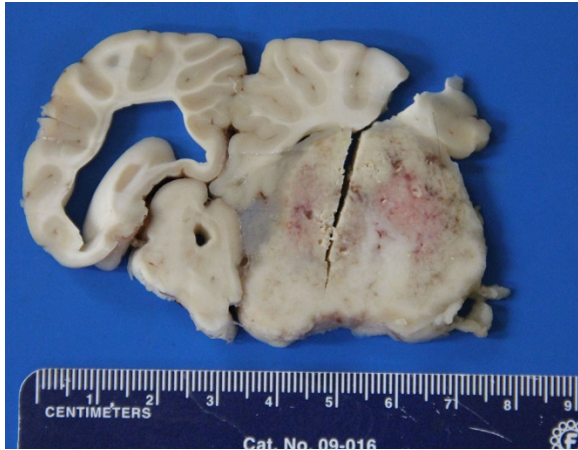
**CASE IV: S1103067 (JPC 4003084).**

**Signalment:** Adult female alpaca (*Vicugna pacos*).

**History:** The animal had a five-week duration of illness that consisted of progressive severe weight loss, and a subacute onset of unilateral blindness on the left, with right head tilt and circling to the right. There were palpably soft areas of the skull that were originally diagnosed as skull fractures. Radiographs were not taken. Treatments included antibiotics, steroids, non-steroidal anti-inflammatory drugs, and supportive therapy. In the absence of response to treatment, a brain tumor was suspected and the owner elected euthanasia.

**Gross Pathology:** The referring veterinarian removed the head and disposed of the remainder of the carcass. The calvarium was deformed with pronounced nodular doming and multifocal marked thinning and translucency over the caudodorsal aspect of the left and, to a lesser extent, the right cerebral hemispheres. The calvarium and intact brain were fixed in formalin and submitted to the CAHFS- San Bernardino laboratory for histologic examination.

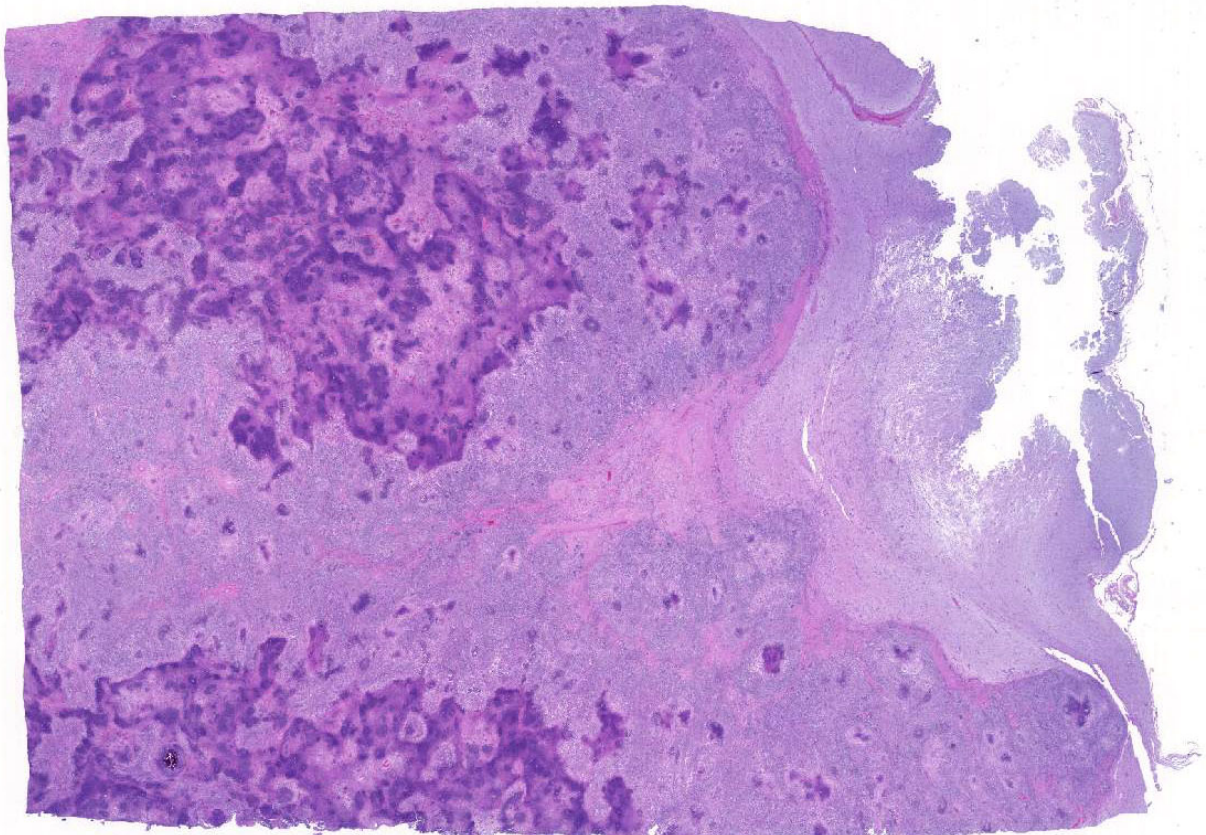




*Cerebrum, alpaca: The right cerebrum is 50% effaced by a large granuloma which effaces the lateral ventricle, crosses the midline and has resulted in expansion of the left lateral ventricle and 3rd ventricle. (Photo courtesy of California Animal Health and Food Safety Laboratory – San Bernardino Branch, University of California Davis, 105 W. Central Avenue, San Bernardino, CA 92408 <http://cahfs.ucdavis.edu/>)*

On removal of the calvarium, a large, firm mass with several, irregular, soft areas were identified in the right cerebral hemisphere.

The caudal half of the right hemisphere was moderately enlarged, misshapen and firm, with focal nodularity of the meningeal surface of the caudolateral aspect of the hemisphere. Transverse sectioning of the rostral half of both cerebral hemispheres revealed bilateral, moderate hydrocephalus (dilated lateral ventricles / reduced thickness of the cerebral grey and white matter). Extending caudally from the optic chiasm to the posterior extremity of the right cerebral hemisphere, was an irregular, nodular, roughly egg-shaped mass with approximate dimensions of 5.5 cm [length] x 3-4 cm [width] x 3.5-4 cm [height]. The mass markedly expanded and distorted the right lateral ventricle, and deformed/ partially replaced the right thalamus. Further caudally, there was partial replacement of the right diencephalon / mesencephalon by the mass. The left thalamus / mesencephalon was laterally displaced and the left ventricle was dilated (hydrocephalus) from the rostral



*Cerebrum, alpaca: The section is 90% effaced by granulomatous inflammation containing geographic outlines of lytic necrosis. The cerebrum at right contains a large area of cavitory necrosis. (HE, 4X)*

to the caudal extremities.

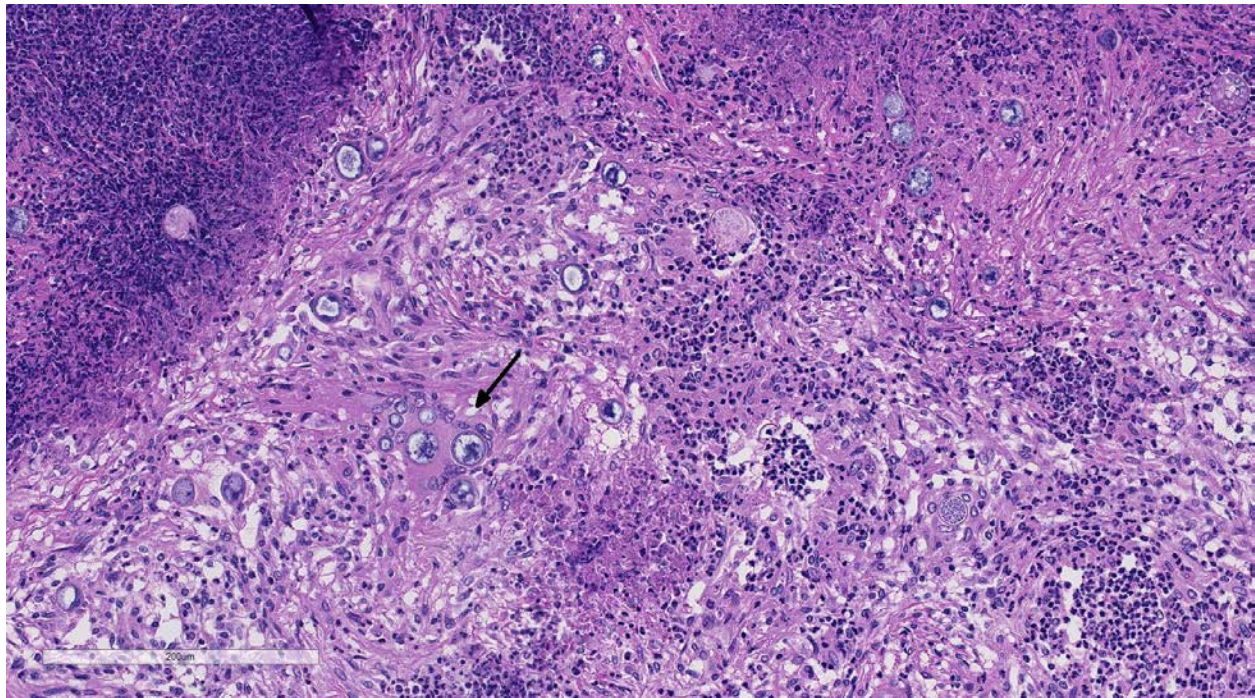
On cross section, the mass was firm, slightly gritty, and mottled, tan/pink/cream with scattered yellow foci of approximately 0.2 cm in diameter.

**Laboratory results:** N/A

**Histopathologic Description:** Brain, cerebral cortex: Affecting approximately 90% of the white matter and disrupting the normal architecture is a well-demarcated, non-encapsulated inflammatory focus. The focus consists of multiple, discrete to coalescent, variably-sized cores of necrosis, viable and degenerate neutrophils, extravasated red cells and /or mineral, interspersed with expanses of granulomatous to mixed inflammation and plump reactive astrocytes. Large numbers of fungal spherules of varying sizes and stages of development and occasional clusters of endospores are scattered throughout the necrotic cores, in few multinucleate giant cells, or free amongst the mixed inflammatory infiltrate. Spherules are round,

range in size from 20-60 um in diameter, and are surrounded by a 3-5 um thick, double contour, hyaline wall. Spherules are empty, or contain either granular to flocculent, basophilic material or 4-5 um oval endospores. In some sections, the inflammatory focus regionally extends to involve the meninges, while in others the border is irregular with peripheral, discrete pyogranulomas. Neuropil bordering the inflammatory focus is either rarified (malacic) with proteinaceous effusion (edema), gitter cell infiltration and astrocyte proliferation, or is irregularly infiltrated by aggregates of lymphoplasmacytic cells.

In other sections (slides not submitted), there is marked dilation of the lateral ventricle accompanied by multifocal, mild to moderate cuffing of subependymal blood vessels by plasma cells. One section of the lateral ventricle contains clumps of cellular debris, purulent exudate, and several small spherules. A moderate lymphoplasmacytic infiltrate is present in the choroid plexus.



*Cerebrum, alpaca: Enmeshed in a bed of spindling epithelioid macrophages, astrocytic processes, and fibroblasts within loosely arranged collagen, and spherules of Coccidioides immitis are present within foreign body-type multinucleated macrophages (arrow), surrounded by aggregates of neutrophils, and scattered throughout areas of lytic necrosis. (HE, 400X)*

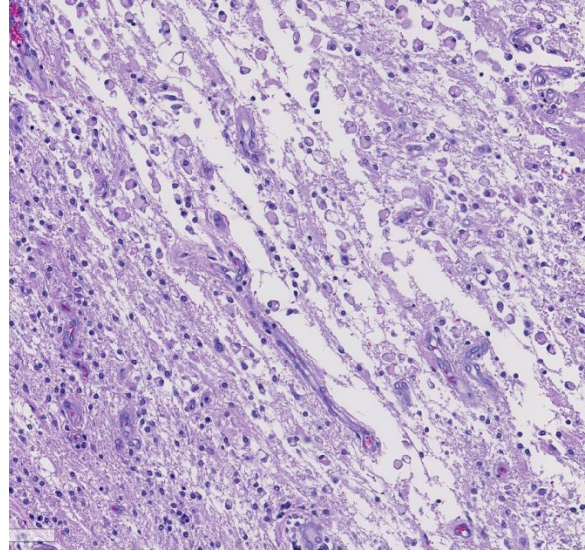
### **Contributor's Morphologic Diagnosis:**

Brain, right cerebral cortex including the lateral ventricles and meninges:

1. Meningoencephalitis, necrotizing and pyogranulomatous, focally extensive, chronic, severe, with numerous intralesional fungal spherules and endospores, etiology consistent with *Coccidioides* spp.
2. Hydrocephalus, bilateral, moderate.

**Contributor's Comment:** The dimorphic soil fungi, *Coccidioides immitis* and *C. posadasii*, are the causative agents of coccidioidomycosis, a systemic fungal disease in man and animals.<sup>8</sup> The disease is endemic in arid regions of southwestern USA, Mexico, Central and South America<sup>9</sup>, and is commonly known as desert fever, valley fever, or San Joaquin Valley fever.<sup>8</sup> Coccidioidomycosis has been reported in a large variety of domestic animals including dogs<sup>2</sup>, cats<sup>17</sup>, horses<sup>10</sup>, llamas<sup>4</sup>, and wild animals including chimpanzee<sup>11</sup>, bottlenose dolphin<sup>13</sup>, free-living California sea lions<sup>7</sup>, Przewalski's horses<sup>16</sup>, and mountain lion.<sup>1</sup>

Fungal mycelia survive well in dry, hot conditions; grow after intense rainfall and release arthroconidia which are disseminated by the wind.<sup>3,9</sup> Inhalation of airborne arthroconidia is the most common route of infection, although local traumatic inoculation has been associated with cutaneous and subcutaneous lesions.<sup>3</sup> The arthroconidia migrate to bronchi and alveoli and transform to yeast forms (immature spherules) of 10-20  $\mu\text{m}$  in diameter. As spherules mature they enlarge up to 100  $\mu\text{m}$  in diameter and are surrounded by a double contour, 4-5  $\mu\text{m}$  hyaline wall. Spherules undergo endosporulation forming numerous uninucleated endospores 2-5  $\mu\text{m}$  in diameter. Mature spherules rupture to release endospores that form new spherules in tissue or mycelia if released to the environment.<sup>3,14</sup> Dissemination to other organs is through



*Cerebrum, alpaca: A large focus of cavity necrosis (right) is present adjacent to the granuloma, suggesting an infarct as a result of vascular interruption. Cellular debris, presumably the majority of which is myelin, is being phagocytized by numerous Gitter cells. (HE, 400X)*

blood or lymphatics, and fungi are believed to reach the central nervous system through leukocytic trafficking and hematogenous spread from primary sites of infection.<sup>3,12</sup>

Three main virulence mechanisms by which *Coccidioides* spp. survive in host environment have been described.<sup>12</sup> These include:

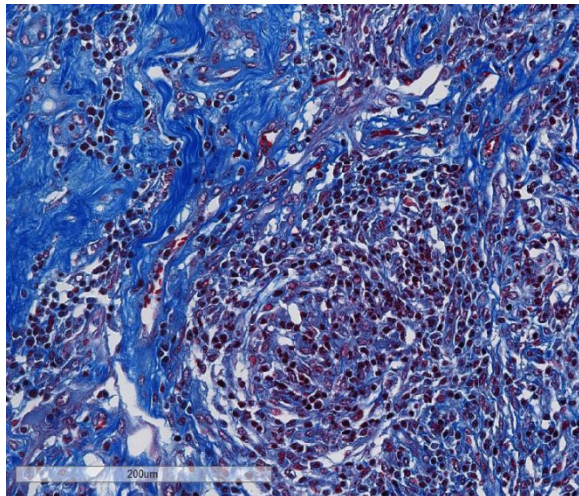
1. Production of dominant spherule outer wall glycoprotein (SOWgp) which modulates the host immune response resulting in compromised cell-mediated immunity
2. Depletion of SOWgp presentation on endospore surface preventing host recognition of the pathogen
3. Induction of host arginase 1 (decreased nitric oxide production) and coccidial urease which contribute to tissue damage at sites of infection.

Successful host immunological response to *Coccidioides* spp. is dominated by cell-mediated immunity<sup>9,12, 15</sup>, particularly Th1, and in general is related to the phase of the

organism involved.<sup>11</sup> Due to the marked increase in size as the spherules mature, phagocytosis is restricted to initial yeast cells from germinated arthroconidia, and endospores released from ruptured spherules.<sup>12</sup> Inhaled arthroconidia elicit an initial response composed primarily of neutrophils with fewer macrophages and lymphocytes; in later stages, a granulomatous response predominates.<sup>1,2</sup>

Although the spherule form shed from lesions is not readily infectious, arthroconidia from mature cultures are easily aerosolized and are highly infectious.<sup>14</sup> As such, *Coccidioides immitis* is designated as Biosafety Level 3, and is classed as a select agent of bioterrorism in the United States due to its high virulence and infectious nature.<sup>6</sup>

**JPC Diagnosis: 1.** Cerebrum and meninges: Meningoencephalitis, pyogranulomatous and necrotizing, focally extensive severe with intra- and extracellular endosporulating yeasts, alpaca (*Vicugna pacos*).  
2. Cerebrum, grey matter: Necrosis, focal, moderate.



*Cerebrum, alpaca.* A Masson's trichrome stains reveals abundant collagen within the granuloma, which is unusual, as fibrocytes are not a normal parenchymal component in the brain. It is likely that fibrocytes penetrated into the granuloma from the adjacent meninges. (Masson's trichrome, 320X)

**Conference Comment:** The contributor provides a striking example and a concise review of the epidemiology, pathogenesis, and virulence factors of these dimorphic, endosporulating fungi. South American camelids, such as llamas and alpacas, are exquisitely susceptible to coccidioidal infection and clinical cases usually present with severe respiratory and/or disseminated infection.<sup>9</sup> In any species, disseminated disease can occur either early, or several months after initial infection.<sup>3,9</sup> In dogs, up to 22% can have disseminated systemic infection without a history of respiratory disease. However, cases of systemic lesions without pulmonary involvement are theorized to reflect clinical resolution of the lung, rather than extra-pulmonary infection.<sup>3,9</sup>

The most common presentation of disseminated disease is lameness due to osteomyelitis.<sup>9,10</sup> This typically occurs late in the disease and is characterized by osteolytic granulomas surrounded by proliferative new bone growth. Painful draining tracts in the overlying skin, palpable bone swelling, and enlarged and reactive regional lymph nodes are additional signs of disseminated disease.<sup>3</sup> However, generalized lymphadenopathy is uncommon in this disease.<sup>3,9</sup> Other clinical signs of disseminated disease are variable and are usually dependent on the organ infected. Animals with central nervous system (CNS) disease, such as this case, typically have seizures, progressive ataxia, and are comatose in severe cases. Other organs affected include: eyes, liver, spleen, kidney, and testes.<sup>3,9</sup> Abortion has also been reported in both horses and an alpaca in Southern California.<sup>5</sup>

This case generated enthusiastic debate among conference participants regarding

whether the profound pyogranulomatous inflammation effacing 90% of the histologic section originated from the cerebrum or the meninges. Participants favoring cerebral origin argued that the cerebrum is lost and replaced by an astrocytic scar with spindled and palisading epithelioid macrophages. Participants favoring meningeal origin noted that the spindled cells are birefringent and likely represent collagen and fibrous connective tissue deposition secondary to chronic pyogranulomatous inflammation. A Masson's trichrome stain revealed abundant blue staining collagen within the granuloma. Given that fibrocytes are not a normal component of the neuropil, it is likely that fibrocytes penetrated into the granuloma from the adjacent meninges. The granuloma was also diffusely immune-negative for glial fibrillary acidic protein (GFAP), supporting a meningeal origin of the granuloma rather than an astrocytic scar in the cerebrum.

Several conference participants also noted a focal area of cavitory necrosis within the cerebrum adjacent to the pyogranuloma. This is likely due to thrombosis of a vessel adjacent to the large granulomatous nodule, creating an infarct in the section of the cerebrum. Unfortunately, none of the conference participants noted vascular thrombi within their tissue sections. The vascular thrombi may be out of the plane of section.

**Contributing Institution:**

California Animal Health and Food Safety  
Laboratory  
San Bernardino Branch  
University of California Davis  
105 W. Central Avenue,  
San Bernardino, CA 92408  
<http://cahfs.ucdavis.edu>

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