CASE I: 096260 (JPC 3167342).

Signalment: 17-year-old, female, French trotter horse (Equus caballus).

History: A 17-year-old female horse was referred to the Veterinary School of Nantes for clinical signs of respiratory difficulty and persistent left sinus deformation.

Physical examination revealed a bilateral deformation of gums with multifocal ulceration. RMI showed maxillary bone and turbinate replacement by a material of fibrous density. The horse was euthanized for humane reasons and a complete necropsy examination was performed shortly after death.

Gross Pathologic Findings: At necropsy, a severe bilateral rostrocaudal osteolysis of maxillary bone was observed. Bone was replaced by a white, firm, fibrous tissue. Other macroscopic findings include a 6 cm diameter, white, firm, homogenous, and well-delimited nodule on the left kidney.

1-1. Maxillary Bone; horse: Severe bilateral rostro-caudal osteolysis of maxillary bone replaced by a homogeneous white, firm fibrous tissue, multifocal ulceration of gums. (Photo courtesy of Department of Veterinary Pathology, ONIRIS (Nantes-Atlantic College of Veterinary Medicine, Food Science and Engineering), Nantes, France. http://www.oniris-nantes.fr)

1-2. Kidney; horse: 6 cm diameter, white, firm, homogeneous, well-delimited nodule. (Photo courtesy of Department of Veterinary Pathology, ONIRIS (Nantes-Atlantic College of Veterinary Medicine, Food Science and Engineering), Nantes, France. http://www.oniris-nantes.fr)
Histopathologic Description: The renal architecture was disrupted by multifocal coalescent granulomas centered on numerous tangential and cross-sections of well-preserved round worms (nematodes). The inflammatory infiltrate mainly consisted of epithelioid cells, multinucleated giant cells, numerous plasma cells, eosinophils and few lymphocytes surrounded by abundant fibrous tissue. Multifocally, foci of dystrophic calcification were adjacent to degenerated nematodes. The nematodes were 250-300 µm length and 15-20 µm diameter, with a smooth thin cuticle, a tapered tail, a uterus containing a dorsoflexed ovary and a long characteristic rhabditiform esophagus with a corpus, an isthmus and a bulb. Smaller nematodes with a length of 150-180 µm and a diameter of 10-15 µm with a rhabditiform esophagus and granular internal basophilic structures were also detected. The nematodes were interpreted respectively as adult and larval stages of *Halicephalobus gingivalis* (formerly *H. deletrix*, *Micronema deletrix*). Numerous eggs with a length of 35 µm and a diameter 20 µm were also observed.
Contributor's Morphologic Diagnosis: Kidney: nephritis, granulomatous, multifocal, coalescent, with intralesional *Halicephalobus gingivalis*.

Contributor's Comment: *H. gingivalis* (formerly *Micronema deletrix*) is a free-living, saprophytic and opportunistic small nematode of the order *Rhabditida*, family *Rhabditidae*. Most species inhabit decaying organic matter and are common in soil, fowl water and manure. *H. gingivalis* infections occur sporadically. This parasitic infection is described at least in 60 cases in horses mainly in the USA, Canada, Japan, in few cases in ponies and humans. One case in a horse was recently described in Switzerland but no case has been described in France before. The pathogenesis, life cycle and route of infection of *H. gingivalis* are poorly understood. Free-living forms of the parasite in the soil are suspected to infect horses via skin or mucous membrane wounds in recumbent animals, and then disseminate hematogenously or to migrate along blood vessels or nerves to internal parenchymal tissues including the central nervous system. Other routes of infection have been suggested, such as: infections from inhalation of the nematodes, possible colostral transfer to foals during nursing (as described in one report involving a Thoroughbred mare with mastitis and a nursing foal with encephalitis). Additionally, the possibility of urogenital route has been considered, as viable organisms were noted in the sperm and urine of two stallions. *H. gingivalis* life cycle is unknown. In tissues, only adult female, larvae and eggs are observed and asexual reproduction by parthenogenesis is suspected. Free male and female worms are found in soil and a sexual reproduction is suspected. The classical granulomatous lesions of *Halicephalobus* in horses are mainly found in the brain, kidneys, oral and nasal cavities, lymph nodes, and spinal cord. They are also frequently found in the adrenal gland and lung. Infrequent sites of granuloma formation include stomach, bone (mandible, femur, maxilla, nasal bones), ganglia, heart, liver, and eye, testicle and prepuce. In human cases, granulomatous lesions are always found in brain and sometimes in the heart and liver.

The other rhabditid parasites infecting the horse are: *Pelodera strongyloides*, *Strongyloides westerii* and *Cephalobus sp*. *Pelodera strongyloides* causes a self-limiting dermatitis normally confined to the ventral abdomen. *Pelodera* is mostly observed in dogs and rarely in sheep. *S. westerii* larvae cause a cutaneous infection but adults and eggs are not found in the skin. In cutaneous and mucocutaneous lesions *Cephalobus* can be distinguished from *H. gingivalis* by its esophagus and stoma shape and blunt posterior end.

**JPC Diagnosis: Kidney:** Nephritis, granulomatous and eosinophilic, focally extensive, severe with adult and larval rhabditid nematodes.

Conference Comment: The contributor provided a very good description and discussion of the classic lesions caused by *Halicephalobus gingivalis* in horses. Conference participants, while discussing the pathogenesis of this disease, elaborated on the role of eosinophils in parasitic infections and reviewed several aspects of eosinophilic inflammation. Eosinophilia (defined as an increase in the number of eosinophils in tissue or blood) is generally observed in parasitic disease, hypersensitivity, and in some neoplasms. Eosinophils can be recruited in cases of both ectoparasitism and endoparasitism. Additionally, they play a role in the late phase of type I (immediate) hypersensitivity reactions (anaphylaxis, allergies, asthma etc.). Neoplastic diseases that often recruit eosinophils include eosinophilic leukemia, mast cell tumor, T-cell lymphoma, lymphomatoid granulomatosis, various carcinomas, fibrosarcoma, and thymoma. Eosinophils are produced and mature in bone marrow. IL-5 is the most important cytokine involved in eosinophil production, influencing eosinophil proliferation, differentiation and maturation, as well as function. GM-CSF and IL-3 also play more minor roles in eosinophilic development.

Eosinophils contain distinct granules, called secondary (aka specific) granules, which are lysosomes that contain several components, including major basic protein, acid hydrolases, eosinophilic cationic protein, and eosinophil-specific peroxidase. Although eosinophils are also capable of phagocytizing and killing bacteria, they are not as adept at this as neutrophils, and generally cannot clear a bacterial infection independently. Their main function is against large organisms (such as helminths) that are too large to be phagocytized. Under the influence of a Th2 biased immune response in a helminth-infected host, eosinophils are recruited predominantly by IL-5, as well as IL-2 and IL-16. These cytokines induce mature eosinophils to emigrate from the blood and respond to chemoattractants (such as eotaxin, aka CCL-11) to home in to the helminth-infected tissue. There eosinophils bind to immunoglobulins (particularly IgE) and complement components (C1q, C3b/C4b, iC3b, C5a) on the surface of their targets. They may also bind to ligands on the parasite surface (Lewis-related molecules and cell-adhesion molecules). Once activated by antigen-IgE complexes, eosinophils secrete cytokines and chemokines (IL-2, IL-4, IL-10, IL-12, IL-16, IL-3, IL-5, eotaxin, TGF-alpha, VEGF, TNG-alpha, among others) and proinflammatory mediators (substance P). Additionally, they degranulate to release their cytotoxic substances with the following effects: Major basic protein is toxic to helminths, as well as tumor and host cells; it also activates platelets, neutrophils mast cells and basophils. Eosinophilic cationic protein is also toxic to helminths and host cells. Eosinophilic specific
peroxidases generate toxic oxygen radicals, providing a further weapon against helminths. Acid hydrolases have digestive functions. Once they degranulate, eosinophils die via apoptosis. Although studies have shown that there may be species variability in the significance of the impact of IL-5 and eosinophils in helminth infections, generally it is accepted that IL-5-induced eosinophilia often plays an important role in the immune response to parasites.

**Contributing Institution:** Department of Veterinary Pathology, ONIRIS (Nantes-Atlantic College of Veterinary Medicine, Food Science and Engineering), Nantes, France
www.oniris-nantes.fr

**References:**
CASE II: V11-1130 (JPC 4001095).

**Signalment:** Eight-year, nine-month-old spayed female, domestic shorthair feline (*Felis catus*).

**History:** The cat presented with a lesion on its abdomen of unknown duration. The referring veterinarian initially suspected a possible spider bite that was too large to debride. The differential diagnosis included neoplasia and inflammation/infection. Culture of the deep tissue tracts yielded growth of coagulase-positive *Staphylococcus aureus*. Convenia® (cefovecin sodium) and injectable corticosteroids were inconsistently administered by the owner. The cat initially appeared tolerant of the constantly growing and purulent lesion that involved over half of its abdomen. When the cat became lethargic, depressed, and inappetent, biopsies of the lesion were taken and submitted for histopathologic evaluation.

**Gross Pathologic Findings:** Extensive cutaneous fistulous tracts involving over half of the abdomen.

**Histopathologic Description:** Haired skin and subcutis, abdomen: Multifocally expanding the dermis and subcutis; separating, surrounding, and replacing adnexa; and elevating the mildly acanthotic and hyperkeratotic epidermis are multiple, densely cellular, up to 2 mm diameter nodules composed of numerous epithelioid macrophages, neutrophils, fewer lymphocytes and plasma cells, and rare mast cells and eosinophils surrounded by reactive fibroblasts and, in some areas, hemorrhage, fibrin, and edema. Multifocal nodules contain variably sized, up to 100 µm diameter extracellular lipid vacuoles (lipocysts) surrounded by a rim of neutrophils; these occasionally contain many 3-5 µm diameter filamentous bacilli. Occasionally, nodules contain a large central area of necrosis characterized by abundant amounts of eosinophilic cellular and karyorrhectic debris.

**Acid–fast stain (Ziehl-Neelsen):** Many intravacuolar and intrahistiocytic acid-fast, 3-5 µm, filamentous bacilli are present.

**Contributor’s Morphologic Diagnosis:** Haired skin and subcutis, abdomen: Dermatitis and panniculitis, pyogranulomatous, nodular, multifocal to coalescing, marked, with many intravacular and intrahistiocytic, 3-5 µm filamentous acid-fast bacilli, domestic shorthair (*Felis catus*) feline.

**Contributor’s Comment:** Classically, three manifestations of feline mycobacteria-associated skin disease are recognized: (1) feline leprosy, (2) cutaneous tuberculosis, and (3) atypical mycobacteriosis (rapidly growing mycobacteria).3,6 The feline leprosy group is comprised of obligate pathogens and can be subdivided into lepromatous (multibacillary) and tuberculoid (paucibacillary) forms.3,6 Affected cats are typically young adults (1-3 years old) and lesions typically occur on the head, limbs, and trunk.3,4,6 Regional lymphadenopathy may be present, but affected cats are otherwise healthy.3,4 The mode of transmission is thought to be by rodent or cat bites, biting insect vectors, or soil contamination of cutaneous wounds.4 The causative agent is presumed to be *Mycobacterium lepraemurium*; however, other potentially causative mycobacteria (*M. visibilis, M. malmoense*) have been isolated from cutaneous lesions clinically consistent with feline leprosy by molecular techniques.4,6 *M. lepraemurium* does not grow in culture using standard techniques, but has been isolated by PCR with DNA sequencing.6 Feline leprosy has no...
known zoonotic potential. Nerve involvement is not a characteristic feature of feline leprosy and is sporadic.\(^4\) The causative agents of cutaneous tuberculosis, *M. bovis* and *M. tuberculosis*, are also considered obligate pathogens. Cutaneous infections are rare. Feline cutaneous mycobacteriosis is most often caused by *M. bovis*.\(^3\) Gastrointestinal and pulmonary infections are more common. Infections of the gastrointestinal tract and mesenteric lymph nodes usually occur following ingestion of milk or meat of infected cattle.\(^3\) Cutaneous infections can occur alone (rare) or in combination with disseminated infection.\(^4,6\) Lesions of feline cutaneous mycobacteriosis typically occur on the face, neck, shoulders, and forepaws. Less commonly the lesions occur on the ventral thorax and tail. Skin lesions can develop from bite wounds or as a result of dissemination from the gastrointestinal tract. Affected cats typically present with a local or generalized lymphadenopathy.\(^3\) The causative agents of cutaneous tuberculosis are zoonotic.

Atypical mycobacteriosis (rapid growing Runyon Group IV mycobacteria) are considered opportunistic pathogens – saprophytes or facultative pathogens that are ubiquitous in soil, water, and decomposing vegetation.\(^3,4,6,8,10\) Included in the atypical group are *M. fortuitum*, *M. phlei*, *M. smegmatis*, *M. chelonae*, *M. abscessus*, *M. flavescens*, *M. thermoresistible*, and *M. xenopi*. The Runyon Group IV mycobacteria have a predilection for lipid rich tissues. Consequently, lesions characteristically occur on the ventral abdomen in the subcutaneous fat of inguinal area in cats.\(^4,5,6\) Female cats are overly represented possibly due to the propensity of spayed females to become obese.\(^3,7\) Studies have shown that introduction of acid-fast bacilli into adipose tissue facilitates their pathogenicity, possibly by providing nutrients for growth and by protecting them from the host immune response.\(^1,4,8,10\) Infections occur via wound contamination or traumatic implantation. Consequently, many cats with atypical mycobacteriosis have a history of previous trauma.\(^4,5\) Animal-to-animal transmission is not thought to occur and affected cats are usually not immunocompromised.\(^4,8\) Atypical mycobacteriosis is not considered zoonotic.

Cutaneous lesions with atypical mycobacteria have been reported primarily in cats.\(^4,6\) The clinical course is prolonged and lesions present as single or multiple cutaneous and subcutaneous nodules, diffuse swellings, plaques, or purpuric macules.\(^4\) Fistulous tracts discharge serous, serosanguinous, or purulent exudates that are not usually malodorous.\(^4,8\) Lesions slowly enlarge and eventually may encompass the entire ventrum and extend up the lateral trunk. Undermining

2-3. Cleared spaces corresponding to residual lipid from degenerating adipocytes (lipocysts) are surrounded by neutrophils and epithelioid macrophages. (HE 360X)
of peripheral skin is characteristic with purple depressions (thin skin) overlying accumulations of pus.\textsuperscript{5} Despite extensive cutaneous involvement, most animals remain active with no systemic signs of illness.\textsuperscript{4,8}

Histologically, the epidermis is usually acanthotic or ulcerated with multinodular to diffuse pyogranulomatous dermatitis and panniculitis.\textsuperscript{5} Thin rims of neutrophils with a wider zone of epithelioid macrophages usually surround varisized lipocyst (clear zones comprised of residual lipid from degenerating adipocytes).\textsuperscript{5,6} Pyogranulomas without central spaces (lipocysts) may be seen as well. Pyogranulomas are often confluent and interspersed with diffuse inflammation composed primarily of macrophages and neutrophils. Chronic lesions may contain peripheral lymphoid nodules and/or fibrosis. Giant cells are usually absent.\textsuperscript{5} Rapidly growing opportunistic mycobacteria may or may not be difficult to find in tissue section and have been described as rare to numerous.\textsuperscript{4,8} When numerous, mycobacteria may be partially visible on hematoxylin and eosin (HE) stained tissue. Fite’s stain (a Fite-Faraco modification of the acid-fast stain) is useful in accentuating difficult-to-see mycobacteria. Mycobacteria are most commonly found in lipocysts due to protective fat, but may also be present in macrophages.\textsuperscript{5}

In summary, pyogranulomas surrounding clear central spaces are very suggestive of a rapidly growing mycobacterial infection with HE stain, but may also be seen in other deep infections (e.g., higher bacteria). Demonstration of acid-fast organisms in lipocysts is highly supportive of the diagnosis; however, this should be confirmed by culture or molecular techniques.\textsuperscript{5}

The differential diagnosis for cutaneous infection with rapid growing mycobacteria includes bite wound abscesses, deep mycotic or bacterial infections, sterile nodular panniculitis, and foreign body reactions.\textsuperscript{5} Deep wedge biopsies are preferred over punch biopsies for diagnosis since inflammation is most intense in the subcutaneous fat.\textsuperscript{4,5}

Cases of feline atypical mycobacteriosis carry a guarded prognosis as treatment can be challenging, especially with chronic lesions.\textsuperscript{5,10} Treatment involves radical surgical debridement followed by long-term (3-6 months) treatment with concurrent administration of multiple antimicrobial agents.\textsuperscript{5,8,10} Identification by culture or molecular techniques is required so that appropriate therapy and zoonotic potential can be determined.\textsuperscript{3,4} Infective mycobacterium can be difficult to culture and repeated cultures may be necessary to yield a positive response.\textsuperscript{8}
**JPC Diagnosis:** 1. Haired skin: Dermatitis, cellulitis and panniculitis, pyogranulomatous, necrotizing, focally extensive, severe, with lipocysts and many intravacuolar and intrahistiocytic 3-5 µm filamentous acid-fast bacilli.  
2. Haired skin: Dermatitis, eosinophilic and mastocytic, subacute, multifocal, mild with bacterial folliculitis and rare intracorneal pustules.

**Conference Comment:** The contributor provides an excellent overview of feline mycobacterial skin diseases. Mycobacterial cell walls are composed of mycolic acids, which not only provide physical protection for the mycobacteria, but also play important roles in the pathogenesis of the diseases they cause. Mycolic acids are glycolipids that regulate the intrahistiocytic growth rate of mycobacteria and influence the host immune response. One important mycolic acid is trehalose 6,6'-dimycolate (TDM, cord factor), which is found in large quantities in many species of *Mycobacterium*, including virulent strains of *M. tuberculosis* as well as saprophytic *M. fortuitum*. TDM appears to regulate the intrahistiocytic growth of mycobacteria by inhibiting fusion between phospholipid vesicles, thus inhibiting phagosome-lysosome fusion during infection. TDM also induces macrophage production of proinflammatory cytokines (e.g., IL-1β, IL-6, TNF-α, IL-12), leading to the development of granulomas. Mycolic acid-containing components of the cell wall also promote angiogenesis and interact with Toll-like receptors. Additionally, their presentation as antigens to CD1-restricted T cells further influence the host immune response. CD1-restricted T-cells are a group of T-cells that recognize lipid antigen (such as fatty acids, phospholipids, glycolipids, isoprenoids, mycolates and lipopeptides) presented by the CD1 family of molecules independent of MHC. CD1a, b and c-restricted T cells recognize several lipid antigens from *M. tuberculosis*. CD1 restricted T-cells are cytotoxic and secrete Th1 cytokines. Hence, the presence of mycolic acids such as TDM in mycobacterial cell walls may partly determine the outcome of mycobacterial infections in host macrophages.

In addition to the prominent pyogranulomatous dermatitis, panniculitis and cellulitis, conference participants noted a second, more subtle lesion consisting of eosinophilic and mastocytic dermatitis; the relationship between the two lesions is uncertain. Additionally, conference participants debated the microscopic presence of fistulous tracts, which would correlate well with the usual gross findings in atypical cutaneous mycobacteriosis.

**Contributing Institution:** Air Force Research Laboratory, 2509 Kennedy Circle, Building 125, Brooks City Base, TX 78235

**References:**

**CASE III:** 090031-35 (JPC 3141966).
Signalment: Seven-year-old, male Nubian-cross goat, caprine (Capra hircus).

History: This castrated adult male goat had lost approximately 35 lbs over two months and, at the time of necropsy, weighed approximately 175 lbs. Over the course of the last week, he exhibited labored, open-mouthed breathing, mucoid nasal discharge, and bruxism. On the day he was euthanized, he had blood and bloody froth coming from his nostrils. He had recently been treated with antibiotics, anthelmintics, and analgesics but continued his downward progression.

Gross Pathologic Findings: This approximately 175-pound male castrated goat was in good nutritional condition with adequate body fat stores. The nasal cavity contained mucoid to mucopurulent discharge and, on the right side of the nasal cavity, from the level of the upper 5th and 6th cheek teeth to the caudal termination of the nasopharynx, a space occupying mass. This mass was firm, measured approximately 5 cm long x 5 cm high x 1-2 cm wide, was partially covered in a mucopurulent, yellow exudate, and was partially adhered to the nasal respiratory mucosal epithelium.

The rumen was filled with brown fluid and watery hay ingesta; expanding the wall of a saccula, there was a 5 cm long x 3 cm wide x 3 cm thick mass with an ulcerated, crateriform center containing greenish-white pus. There were multiple 2-4 mm in diameter erosions around the central crater. On cut section, the crateriform ulcer extended into the rumen wall forming a pus-filled pocket which was surrounded by an approximately 1-2 cm thick band of white, firm, glistening tissue. Adhered to the adventitial surface of the caudal vena cava in the mid-abdomen was a 1.5 cm in diameter, fluid-filled translucent, sac-like structure. Multiple lymph nodes including the mediastinal, mesenteric, and axillary, as well as several subcutaneous lymph nodes, were bright green and enlarged up to three times normal size.

Laboratory Results: Immunohistochemistry was performed on sections of the rumen mass with the neoplastic cells showing the following results:

Positive: Vimentin; smooth muscle actin; S100 protein; desmin, multifocally positive.

Negative: Glial fibrillary acidic protein (GFAP); myoglobin; CD117a (c-kit); discovered on GIST-1 (DOG-1).

Microorganism stains:
Lilli Twort (Gram stain): Gram-positive coccobacilli in small colonies on the periphery of large colonies of Gram-negative filamentous bacteria within the necrotic exudate.

Periodic Acid-Schiff (PAS): Positive for long, filamentous bacterial rods forming club-shaped colonies; positive for fungal hyphae within the ulcer’s necrotic core.

Histopathologic Description: Rumen: Expanding the muscularis externa and elevating the multifocally ulcerated and attenuated rumen papillae, there is a well-circumscribed, unencapsulated, highly cellular neoplasm composed of cells arranged in interlacing streams and bundles, often forming a herringbone pattern.
pattern, and separated by interconnecting rays of
fibrovascular matrix. Neoplastic cells range from
tightly packed to loosely arranged spindle cells with
indistinct cell borders and a moderate amount of
eosinophilic and finely fibrillar cytoplasm. Nuclei are
irregularly oval to elongate, with blunt ends, and are
centrally located with finely stippled to vesiculate
chromatin and indistinct nucleoli. Longitudinally
arranged neoplastic cells are often rectangular,
interlacing and surrounded by a thin rim of clear space.
Mitotic figures average one per high power field, and
there is mild anisocytosis and anisokaryosis. There are
rare karyomegalic cells present. There is a large, 1 cm
in diameter, necrotic core composed of a densely
packed eosinophilic coagulum containing high
numbers of viable and degenerate neutrophils and
eosinophils mixed with necrotic cellular debris and
large, dense club-shaped colonies of filamentous
bacteria. Also present in scattered clusters throughout
the coagulum are filamentous, 3-5 m wide, septate
fungal hyphae which exhibit acute angle, dichotomous
branching. In some sections there is mineralized debris
with or without closely associated fungal hyphae.
There are smaller foci of necrosis scattered throughout
the neoplasm as well as lymphocytes and plasma cells
frequently distributed along fibrovascular tissue.
Multifocally, rumenal papillae are ulcerated or missing
entirely and the exposed, subjacent neoplastic cells are
covered by inflammatory cells and necrotic debris.
Rumen papillae often have clusters of lymphocytes and
plasma cells throughout the submucosa. The line of
demarcation between the splenic capsule and serosa of
the rumen is obscured by infiltrating neoplastic spindle
cells, lymphocytes, plasma cells, hemorrhage, and
fibrosis. There is mild lymphoid depletion within the
white pulp of the spleen.

3-3. Rumen, goat: The ulcerated rumen wall is transmurally effaced by
a densely cellular mesenchymal neoplasm. (HE 4X)

3-4. Rumen, goat: Neoplastic cells are spindled and elongate, with indistinct cell borders and a moderate amount of a finely fibrillar eosinophilic
cytoplasm. Nuclei are centrally placed, oval to elongate, with finely stippled chromatin and 1-3 small basophilic nucleoli. There is mild anisokaryosis,
and mitotic figures are rare. (400X)
Contributor’s Morphologic Diagnoses:
1. Rumen: Leiomyosarcoma, goat, caprine.
2. Nasal mucosa: Rhinitis, granulomatous, focally extensive, severe, with fungal hyphae and granulation tissue (histoslides not submitted).
4. Lung: Pneumonia, interstitial, granulomatous, multifocal, mild, with intralesional nematode larvae, morphology consistent with Muellierus capillaris (histoslides not submitted).
5. Spleen, white pulp: Lymphoid depletion, diffuse, mild (histoslides not submitted).

Contributor’s Comment: Leiomyomas and leiomyosarcomas are neoplasms of smooth muscle cell origin. Morphologic features of this neoplasm that support the diagnosis include the arrangement of interlacing fascicles or bundles of spindle-shaped cells occurring in both long and transverse section and often forming a herringbone pattern. Typical cytologic features include cells which resemble smooth muscle with a moderate amount of eosinophilic, fibrillar cytoplasm and elongate, oval nuclei with characteristic blunt ends. Paranuclear cytoplasmic vesicles, a feature often described in soft-tissue leiomyomas in humans, were not a feature of the cells in this neoplasm. In spite of a lack of either vascular or lymphatic invasion by neoplastic cells, the relatively brisk mitotic rate (one per high power field), cellular atypia, areas of necrosis, and mildly infiltrative neoplastic cells along the tumor’s margins favor a diagnosis of leiomyosarcoma over leiomyoma. Metastases to regional lymph nodes were not identified.

Smooth muscle tumors (leiomyoma and leiomyosarcoma) of the alimentary system are the most common type of gastrointestinal stromal tumor reported in domestic animals. In dogs, there are reports of leiomyomas arising from the wall of the gall bladder and outer muscle coats of the distal esophagus and stomach as well as reports of leiomyosarcomas arising throughout the digestive tract including the tongue, liver and spleen. Likewise for cats, there are reports of leiomyomas and leiomyosarcomas throughout the gastrointestinal tract. Additionally, there are rare reports of leiomyomas and/or leiomyosarcomas arising in the stomach, duodenum, jejunum, ileocecal junction, rectum and omentum of horses as well as reports of a leiomyoma in the spiral colon of a cow and the omasum of a goat. In general, leiomyomas of the digestive tract are more likely to be located in the upper rather than the lower tract. To the best of our knowledge, this is the first report of a primary leiomyosarcoma arising from the rumen of a goat.

In contrast to the alimentary system, leiomyomas and leiomyosarcomas are much more commonly reported in the urinary and reproductive tracts of laboratory and domestic animals. As such, there are reports in the veterinary literature of leiomyomas/leiomyosarcomas of the uterus in rats, rabbits, and guinea pigs; urinary bladder, vagina and uterus of cows; the scrotum, vagina, cervix, uterus, and urinary bladder of goats; the female genital tract, testis and urinary bladder of horses; and the urinary bladder, vagina, uterus, cervix and prostate gland of dogs and cats.

Immunohistochemistry can be useful in differentiating and aiding in the diagnosis of neoplasms which have similar cellular morphology. In this case, gastrointestinal stromal tumor (GIST) and peripheral nerve sheath tumor were considered differential diagnoses.

Leiomyomas/leiomyosarcomas typically express vimentin, desmin, and smooth muscle actin (SMA). GISTs are believed to arise from primitive mesenchymal cells and, as such, can express vimentin, smooth muscle actin (SMA), and S-100 protein to varying degrees. Additionally, in humans, GISTs also commonly express both the tyrosine kinase receptor CD117 (c-kit), and/or the chloride channel protein, discovered on GIST-1 (DOG1). Peripheral nerve sheath tumors are composed of spindloid cells with characteristics of Schwann cells and are typically vimentin, S-100 protein, and glial fibrillar acidic protein (GFAP) positive and SMA negative. The neoplastic cells of the tumor described in this case expresses vimentin, desmin, SMA, and it does not express CD117, DOG1, or GFAP. These immunohistochemical findings support the diagnosis of a smooth muscle origin neoplasm; however, well-differentiated smooth muscle tumors do not typically express S100 protein as was seen in this neoplasm. There is a small percentage of human cases of leiomyosarcomas which do express immunoreactivity for S100 protein.

Additional features of this lesion include large, up to 300 µm in diameter, radiating, club-shaped colonies of filamentous bacteria scattered throughout the necrotic exudate, often associated with mineralized debris (“sulfur granules”). Gram-negative and strong PAS staining of the large colonies of filamentous bacteria suggests infection by Fusobacterium necrophorum, a normal inhabitant of the alimentary tract and a common cause of necrobacillary rumenitis secondary to rumenal acidosis. Differentials for large colonies of filamentous bacteria include the higher forms of bacteria such as Actinomyces sp., Nocardia spp., or Arcanobacterium pyogenes; however, all three generally stain gram-positive. Additionally, with the aid of Gram stain, small colonies of gram-positive
coccobacilli, suggestive of Corynebacterium pseudotuberculosis, are also identified within the necrotic exudate.

Multifocally concentrated along the deep margin of the exudate are clusters and individually scattered fungal hyphae. These hyphae have a width of approximately 3-5 m with regularly septate parallel walls and dichotomous acute angle or Y-shaped branching consistent with Aspergillus sp. Based on microscopic morphology, differential diagnoses for Aspergillus sp. include Fusarium spp., Pseudoallescheria boydii and Candida sp.

The yellow, firm, tissue-like mass partially adhered to the nasal respiratory mucosal epithelium was several centimeters long and partially occluded the respiratory passages on the right side of the head. This mass was composed of necrotic debris and a thick mat of fungal hyphae also consistent with Aspergillus sp. infection. Additionally, several lymph nodes were greatly enlarged and contained bright green pus suggestive of infection by Corynebacterium pseudotuberculosis, a known pathogen of this herd.

JPC Diagnosis: Rumen: Leiomyosarcoma.

Conference Comment: The contributor provided an excellent summary of both the primary lesion (leiomyosarcoma) as well as the additional findings of bacteria, fungi, inflammation, and mucosal ulceration in this specimen.

Conference participants began their discussion with a review of the histological morphology of ruminant forestomachs:

- **Forestomach**
  - Papillar structure
  - Presence/Absence of muscularis mucosae
  - Rumen
  - Long, finger or paddle-like papillae covered by stratified squamous epithelium
  - Muscularis mucosae is absent; no muscle is observed microscopically within ruminal papillae
  - Reticulum
  - Conical papillae project from crests of the honeycomb-like folds, as well as from the bottom of the wells
  - Muscularis mucosae is present in the tips of the folds, but is discontinuous down the rest of the fold
  - Omasum
  - Papillae project from long folds
  - Muscularis mucosae is continuous projecting into omasal fold

Conference participants went on to discuss the criteria for malignancy, asking the following questions:

1. Is there evidence of metastasis?
2. Is there evidence of local vascular or lymphatic invasion?
3. Is the neoplasm infiltrative?
4. Are there necrotic foci within the neoplasm?
5. Do neoplastic cells display cellular features of malignancy—Are the cells anaplastic?
6. Are the cells pleomorphic?
7. Is the chromatin clumped? Are the nucleoli large and/or irregular?
8. Is there a high mitotic rate? Are there bizarre mitoses?
9. Is there loss of cellular polarity or orientation?
10. Are the cells forming multinucleated cells?

These questions must be addressed whenever evaluating a neoplasm to differentiate benign from malignant tumors. The most important features of malignancy are the ability to metastasize systemically and/or the ability to invade local tissue. Malignant tumors owe their invasive nature to enhanced cell motility, increased protease production, and alterations in cell adhesions. Furthermore, malignant tumors tend to grow independent of exogenous growth factors and are resistant to environmental growth inhibitory signals; thus they have virtually unlimited growth potential. Malignant cells are also more adept at avoiding apoptosis and escaping the cytotoxic immune response and inducing angiogenesis than benign tumors, further enhancing their proliferative abilities.

In this case, the infiltrative nature of the tumor, as well as necrotic foci and cellular features of malignancy such as karyomegaly prompted a diagnosis of leiomyosarcoma rather than leiomyoma.

**Contributor:** U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Pathology Division, 1425 Porter Street, Fort Detrick, MD 21702-5011 http://www.usamriid.army.mil/

**References:**

*Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.*

**Opinions, interpretations, conclusions, and recommendations above are those of the author and are not necessarily endorsed by the U.S. Army or Department of Defense.**
CASE IV:  7-16-12 (JPC 4019384).

**Signalment:**  Adult female wild turkey (*Meleagris gallopavo*).

**History:**  Several individuals within a single flock of turkeys reported to be exhibiting clinical signs, including falling out of the trees where they roost and circling on the ground. An unspecified number were found dead.

**Gross Pathology:**  Within the oral cavity of affected animals, and largely effacing the normal mucosal surfaces, are numerous variably sized and irregularly shaped, multifocal to coalescing, proliferative, yellow-tan to pale yellow-green, caseous, nodular masses, which are variably covered in necrotic debris and fibrin.

**Histopathologic Description:**  Oral cavity and tongue (sections vary): Within the sections examined are several areas of moderate to severe, locally to regionally extensive epithelial hyperplasia with occasional formation of variably sized and shaped papillary and frond-like projections. Many affected regions are characterized by variable degrees of erosion and superficial, adherent mats and masses of abundant amorphous eosinophilic necrotic material, cellular debris, blood, and occasional colonies of mixed bacteria. Individual epithelial cells in affected regions frequently have swollen, pale cytoplasm and there are many prominent, large, round to frequently ring-shaped, eosinophilic cytoplasmic inclusion bodies (Bollinger bodies). Affected cells also contain variably sized clear cytoplasmic vacuoles. Scattered individual cell necrosis is present. Similar hyperplastic and cytoplasmic changes occasionally extend into the epithelium of the underlying glandular tissue (not present in all slides). Within the underlying submucosal tissue is an inflammatory response composed predominantly of heterophils and macrophages, with smaller numbers of lymphocytes. Some sections contain deeper skeletal muscle, which contains rare, round to oval, intra-sarcoplasmic protozoal cysts characterized by a thin outer wall and abundant, central, crescent-shaped, basophilic bradyzoites (*Sarcocystis*).

**Contributor’s Morphologic Diagnosis:**  Oral cavity: Severe, multifocal to locally extensive, hyperplastic, necrotizing and erosive stomatitis, heterophilic and histiocytic, with epithelial ballooning degeneration and intracytoplasmic inclusion bodies, etiology consistent with avian poxvirus.

**Contributor’s Comment:**  The gross and histologic appearances of the lesions in the affected animals are typical of avian pox. This is a viral disease of birds that occurs nearly worldwide, with the exception of the polar regions. The avipoxviruses consist of a large, incompletely described group of viruses that affect both domestic and wild birds, and exhibit significant variability in host-specificity.1,5,8 Poxviral disease has been reported in up to 278 species of birds and across at least 20 orders, and tends to be more prevalent in warmer and temperate regions of the globe.8 At this time, the International Committee on Taxonomy of Viruses only recognizes 10 distinct avipoxviruses, but 3 other viruses are tentatively recognized, and there are many additional viruses that have not yet been fully characterized.9 The primary source of infection is infected birds, and transmission occurs both by means of biting insects serving as mechanical vectors, and through direct contact.8

Clinical disease in all affected birds occurs in two main forms: the cutaneous form, in which multiple nodular and proliferative lesions develop on the skin, and the...
4-3. Oral cavity, turkey: The glandular submucosa of the oral cavity is covered by markedly hyperplastic epithelium as well as a brightly eosinophilic necrotic coagulum. (HE 110X)

4-4. Oral cavity, turkey: Epithelial cells within the hyperplastic mucosa often undergo ballooning degeneration and contain a 15-30 m, eosinophilic, intracytoplasmic inclusion (Bollinger body). (Arrow) (HE 260X)
diphtheritic form, which is characterized by extensive necrotizing foci in the oral cavity and upper respiratory tract.\(^8\) The diphtheritic form is less commonly observed, especially in wild birds, but frequently results in increased morbidity and mortality. This particular case represents an example of the diphtheritic form.

Avipoxviruses are large, oval-shaped viruses with a double stranded DNA genome. Due to their large size (up to 250 by 350 nm), and unlike other DNA viruses, poxviruses replicate in the cytoplasm of infected cells where they induce a cytopathic effect and form characteristic eosinophilic inclusion bodies (Bollinger bodies).\(^9\) Avipoxviruses have classically been viewed as highly species-specific, but there is great variability in this, with some isolates inducing severe disease in non-host species, and other isolates causing little to no clinical disease.\(^6,9\)

In North American wild turkeys, poxviral infection has been observed, and is the most commonly diagnosed viral disease in several states, but is geographically centered on the southeastern portion of the United States.\(^2,3\) One report describes a localized outbreak within a single flock in Oregon, but in general this disease is not frequently observed in western or northern states.\(^7\) This particular outbreak is unusual in that it occurred in the northwestern region of the continent, during mid-winter, when insect vectors are at their lowest numbers. Given these considerations, there may have been some other point source of infection, potentially including contact with infected domestic poultry.

Further diagnostics to specifically identify the virus within these affected animals were not performed. As previously mentioned, avipoxviruses have traditionally been regarded as highly species specific, and clinical disease in turkeys (wild or domestic) would be expected to be caused by infection with turkeypox virus. However, a recent report describes infection of turkeys with the closely related fowlpox virus, calling the extent of species-specificity into question and leaving scars. Mucosal lesions often develop into ulcers rather than pustules. In addition to these cutaneous lesions, some poxviruses (such as Sheeppox virus, Ectromelia virus, Monkeypox virus, and Variola virus) also cause severe systemic disease. Following is a list of important poxviruses in veterinary medicine:\(^7\)

### Genus Poxvirus

**Orthopoxvirus**
- Camelpox virus
- Cowpox virus
- Ectromelia virus (mousepox)
- Monkeypox virus
- Vaccinia virus (buffalopox virus, rabbitpox virus), Uasin Gishu disease virus*

**Parapoxvirus**
- Bovine popular stomatitis virus
- Contagious ecthyma virus (Orf virus)
- Pseudocowpox virus, Auzduk disease virus*
- Camel contagious ecthyma virus
- Chamois contagious ecthyma virus*, Sealpox virus*

**Avipoxvirus**
- Fowlpox virus
- Pigeonpox virus, many other avian poxviruses

**Capripoxvirus**
- Goatpox virus, Lumpy skin disease virus, Sheepox virus
- Leporipoxvirus
Myxoma virus, Rabbit fibroma virus (Shope fibroma virus)
Suispoxvirus
Swinepox virus
Molluscipoxvirus
Molluscum contagiosum virus
Yatapoxvirus
Tanapox virus, Yaba monkey tumor virus
*Unassigned members of the genus*

**Contributing Institution:** Montana Veterinary Diagnostic Laboratory, PO Box 997, Bozeman, MT 59771
http://liv.mt.gov/lab

**References:**