



WEDNESDAY SLIDE CONFERENCE 2012-2013

Conference 2

26 September 2012

CASE I: 05-0560 7/ 05-0619 5 (JPC 3139510).

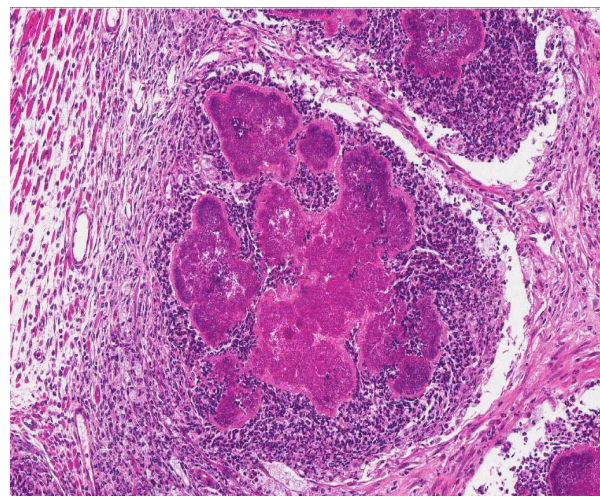
**Signalment:** 6-week-old male Swiss Webster mice (*Mus musculus*).

**History:** Multiple animals were euthanized due to visible swellings and masses around the head and mouth. Development of a head tilt was noted in some.

**Gross Pathologic Findings:** All mice were in good body condition at postmortem examination. Multiple, small white nodules (ranging from 2-10mm diameter) were present subcutaneously around the head, especially in the parotid and submandibular areas. On coronal sectioning of the head after fixation and decalcification, similar nodules effaced and expanded many tissues multifocally from the rostral to caudal



1-1. Head at level of olfactory bulbs, mouse: Unilaterally, the dermis, subcutis, skeletal muscle and lamellar bone of the mandible are replaced by chronic suppurative inflammation centered on bacterial colonies (botryomycosis). (HE 40X)



1-2. Head, mouse: Bacterial colonies are embedded in aggregated brightly eosinophilic protein (Splendore-Hoeppli material) in turn surrounded by degenerate neutrophils, macrophages, and granulation tissue. (HE 120X)

aspect of the head. Nodules were firm and contained tan-brown fluid on cut section.

**Laboratory Results:** *Staphylococcus aureus* was cultured from the lesions.

**Histopathologic Description:** Multiple coronal sections of the head are submitted. Lesions and anatomic structures captured subsequently vary on different sections. Multifocal to coalescing, large, discrete inflammatory cell aggregates bilaterally, efface and expand the ventrolateral aspects of the head, predominantly the mandibular bone, molar teeth, skeletal (masseter) muscle, adipose tissue and skin. Aggregates are composed of central foci of 2-3µm diameter cocci admixed with hyalinized, amorphous material that frequently forms peripheral, radiating, club-like projections (Splendore-Hoeppli material). Within and encircling these cores there are many neutrophils admixed with necrotic cell debris and basophilic mineralized foci. These foci are in turn encircled by epithelioid macrophages, lymphocytes, fibroblasts and collagen fibers. Additional associated microscopic changes include: thickening of mandibular bone by subperiosteal woven bone formation, bone lysis and increased numbers of osteoclasts, odontoclastic resorption of molar teeth, and skeletal myocyte loss/atrophy. Epidermal parakeratotic hyperkeratosis and sub-epidermal pustule formation are additionally seen.

**Contributor's Morphologic Diagnosis:** Multifocal to coalescing, necrosuppurative osteomyelitis, myositis, panniculitis, dermatitis and cellulitis, with Splendore-Hoeppli material and intralesional bacterial cocci, bone lysis and subperiosteal woven bone formation.

**Contributor's Comment:** Splendore-Hoeppli material is the bright eosinophilic, club-shaped material that typically radiates around bacterial colonies in histologic sections.<sup>1,4,5</sup> Classically this phenomenon has been described in cases of chronic staphylococcal infection which is associated with formation of grossly visible bacterial colonies that resemble granules. The term botryomycosis is used to refer to such chronic infections where Splendore-Hoeppli material is seen. This condition usually affects the skin and subcutis and is typically initiated by a skin wound, resulting in lesions that present as local, firm nodules, ulcers or sinuses communicating with deep abscesses.<sup>4,5</sup> Ultimately these lesions may be walled off by fibrous connective tissue and can coalesce to form aggregates of granulomas within the subcutis and surrounding tissues.<sup>4,5</sup> Botryomycosis may also involve other organs such as the lung, and although it can affect immunocompetent animals, immunosuppression is considered to be a predisposing factor to its development.<sup>1,2,4,5,8</sup>

In addition to staphylococci (*S. aureus*, *S. hominus*, *S. xylosum*), other bacterial agents have also been isolated from cases of botryomycosis in rodents, including, *Streptococcus intermedius*, *Pseudomonas aeruginosa*, *Proteus* spp., *Escherichia coli* and *Nocardia asteroides*.<sup>1,3,4,8,11</sup> In other animals and in people however, Splendore-Hoeppli material has been reported to arise in association with infections due to fungi and other parasites, and has also been known to surround biologically inert substances such as suture material.<sup>1,3,4,5,7,12</sup>

Although the exact nature of this reaction is unknown, it is considered to be a localized immunological response to an antigen-antibody precipitate related to fungi, parasites, bacteria or inert materials.<sup>5</sup> The characteristic formation of the Splendore-Hoeppli reaction around infectious agents or biologically inert materials probably represents some attempt at containment of the agent on the part of the host. It likely prevents phagocytosis and intracellular killing of the injurious agent leading to chronicity of infection, so whether this degree of immune responsiveness is actually helpful or harmful remains to be determined.<sup>5,6</sup> Similarly the nature of the intense eosinophilic nature of this material remains poorly understood, but is thought to be due to its composition of antigen-antibody complexes (immunoglobulins and major basic proteins) combined with cell debris from inflammatory cells (lymphocytes, plasma cells, eosinophils and macrophages) and fibrin.<sup>5</sup> Composition of the reaction likely depends on the type of causative agent, and one hypothesis is that Splendore-Hoeppli material may be derived from host leukocytes that aggregate in response to the bacteria, parasites or inert foreign materials that can initiate the reaction.<sup>5,6</sup>

Morphological mimickers of the Splendore-Hoeppli reaction are said to include flame figures (as seen in conditions such as feline eosinophilic granuloma complex, insect bites and drug eruption), tophaceous lesions of gout, asteroid bodies (as may be rarely seen in some granulomatous lesions in animals) and actinomycotic sulfur granules (the radiating structures of Splendore-Hoeppli reaction resemble these sulfur granules). The absence of central branching filaments in Splendore-Hoeppli reaction helps to distinguish this from true actinomycotic sulfur granules.<sup>5</sup>

Flame figures represent degranulated eosinophils that form aggregates of granular necrotic material surrounded by collagen, and these foci are often basophilic with peripheral macrophages.<sup>5</sup> Gout tophi appear as variably sized deposits of amorphous, amphophilic material with parallel, acicular clefts; however, these crystals often have a brownish color and are doubly refractile with polarized light.<sup>5</sup>

Asteroid bodies are stellate eosinophilic inclusions seen within giant cells of granulomas occasionally.<sup>5</sup> This reaction is histologically opposite to that which is seen in the Splendore-Hoeppli reaction, i.e., a central stellate acellular region that is surrounded by inflammatory cells.<sup>5</sup>

**JPC Diagnosis:** Skin, subcutis and maxilla: Cellulitis, dermatitis, myositis and osteomyelitis, pyogranulomatous, multifocal to coalescing, severe, with large colonies of cocci and Splendore-Hoeppli material.

**Conference Comment:** There was considerable slide variation in this case, with the bone involvement totally absent in some slides. Also, bone marrow was visible in some slides, and it was noted that within the marrow there was myeloid hyperplasia, with the majority of cells being granulocytes. Conference participants discussed the various causes of botryomycosis as listed by the contributor, with the addition of a recent case of subcutaneous botryomycosis in a Texas Longhorn steer caused by *Bibersteinia trehalosi*.<sup>10</sup> Participants also noted that in addition to immunosuppressed mice, urokinase-type plasminogen activator-deficient mice are particularly susceptible to developing botryomycosis.<sup>9</sup> This is presumed to be due to their lack of urokinase-type plasminogen activator (uPA). Plasminogen activators are serine proteinases that cleave plasminogen to form plasmin in the fibrinolytic system. Plasmin is a serine proteinase that is involved in several physiological as well as pathological processes.<sup>1</sup> In this case, it is its impaired role in the proteolysis of fibrin and extracellular matrix (ECM) that contributes to the increased susceptibility of uPA deficient mice to developing botryomycosis.

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**CASE II: C6883 H0728 (JPC 4003092).**

**Signalment:** Adult rabbit of unknown age and breed.

**Gross Pathology:** The rabbit showed severely swollen eyelids.

**Histopathologic Description:** Complete eyelid with epidermis and conjunctiva: The epidermis is generally hyperplastic and invades the dermis at the conjunctival-epidermal junction in a lobular pattern, extending into the lamina propria of the palpebral conjunctiva. The hyperplasia is predominantly acanthosis, but the non-invading epidermis exhibits orthokeratotic hyperkeratosis. Keratinocytes within the invading portion of the epidermis show widespread hydropic degeneration. Some keratinocytes contain a 10-15 µm long, eosinophilic cytoplasmic inclusion body surrounded by a clear halo (Splendore inclusions or bodies).

In the dermis, multiple large, stellate or polygonal cells with finely granular, basophilic cytoplasm and one or more large nuclei with finely granular to fragmented chromatin and one clear nucleolus are present. Mitoses are less than 1 per HPF. There is a slight infiltration of eosinophils, macrophages and some lymphocytes and plasma cells. The surrounding collagen fibers are loosely arranged and interspersed with a moderate amount of basophilic amorphous substance (proteoglycans).

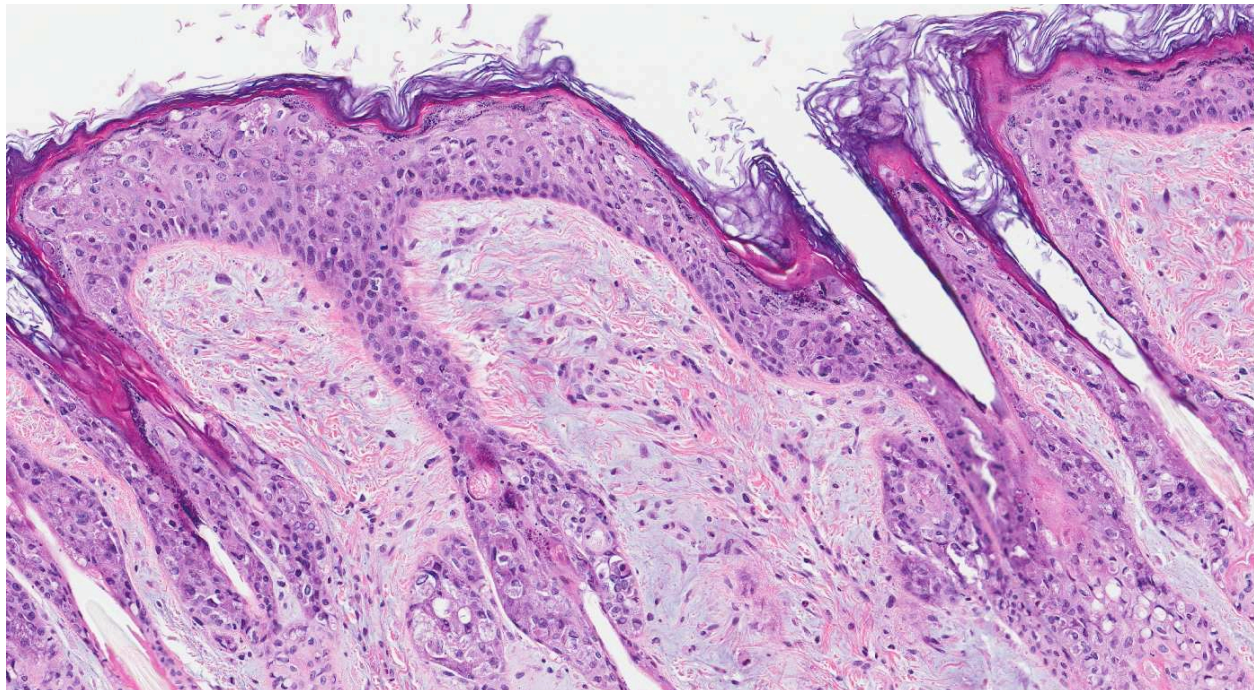
Part of the conjunctival mucosa is desquamated due to autolysis.

**Contributor's Morphologic Diagnosis:** Proliferative dermatitis with myxomatous changes.

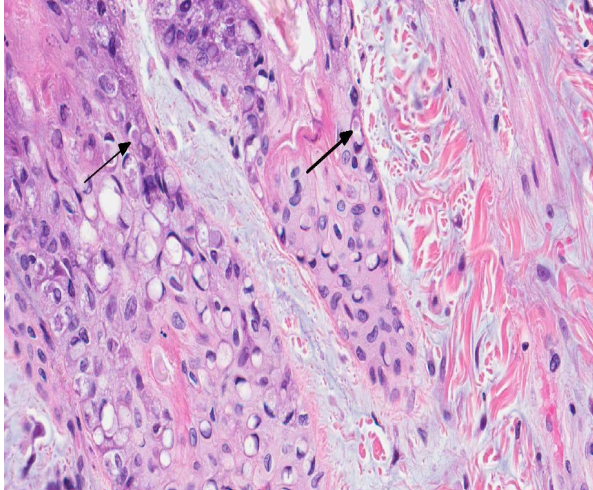
**Contributor's Comment:** Myxomatosis is a disease of wild and domestic rabbits or, rarely, hares caused by the myxoma virus, which belongs to the Leporipoxvirus genus of the family Poxviridae. Transmission of the virus occurs through direct contact or via vectors such as mosquitos or fleas.<sup>1,4</sup>

Three to five days after infection, affected animals first show signs of fever, depression and loss of appetite. Subsequently, edematous or firm skin nodules (mostly around the eye, mouth, nose, ear or genitalia), conjunctivitis and severe depression are seen. Death occurs within one to two weeks after the first symptoms appear.<sup>5</sup>

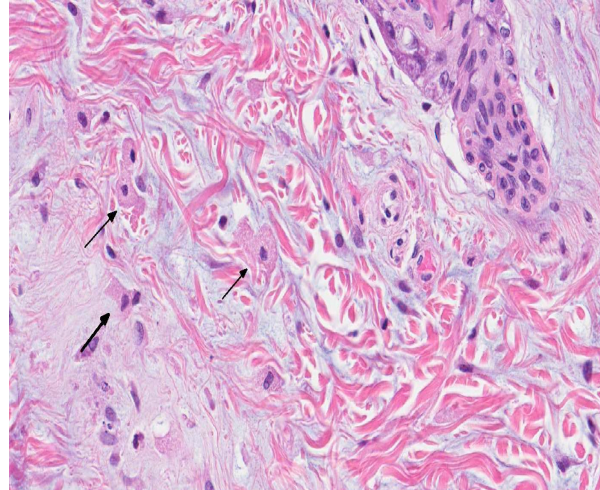
Histologically, one of the first apparent changes is an increase in number and size of the epidermal cells. After this, granular, pink cytoplasmic inclusions appear in these cells. The disease progresses until cell lysis occurs and epidermal vesicles develop. In the dermis, large stellate or polygonal cells with swollen nuclei and fragmented chromatin, polymorphonuclear leukocytes and multinucleated cells appear. Histological changes can also be seen in other organs. The lymph nodes, for example, first become activated,



2-1. Haired skin, eyelid, and rabbit: There is moderate hyperplasia of the epidermis (particularly surrounding follicles), with disorganization and lack of normal epidermal stratification. The superficial dermis is expanded by an accumulation of amphophilic ground substance which surrounds low numbers of plump stellate to spindle cells. (HE 180X)



2-2. Haired skin, eyelid, rabbit: Epithelial cells exhibit intracellular edema (ballooning degeneration) and occasionally contain a 10  $\mu$ m, intracytoplasmic viral inclusion that peripheralizes the nucleus (arrows). (HE 360X)



2-3. Haired skin, eyelid, and rabbit: A population of plump, vacuolated mesenchymal cells populates the myxodematous areas of the dermis ("myxoma cells"). (HE 360X)

while, as the disease progresses, lymphocytes are replaced with the large stellate or polygonal cells previously described. The same cellular infiltration can be seen in the spleen.<sup>5</sup>

**JPC Diagnosis:** Eyelid: Atypical mesenchymal proliferation, diffuse, moderate, with epithelial ballooning degeneration and necrosis, intraepithelial eosinophilic intracytoplasmic viral inclusions, and mild myxedema.

**Conference Comment:** Conference participants discussed the interesting history of myxomatosis, which was first recognized in European rabbits (*Oryctolagus cuniculus*) in a South American laboratory in the late 1800's. Myxomatosis was first recognized in California in 1930, and remains enzootic in the western United States to this day. In the 1950s, the virus was implemented as a biological control in Australia, where it was introduced to cull the ballooning population of nonnative European rabbits. Mortality rates in the European rabbits at the time of introduction were as high as 99%; however, mortality dropped to around 25% in the years that followed, likely due to natural selection of resistant rabbits as well as the emergence of less virulent viral strains. In 1953, myxomatosis was introduced into the wild rabbit population in France, reportedly by a disgruntled individual; from there the virus spread to other parts of Western Europe, including England.<sup>3</sup>

Conference participants also discussed the pathogenesis of myxomatosis. As the contributor stated, myxoma virus is spread via arthropod vectors. The virus replicates in the host at the site of inoculation, resulting in a subcutaneous myxoid mass. In susceptible animals, the virus can then spread via

cell-associated viremia (also known as leukocyte trafficking) and cause systemic disease. In less susceptible rabbit species, such as *Sylvilagus brasiliensis* and *S. bachmani*, viremia does not develop, and lesions are generally restricted to localized cutaneous fibromas.

A closely-related leporipoxvirus, rabbit fibroma virus (RFV, also known as Shope fibroma virus), occurs naturally in the eastern cottontail (*Sylvilagus floridanus*) and causes similar lesions, referred to as "Shope Fibromas", in both cottontails and European rabbits.<sup>2</sup> Shope fibromas appear grossly as flattened subcutaneous tumors on the legs, feet, and occasionally the face. Histologically, Shope fibromas are composed of a localized proliferation of reactive fibroblasts that may contain large intracytoplasmic eosinophilic inclusion bodies. The inclusion bodies can also be found in the cells of the overlying epidermis. These well-circumscribed fibrous masses are generally easily distinguishable from myxomatosis, which is characterized by proliferation of stellate mesenchymal cells on a mucinous matrix with epithelial hypertrophy. Participants noted that this case features a comparatively milder atypical mesenchymal spindle cell proliferation, and more severe epithelial proliferation and loss of normal epithelial stratification than is usually observed in this disease.

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**References:**

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**CASE III: 07003965 (JPC 3125786).**

**Signalment:** Adult female C57BL/6 mouse (*Mus musculus*).

**History:** This mouse was treated with six doses of 7,12-dimethylbenz[a]anthracene (DMBA) at 10mg/ml to induce mammary carcinomas.

**Gross Pathologic Findings:** On gross examination, there was an irregular, expansile, 13x18x18mm mass on the seventh and eighth mammary glands within the right axillary region. The mass was pale tan on cut surface with multifocal 2-4mm foci of hemorrhage and necrosis. Internally, the lungs were multifocally mottled pink to dark red, and there were two 1-2mm white foci on the right caudal lung lobe, and two 3-4mm white foci on the left lung lobe.

**Histopathologic Description:** Lung: In sections of lung, there were one to multiple, expansile, well-demarcated, unencapsulated proliferations of well-differentiated epithelial cells compressing the adjacent pulmonary architecture and collapsing subjacent alveolar spaces. The masses were composed of lobules, cords, and papillary projections of cuboidal to columnar epithelial cells with moderate faintly eosinophilic amorphous cytoplasm and a central round nucleus with coarsely clumped to condensed chromatin and an occasional nucleolus, supported by a fine fibrovascular stroma. Mitotic figures were not observed. Adjacent to and within the surrounding alveolar spaces were small to large numbers of large macrophages with moderate to abundant brightly eosinophilic cytoplasm containing refractile needle-shaped to acicular crystalline material. Occasional to frequent multinucleate giant cells were present,

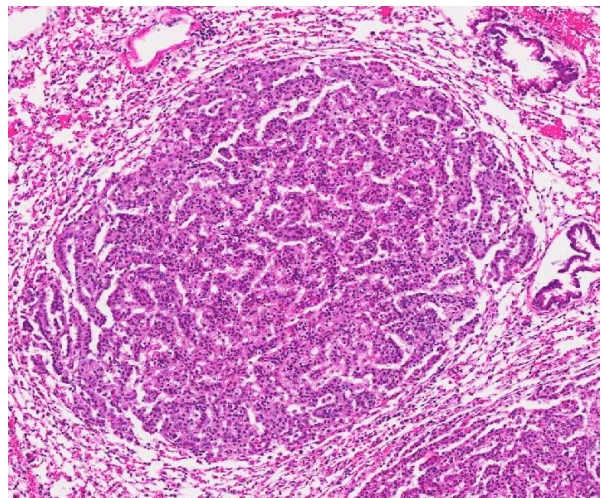
containing similar crystalline material. In some sections, large acicular crystals were present within small bronchioles, and there were multifocal inflammatory aggregates composed of small to moderate numbers of lymphocytes and plasma cells scattered randomly throughout the lung parenchyma.

**Contributor's Morphologic Diagnosis:** Lung: Pulmonary adenomas; Mild to moderate, chronic, multifocal to coalescing, accompanied by histiocytic to granulomatous interstitial pneumonia with intracytoplasmic eosinophilic crystalline material.

**Contributor's Comment:** Eosinophilic crystalline pneumonia is an idiopathic disease that occurs in several different strains of mice, most commonly in those of a C57BL/6 background.<sup>4,8</sup> This disease varies from mild and subclinical to severe, sometimes resulting in dyspnea and death. This disease may occur alone or in conjunction with other pulmonary lesions such as pulmonary adenomas (as in this case), lymphoproliferative disease, allergic lung disease, and parasitic or fungal infections.<sup>1,3</sup> The crystals that are found within the cytoplasm of macrophages and multinucleate giant cells, as well as often free within alveolar spaces and bronchioles, are reminiscent of Charcot-Leyden crystals morphologically, which are products of eosinophil breakdown and eosinophil-related diseases in humans.<sup>3,8</sup> However, crystals in ECP are composed primarily of Ym1 protein, a chitinase-like protein that is secreted by activated macrophages. The exact function of Ym1 protein is poorly understood, but is believed to play a role in host immune defense, eosinophil recruitment, and cell-cell and cell-matrix interactions consistent with tissue repair.<sup>2,3</sup>

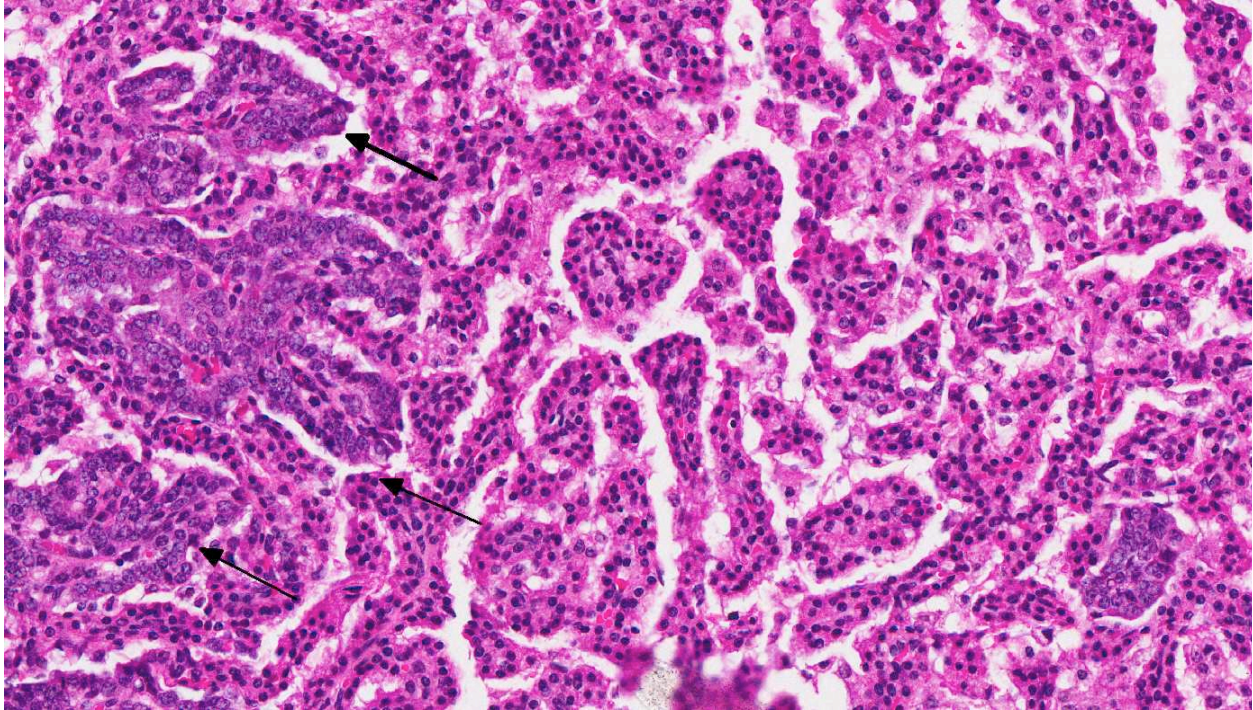


3-1. Lung, mouse: There are several expansile proliferative lesions within this section of lung. (HE 50X)

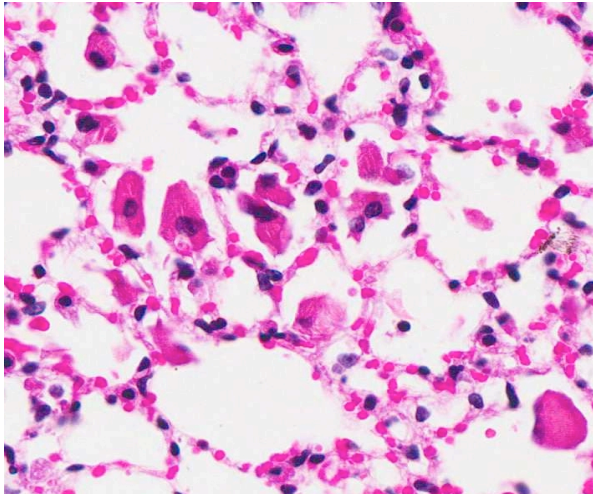


3-2. Lung, mouse. Lesions often exhibit a combination of lepidic (growing in a "scale-like" fashion along preexisting alveolar septa – seen at bottom left) and expansile growth, compressing adjacent alveoli (at upper right). (HE 120X)





3-3. Lung, mouse: In some neoplasms, neoplastic cells exhibit pleomorphism, a higher nuclear/cytoplasmic ration, and finely stippled chromatin (suggestive of malignant transformation) (arrows). (HE 400X)



3-4. Lung, mouse: Multifocally, alveoli contain variable numbers of macrophages which contain numerous brightly eosinophilic spicules ("acidophilic pneumonia"). (HE 400X)

**JPC Diagnosis:**

1. Lung: Pulmonary (bronchioalveolar) carcinoma.
2. Lung: Pulmonary (bronchioalveolar) adenoma.
3. Lung: Bronchioalveolar hyperplasia, multifocal.
4. Lung: Pneumonia, interstitial, histiocytic, focally extensive, marked, with intracytoplasmic eosinophilic crystalline inclusions.

**Conference Comment:** The slide variation with this case initiated an excellent discussion amongst conference participants regarding the spectrum of lung

lesions observed in various sections. The severity of eosinophilic crystalline pneumonia ranges from mild alveolar histiocytosis in some sections to granulomatous pneumonia in others. In some sections, eosinophilic crystals were visible within epithelial cells lining bronchi as well. Additionally, there are also several proliferative bronchoalveolar lesions comprising a continuum from what conference participants considered benign hyperplasia to adenoma to carcinoma. Within nodules of more quiescent hyperplastic/adenomatous cells, there are occasionally foci of cells with a more malignant appearance that were interpreted as representing malignant transformation.

Discussion also centered on the current variation in classification of and terminology for lung tumors in the mouse, arising from the lack of a consensus on histologic subtype.<sup>6</sup> In some instances, neoplasms showing lepidic growth along preexisting alveolar structures have been referred to as bronchioalveolar adenoma if < 3mm and bronchioalveolar carcinoma if > 3mm. However, Percy and Barthold, in the 3<sup>rd</sup> edition of *Pathology of Laboratory Rodents and Rabbits*, use the terminology "pulmonary" rather than "bronchioalveolar" when describing these neoplasms in mice.<sup>5</sup> Interestingly, in human pathology, the term "bronchioalveolar carcinoma" has recently been dropped from the terminology, and the new (2011) classification scheme uses the terms "atypical adenomatous hyperplasia" for preinvasive hyperplastic



lesions, “adenocarcinoma in situ” and “minimally invasive adenocarcinoma” for small (<3 cm) solitary adenocarcinomas with pure or predominant lepidic growth, respectively, and < 5 mm invasion, and “invasive adenocarcinoma” for tumors with > 5 mm invasion.<sup>7</sup>

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**References:**

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**CASE IV: 09003277 (JPC 3165092).**

**Signalment:** 1-year-old male Armenian hamster (*Cricetulus migratorius*).

**History:** Over the course of a month, 8 male Armenian hamsters, ages 5 months to 1 year, presented to necropsy either moribund or dead from an illness of short duration. The hamsters were housed individually in an isolation cubicle and were manipulated only in a BSL-2 safety cabinet. The hamsters were used for monoclonal antibody production and were immunized with KLH-peptides (Keyhole Limpet Hemocyanin-conjugated peptides) emulsified in Titermax.

The hamsters routinely developed chronic dermatitis, most severe over the shoulders and dorsal neck. This is the injection location of the immunizations that were given 4-5 times over a 2-4 month period. All hamsters were purchased from the same vendor and often arrived with a history of receiving ivermectin treatment for demodicosis. Treatment for the chronic dermatitis over the cervical dorsal area consisted of triple antibiotic ointment (Vetropolycin®) applied three times per week topically.

**Gross Pathologic Findings:** At necropsy the hamsters were in good body condition, although all had varying degrees of chronic dermatitis localized to the dorsal cervical area, shoulders and extending to the base of the ears. Dermatitis was sometimes mild, with hyperemia and patchy hair loss or characterized by punctate excoriations with crust formation and associated alopecia. Approximately half of the hamsters had small, dermal pyogranuloma formation in these areas.



4-1. Cecum, colon, and hamster: The ceca and colon are congested, with serosal erythema and petechiations. Photograph courtesy of Memorial Sloan-Kettering Cancer Center.

All of the hamsters had formed fecal pellets in the rectum and stomachs distended with food. The cecum and large intestine contained pasty ingesta. Several hamsters presented with congested ceca and proximal colons, characterized by serosal erythema and petechia. No other gross abnormalities were detected.

**Laboratory Results:** *Clostridium difficile* toxins A/B identified by ELISA in the cecal contents.

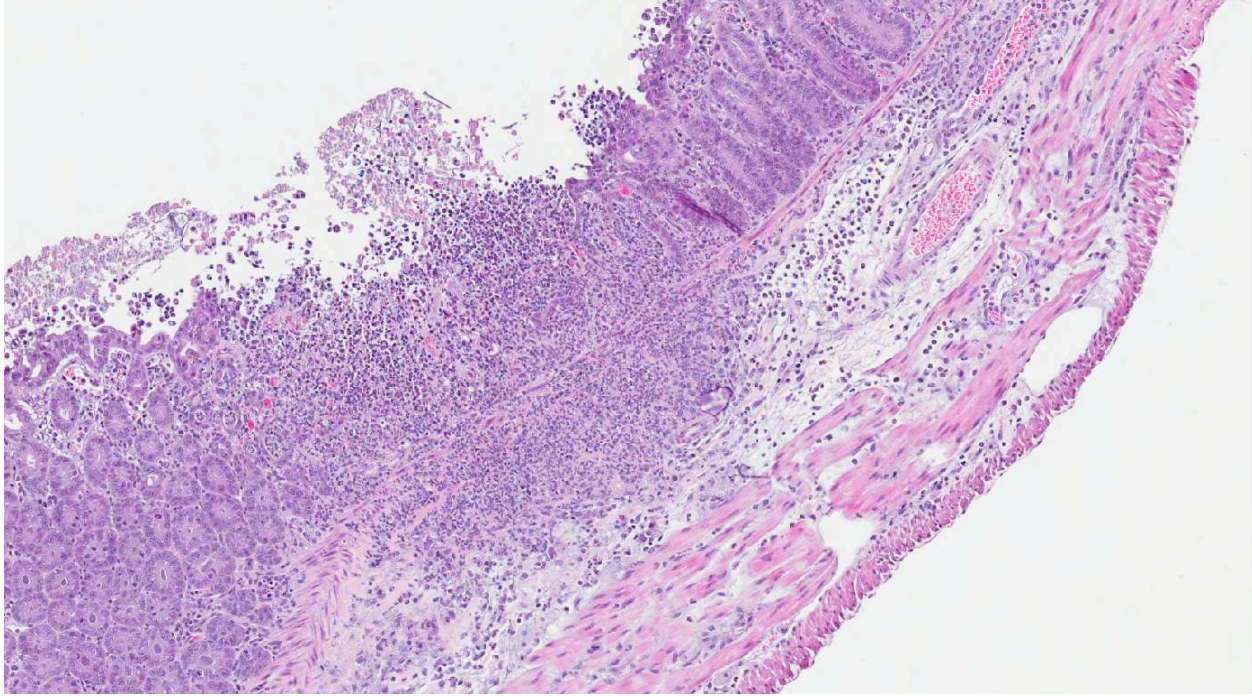
**Histopathologic Description:** Cecum: Multifocally there is necrotizing fibrinopurulent mucosal inflammation, ranging from attenuation of surface enterocytes and patchy focal mucosal erosions which exude fibrin and neutrophils, to extension into the mildly hyperplastic glands, filling them with necrotic debris. The submucosa is multifocally infiltrated by macrophages, neutrophils, lymphocytes and scattered eosinophils, within an edematous stroma. Rarely inflammation is transmural and extends to the serosal surface. Lymphatics are dilated, and multifocally contain edema and viable and karyorrhectic inflammatory cells. Superficial mucosal vessels are multifocally congested and occasionally associated with fibrinoid necrosis. There is rare overlying pseudomembrane formation.

**Contributor's Morphologic Diagnosis:** Cecum: Typhlitis, fibrinopurulent, necrotizing, subacute, mucosal to transmural, multifocal, moderate to marked.

**Contributor's Comment:** *C. difficile* causes antibiotic-associated disease in a variety of laboratory animal species, but since the 1970s the hamster's renowned sensitivity has made it a model for human disease. A variety of antibiotics can initiate the clinical syndrome by rendering the hamster susceptible. Hamsters acquire *C. difficile* infection through direct contact, fomites contaminated with fecal material, and a recent report describes *C. difficile* present in the air surrounding human patients with the disease.<sup>2</sup>

Overgrowth of *C. difficile* occurs following the disturbance of the normal Bacteroides and *Lactobacillus* flora.<sup>5</sup> Clindamycin and ampicillin antibiotics are reported to directly enhance *C. difficile* colonization by increased expression of adherence factors beneficial for *C. difficile* colonization and adherence.<sup>3</sup> Typhlitis and enterotoxemia develop secondary to the elaboration of toxins A and B, responsible for the tissue damage and influence of the immune response, resulting in watery or hemorrhagic diarrhea, dehydration and death within 2-10 days.<sup>5</sup> Two A exotoxins, an enterotoxin, and the cytotoxin B are produced by *C. difficile*. Epithelial tissue damage from the toxins is likely following the glycosylation and inactivation of Ras GTPases, disabling signaling





4-2. Colon, hamster: The colonic mucosa is multifocally ulcerated with transmural infiltration of large numbers of neutrophils. (HE 35X)

pathways.<sup>4</sup> Toxins also glycosylate Rho, which regulates the actin cytoskeleton, resulting in the opening of tight junctions, and eventual cell apoptosis.<sup>4</sup> The toxins also cause release of proinflammatory mediators, attracting neutrophils, and activate secretion stimulated by the enteric nervous system.<sup>4</sup> Demonstration of the toxins is necessary for interpretation of the diagnostic significance of *C. difficile*.

This case is similar to a recently reported fatal clostridial typhlitis of a Syrian hamster treated with topical antibiotic ointment, containing polymyxin B, neomycin and bacitracin.<sup>1</sup> That case surmised that self-grooming may have resulted in unintentional oral exposure of the antibiotic, as well as possible absorption through the skin wound. Neomycin has been demonstrated to induce antibiotic-associated diarrhea in hamsters, and the other antibiotics are not known to induce diarrhea or colitis. Unlike the other antibiotics, neomycin can be absorbed through abraded skin or wounds. The Vetropolycin® applied repeatedly to the dorsal cervical region of the hamsters in this case contained the same mix of antibiotics: polymyxin B, neomycin and bacitracin.

This case cautions against the topical clinical application of antibiotics to hamsters. Antibiotic therapy in hamsters is challenging, and trimethoprim sulfonamides, fluoroquinolones and chloramphenicol are generally safer antibiotic choices.

**JPC Diagnosis:** Cecum, colon: Typhlocolitis, necrotizing, multifocal, moderate.

**Conference Comment:** As stated by the contributor, demonstration of *Clostridium* toxin is necessary to evaluate the significance of observing *C. difficile*, as it is the toxins, rather than the mere presence of the bacteria that elicits disease. The primary toxins, TcdA and TcdB, belong to the family of large clostridial toxins (LCTs), along with lethal and hemorrhagic toxins from *C. sordelli*,  $\alpha$ -toxin from *C. novyi*, and large cytotoxin from *C. perfringens*. LCTs inactivate host Rho and Ras GTPases, which are responsible for regulating many cellular processes, including maintenance of the cytoskeleton. More specifically, TcdA and TcdB intoxication results in loss of cytoskeletal structure and rounding of the affected cell, commonly referred to as the cytopathic effect. TcdB has more potent cytotoxic effects, and is therefore referred to as the “cytotoxin.” TcdA also induces fluid accumulation in the intestinal tract, earning it the title “enterotoxin.”<sup>6</sup> TcdA and TcdB cause cell death through a variety of mechanisms including both apoptosis (p-53 dependent, p-53 independent, caspase-dependent and caspase-independent) and necrosis. Other effects on cells exposed to these toxins include disruption of cell-cell junctions and increased secretion of cytokines TNF, IL-1, IL-6, and IL-8. IL-8 is an important factor in the recruitment of neutrophils, which play a major role in the host response to *C. difficile* infection.

Both TcdA and TcdB are composed of two subunits: A and B; subunit A is an N-terminal glucosyltransferase domain (GTD), and subunit B is responsible for the delivery of subunit A into the target cell. Repetitive oligopeptides on subunit B bind to sugar moieties on host cell surfaces; the toxins then enter the cell through clathrin-mediated endocytosis, and endosomal acidification induces structural changes that create a pore in the host membrane. Next subunit B is cleaved via proteolysis to release GTD into the cytosol, where it inactivates host cell GTPase by glucosylation, leading to the previously-described deleterious effects on the cell.<sup>6</sup>

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