Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2017-2018

C o n f e r e n c e 18

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CASE I: 1704415 (JPC 4101081).

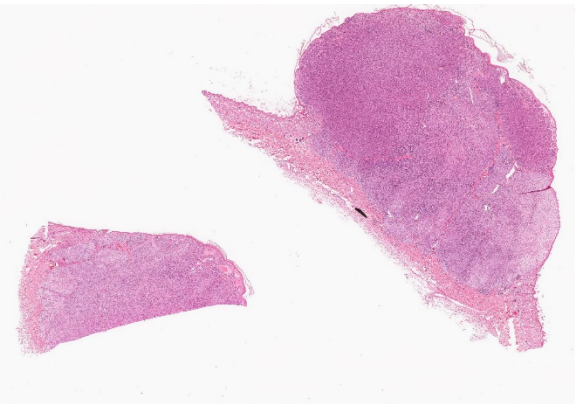
Signalment: 21-year-old, female, American Quarter Horse (*Equus caballus*), equine.

History: The mare had a well-circumscribed hairless mass in the right nuchal region. The mass was reported to be pruritic and was excised surgically and submitted whole for histopathologic evaluation.

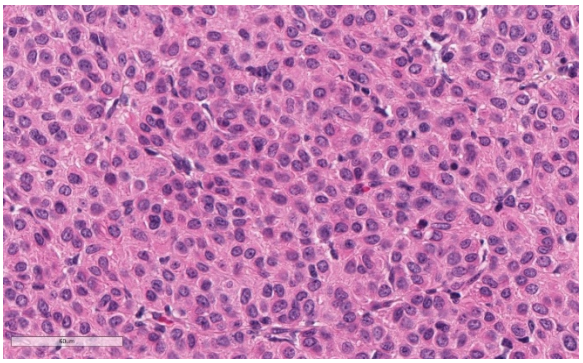
Gross Pathology: The submitted formalin-fixed sample consisted of haired skin with an approximately 14 mm diameter, slightly firm, tan, raised and hairless mass that markedly expanded the superficial dermis and elevated the epidermis.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.):
None provided.

Microscopic Description:
The superficial dermis is focally expanded by an unencapsulated, multilobulated and densely cellular neoplasm that replaces adnexal units and elevates the epidermis. The neoplastic cells have variable arrangements, ranging from dense sheets and nests to trabeculae or thin ribbons separated by a delicate fibrovascular stroma. Multifocally throughout the neoplasm, cells closely abut or palisade along vascular channels and impinge on the lumina without disrupting the endothelium. The cells are cuboidal to polygonal with variably distinct cell borders,



Haired skin, horse. Glomus tumor, horse, haired skin. A multilobulated, highly cellular neoplasm expands the dermis and elevates the epidermis. (HE, 7X) (Photo courtesy of: University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology http://www.vet.upenn.edu/research/academic-departments/)



Haired skin, horse. Typical rounded glomus cells with pale eosinophilic cytoplasm and a round nucleus with finely stippled chromatin and one inconspicuous nucleolus. (HE, 400X) (Photo courtesy of: University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology http://www.vet.upenn.edu/research/academic-departments/)

a moderate amount of pale eosinophilic cytoplasm, and a round to ovoid or irregular nucleus with finely stippled chromatin and a small inconspicuous nucleolus. Occasionally, the cells have abundant hypereosinophilic cytoplasm that peripheralizes the nucleus (epithelioid-type cells). Anisocytosis and anisokaryosis are mild to moderate and mitotic figures are infrequent, with an average of 0 to 1 per single high power field (12 per 50 consecutive 40X high power fields). Binucleation and individual cell necrosis are occasionally observed. Low numbers of lymphocytes and plasma cells multifocally infiltrate the intervening stroma. The overlying epithelium is mildly hyperplastic with orthokeratotic hyperkeratosis and is focally ulcerated.

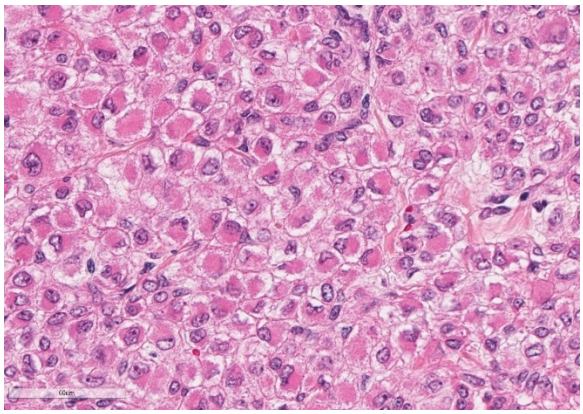
A periodic acid-Schiff (PAS) stain highlights a thin PAS-positive basement membrane surrounding individual or small nests of cells.

Immunohistochemistry:
The neoplastic cells exhibit diffuse and strong positive cytoplasmic immuno-reactivity for alpha-smooth muscle actin (α -SMA), vimentin, and desmin, and are negative for pancytokeratin (AE1/AE3) and CAM 5.2.

Contributor's Morphologic Diagnosis:
Horse, cutaneous mass: glomus tumor

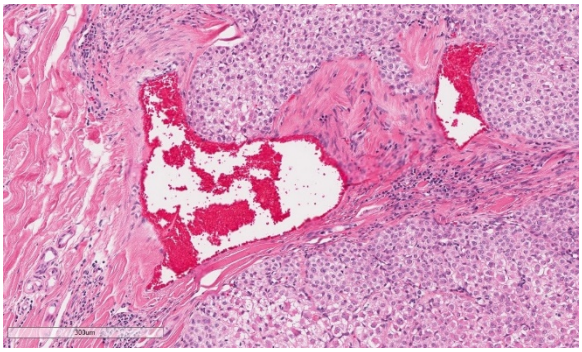
Contributor's Comment: Glomus tumors (GT) are rare, typically benign neoplasms that are thought to arise from the modified perivascular smooth muscle cells of the glomus body, a structure that plays a role in body temperature regulation by allowing arterio-venous shunting of blood and is most commonly found in the subungual regions, subcutis of the extremities, or other specific locations such as the precoccygeal region.[5,9]

Glomus tumors in humans are usually solitary tumors that appear as small circumscribed nodules in the deep dermis or subcutis of the upper and lower extremities, with the subungual region of the finger being the most common location. Many other, less common locations have been described, such as the gastrointestinal tract, penis, urinary bladder, lungs etc.[5]



Haired skin, horse. Epithelioid-type glomus cells with abundant eosinophilic cytoplasm and a peripheral nucleus. Binucleation is occasionally noted. (HE, 400X) (Photo courtesy of: University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology http://www.vet.upenn.edu/research/academic-departments/)

The clinical diagnosis in humans is based on the typical red-blue appearance, combined with a history of paroxysmal pain from cold exposure and light touch, regardless of the size of the tumor.[4,5] Depending on their histologic appearance, GT are classified as classic (or sporadic/solid), glomangiomas, glomangiomyomas, glomangiomatosis and symplastic GT, which have a higher grade of nuclear atypia.[4] The classic GT are by far the most common type and account for approximately 75% of tumors.[5] Although predominantly benign, a small subset of tumors displays clinical or histological features of malignancy.[3] Folpe et al., in 2001, proposed the following classification system for this subset of tumors. Malignant GT, or glomangiosarcoma, are tumors larger than 2 cm in diameter and located deep in the subcutis and underlying tissues, or that have atypical mitotic figures, or a moderate to high nuclear grade and ≥ 5 mitotic figures/50 consecutive 40x high power fields. Symplastic GT have a high nuclear grade in the absence of other malignancy features. Tumors that lack the criteria for malignant GT or symplastic GT, but exhibit one of the following characteristics, high mitotic activity and superficial location, or large size, or deep location only, are classified as GT of uncertain malignant potential. Finally, glomangiomatosis are tumors resembling diffuse angiomatosis with increased numbers of glomus cells. Only malignant GT were associated with metastasis, which included the brain, bone, lung, liver, small intestine, mediastinal lymph nodes, and bowel mesentery.[3]

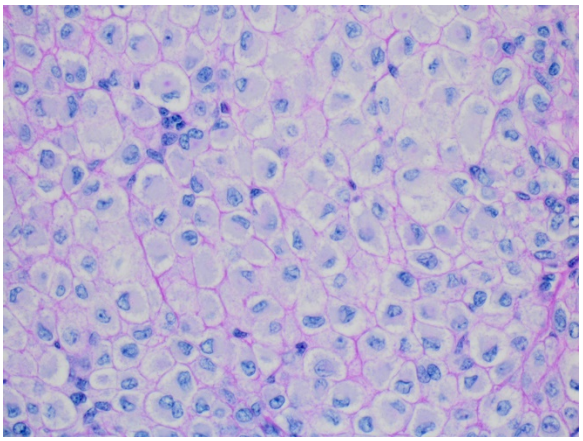


Haired skin, horse. Neoplastic cells multifocally impinge on vascular lumina beneath an intact endothelial lining (HE, 100X) (Photo courtesy of: University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology, http://www.vet.upenn.edu/research/academic-departments/)

Histologically, classic glomus tumors are well-defined, multilobulated, often partially encapsulated neoplasms composed of nests of small rounded cells often closely associated to capillaries and supported by a hyalinized or myxoid stroma. Cells have eosinophilic cytoplasm and a round, centrally located nucleus.[4] A more oncocytic or epithelioid appearance of cells is occasionally reported.[4] Cellular and nuclear pleomorphism and mitoses are not prominent features.[4] Immunohistochemical staining is consistently positive for α-smooth muscle actin and vimentin.[8]

Similarly to humans, glomus tumors in domestic animals are rare and typically benign, and have been described in non-human primates, cats, dogs, cows, and horses.[7,10,11] In horses, these tumors have been reported to occur in the subcutis of the head and neck and in the foot.[1,2] A single case of glomus tumor with neuroendocrine differentiation was reported in the maxilla of a 13-year-old Icelandic crossbred mare.[10] Most cases reported in horses displayed features of malignancy, either by local invasion, or by cytological criteria, according to the human classification of malignant glomus tumors.[1-3,10] However, no metastasis to the local lymph nodes or distant sites were reported.

In the case presented herein, the preliminary diagnosis of a trichoblastoma was ruled out based on negative immunoreactivity for



Haired skin, horse. A thin basement membrane surrounds individual or small groups of neoplastic cells. (PAS, 400X) (Photo courtesy of: University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology, http://www.vet.upenn.edu/research/academic-departments/)

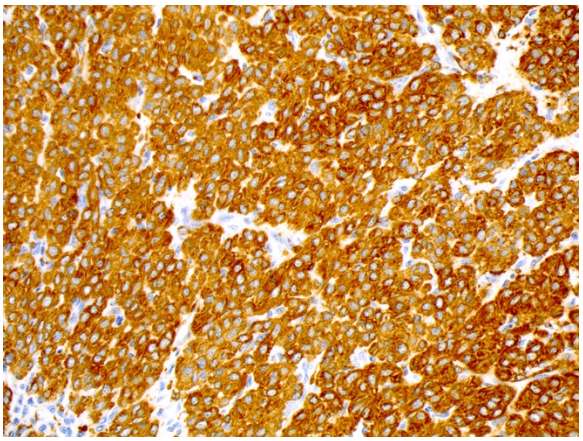
pancytokeratin (AE1/AE3) and CAM 5.2, while the positive immunoreactivity for alpha-smooth muscle actin (α-SMA), vimentin, and desmin instead supported a diagnosis of glomus tumor. Given the mitotic activity and nuclear pleomorphism, the tumor in this horse would be classified as a malignant glomus tumor (glomangiosarcoma) based on the human classification criteria. The malignant behavior of glomus tumors reported in horses was typically associated with increased cellular pleomorphism and the difficulty to achieve a complete surgical excision.[1,2,10] In the present case, the small circumscribed nature of the neoplasm and complete surgical excision suggest a favorable prognosis.

JPC Diagnosis: Haired skin: Glomus tumor, American Quarter Horse (*Equus caballus*), equine.

Conference Comment: Glomus tumors arise (not surprisingly) from glomus cells which are specialized modified smooth muscle cells controlled by the sympathetic nervous system. They are typically associated with arteriovenous shunts or anastomoses that act to regulate flow within the shunt vessels and influence temperature regulation. In humans, these are frequently found in extremities.[6] In animals, glomus tumors are most frequently reported in dogs where they also tend to occur along extremities. Additionally, there have been isolated reports in cats, horses, non-human primates, and several cows.[2,7,9,11] In horses, there appears to be a predisposition for the head or neck (as seen in this case); however, examination of additional cases is necessary to determine whether there is indeed a true site predilection.[2] A recent case described in a Holstein-Friesian cow identified a large primary glomus tumor within the liver.[7]

Microscopically, glomus tumors are well-demarcated, dermal or subcutaneous masses with a fibrous capsule that often contains subcapsular vessels and nerve fibers. In domestic animals and humans, most are considered round cell type, or glomus tumor proper, in which round cells are densely packed with centrally located, prominent nuclei and moderate amounts of pale eosinophilic cytoplasm. Rarer are the spindle cell type in which cells are more elongated with oval nuclei. In both types, mitotic figures and multinucleated cells are few, and each cell or groups of cells is surrounded by a PAS-positive basement membrane. Ultrastructurally, the basement membrane can be seen as well as actin-like filaments, cytoplasmic dense bodies, pinocytotic vesicles, and glycogen granules.[6]

Differentials for the round cell type of glomus tumor include: Merkel cell tumors, plasma cell tumors, nonepitheliotropic lymphomas, histiocytomas, mast cell tumors, and transmissible venereal tumors (TVTs). In general, all of the above differentials lack the association with peripherally located blood vessels and nerve fibers that are prominent in



Haired skin, horse. Neoplastic cells exhibit strong positive cytoplasmic immunoreactivity to α-SMA. (IHC for α-SMA, DAB chromogen, 200X) (Photo courtesy of: University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology, http://www.vet.upenn.edu/research/academic-departments/)

glomus tumors. Additionally, Merkel and plasma cell tumors have prominent "packeting" of cells which are separated by a fine stroma and not surrounded by basement membrane. The location of the nuclei can separate glomus tumors from plasma cell tumors and histiocytomas. Also, histiocytomas have a characteristic "top heavy" appearance and often have intraepidermal neoplastic cells. Mast cells can be clearly identified using toluidine blue or Giemsa stains to highlight metachromatic cytoplasmic granules. Finally, lymphomas and TVTs are most difficult to distinguish based on H&E cytologic characteristics alone. Due to its smooth muscle origin, glomus cell tumors retain positivity for vimentin, α-smooth-muscle actin, and pan-muscle-specific actin which will be negative in lymphomas and TVTs.[6]

Contributing Institution:
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http://www.vet.upenn.edu/research/academic-departments/

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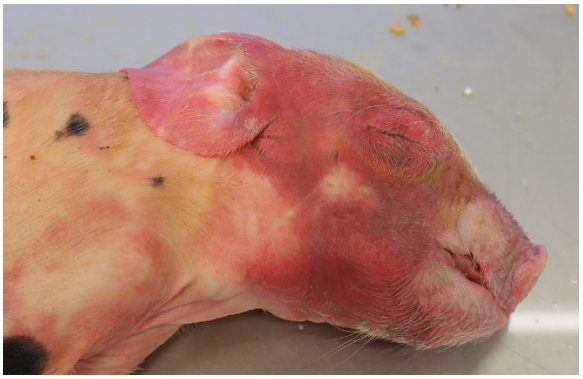
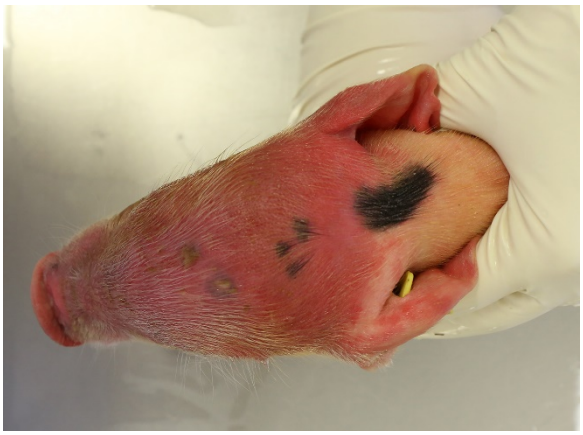
CASE II: NP-20/17 (JPC 4102669).

Signalment: 3-day-old male piglet (*Sus scrofa domesticus*), porcine.

History: This was one of two piglets that came from a farm of 1100 sows and 3000 suckling piglets. 24-48 hours post-birth the piglets showed diffuse inflammation and cyanosis of the head, lethargy and they died 12 to 24 hours after the first clinical signs appeared. The clinical signs affected one or few of the piglets from a same litter and all the affected animals died. The problem occurred in litters of both gilts and sows. Teeth shaving or trimming of piglets is not a general procedure in this farm.



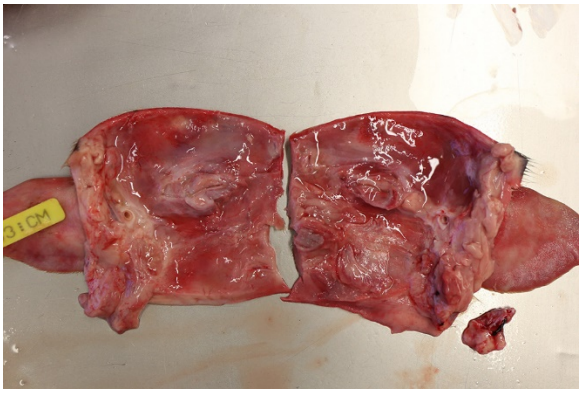
Face and neck, skin, piglet. There is diffuse erythema and swelling of the skin and the subcutaneous tissue of the face, the head and the cranial portion of the neck. (Photo courtesy of: Veterinary Pathology Department, Veterinary Faculty, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.)



Subcutaneous tissue of the face and submandibular lymph node. The subcutaneous tissue and the hypodermis, and regional lymph nodes are markedly edematous. (Photo courtesy of: Veterinary Pathology Department, Veterinary Faculty, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.)

Gross Pathology: Both piglets submitted for necropsy presented an intense and diffuse swelling of the skin and the subcutaneous tissue of the face, the head and the cranial portion of the neck. The skin also had a diffuse reddish coloration. In the frontal aspect of the face, in the cutaneous surface there were two small irregular epithelial erosions covered with crusts. After removing the skin of the head and neck there was moderate amount of clear, fluid to gelatinous material in the subcutaneous tissue and the hypodermis, interpreted as edema. The regional lymph nodes (submandibular and retropharyngeal lymph nodes) appeared diffusely edematous and reddened. In both animals teeth were intact. In the rest of the body there were not significant alterations. The stomach was filled with clotted milk.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.):



Face and neck, skin, piglet. In the surface frontal part of the face there are two small irregular epithelial erosions covered with crusts. (Photo courtesy of: Veterinary Pathology Department, Veterinary Faculty, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.)

Microbiology: *Pasteurella multocida* was isolated on bacterial culture of the skin and subcutaneous tissue, liver and spleen of both animals.

Tissue gram stain: Abundant numbers of coccobacillary gram-negative bacteria in the deeper dermis and subcutaneous tissue. There were also large quantities of gram positive bacterial cocci on the epidermal surface.

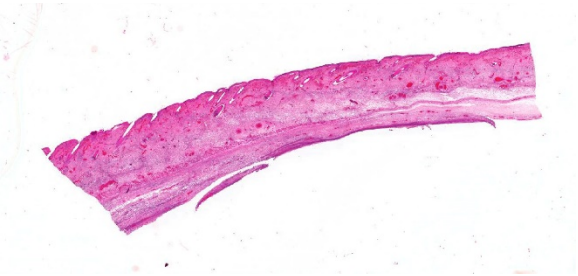
Microscopic Description:
Multifocally, the skin shows areas of loss of continuity of the epidermis (ulcers) and substitution by moderate amounts of necrotic debris, degenerated neutrophils, extravasated erythrocytes and occasional aggregates of cocci (serocellular crust). The dermis is entirely expanded by abundant interstitial edema and hemorrhage, together with large amount of inflammatory infiltrate composed mainly by neutrophils and in lesser extend macrophages and lymphocytes that reach the subcutaneous muscle and adipose tissue. In these localizations, there are abundant coccobacilli distributed diffusely and forming small clusters. Additionally the deep dermis there are multifocal areas of necrosis composed by eosinophilic amorphous material with cellular debris and abundant bacterial colonies. The blood vessels are markedly congested and contain abundant bacterial emboli in their lumen.

Contributor's Morphologic Diagnosis:

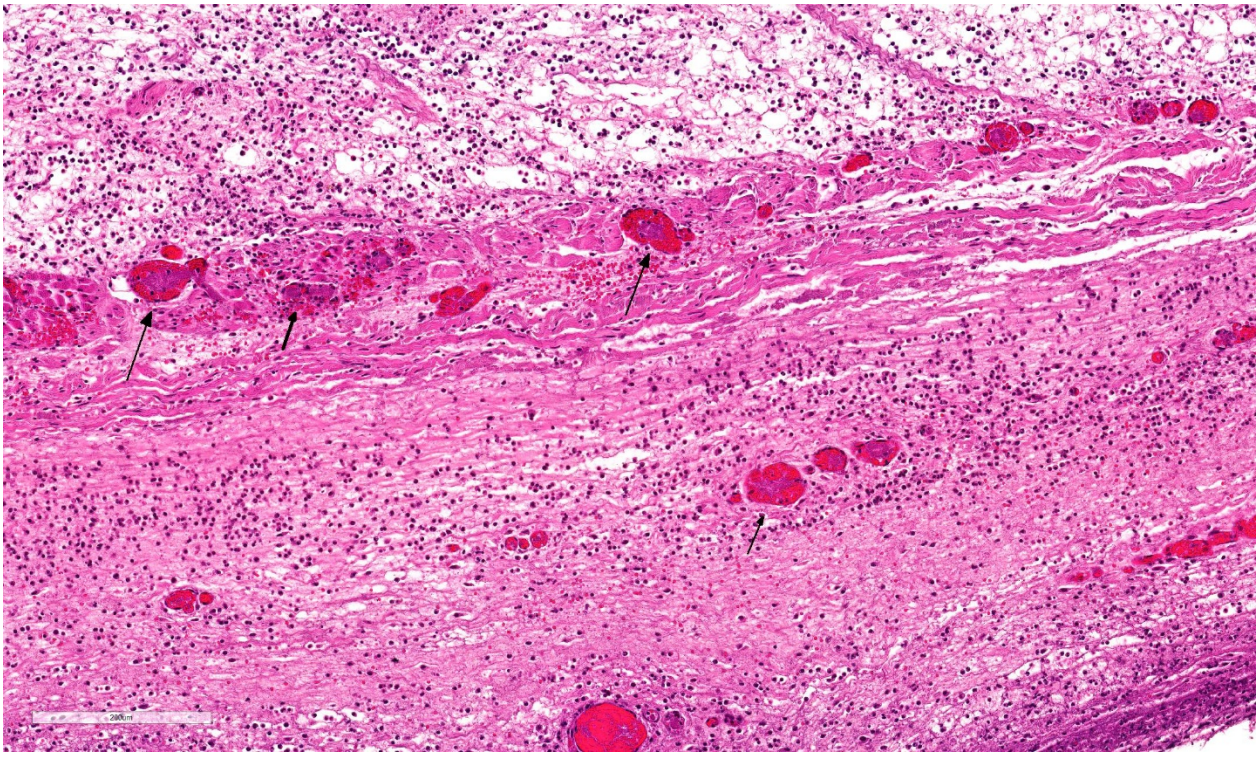
1. Haired skin: Dermatitis, cellulitis and panniculitis, necrotizing and suppurative, diffuse, acute, severe with gram-negative bacteria consistent with *Pasteurella multocida*.
2. Haired skin: Dermatitis, ulcerative and suppurative, multifocal, acute, severe with gram-positive bacteria.

Contributor's Comment: This case is consistent with a condition known as facial necrosis (facial pyemia),[2,8] a common condition in suckling pigs less than one week of age characterized by bilateral necrotic ulcers that are often covered by hard brown crusts and that extent from the side of the face to the lower jaw area, especially on the lips and cheeks.

The condition is the result of infection of wounds inflicted by piglets on each other with their sharp teeth while suckling from the sow. Lacerations to the sides of the face become infected with organisms such as *F. necrophorum*, *Streptococcus* spp., and *B. suis*, among others.[2,5]



Facial skin, piglet. The dermis and subcutis is markedly thickened by edema and hemorrhage, and vessels are surrounded by a cellular infiltrate. (HE, 6X)



Facial skin, piglet. The deep dermis, panniculus carnosus, and subcutis are markedly expanded by edema, fibrin, and innumerous viable and degenerate neutrophils. Blood vessels contain large colonies of bacilli (arrows). (HE, 155X).

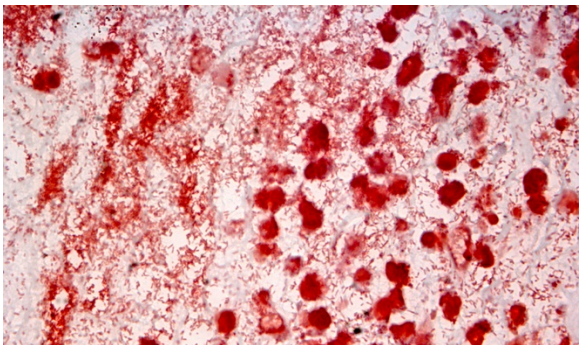
In this case, none of the above bacteria was cultured but *Pasterella multocida* was isolated in pure culture. This case represents an especially severe form of the disease, complicated by cellulitis and panniculitis. This may be associated with the effects of *Pasteurella multocida* dermonecrotic toxin.[12]

Facial necrosis is commonly seen in large litters and especially in the disadvantaged weaker piglets and when milk letdown is slow such as when sows suffer from mastitis, metritis, agalactia syndrome (MMA syndrome). In these cases there is an increase in competitition between piglets and lacerations and bites do occur.

Since 2013, there is a European legislation that regulates the shaving or trimming of teeth in suckling piglets. The aim of the regulation is to avoid teeth shaving as a general procedure, but it can be still considered in specific or individual litters when a facial necrosis situation suddenly occurs.

Facial necrosis occurs during the first few days of life and any number of piglets in a litter can be affected. Initially, lesions can be seen as striated lacerations caused by bites from other piglets. The lesions become infected, resulting in shallow ulcerations covered with hard browns crusts and can predispose to outbreaks of exudative epidermitis.

In this case there was a septicemic pasteurellosis since *Pasteurella multocida* was isolated from skin, liver and spleen. Septicemic pasteurellosis has a sudden onset with mortality ranging from 5% to 40%. Clinical signs include high fever, dyspnea, prostration, edema of the throat and lower



Facial skin, piglet. There are innumerable gram-negative bacilli within the tissue, admixed with degenerate neutrophils. (Photo courtesy of: Veterinary Pathology Department, Veterinary Faculty, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.) (Gram stain, 400X)

jaw and purplish discoloration of the abdomen, suggesting endotoxic shock.[5,10,12]

JPC Diagnosis: Haired skin and subcutis: Dermatitis and cellulitis, necrosuppurative, diffuse, severe with vasculitis, thrombosis, and numerous intravascular and extracellular bacilli, piglet (*Sus scrofa domesticus*), porcine.

Conference Comment: *Pasteurella multocida*, a gram-negative nonmotile cocco-bacillus, was first identified in 1878 as the causative agent of fowl cholera. It was isolated by Louis Pasteur in 1880, in whose honor it is named.[9] Strains of *P. multocida* are currently divided into five serogroups (A, B, D, E, F) based on capsular composition and somatic serovar.[6] *P. multocida* is a ubiquitous pathogen and is often part of the normal respiratory flora in mammals, but can cause a wide range of diseases such as: fowl cholera in poultry, atrophic rhinitis in pigs[12], pneumonia in various species, and bovine hemorrhagic septicemia in cattle and buffalo.[7] In humans, it is a common cause of zoonotic infections following animal bites or scratches.

As a gram-negative bacterium, *P. multocida* expresses the variable carbohydrate surface molecule, lipopolysaccharide (LPS) and a polysaccharide capsule which has been shown to aide in resistance to phagocytosis by host immune cells (serotypes A and B) as well as complement-mediated lysis (serotype A).[1,4] Strains that cause atrophic rhinitis (serotype D) also produce *P. multocida* toxin (PMT) which is a dermonecrotic toxin encoded on bacteriophages that initially stimulates osteoblasts. Then, as toxin levels increase, PMT blocks the function of osteoblasts and increases the activity of osteoclasts, leading to osteolysis of nasal turbinates.[12]

In a laboratory setting, *P. multocida* will grow at 37 °C on blood, chocolate, or high-strength agars, but will not grow on MacConkey agar. Bacterial growth is accompanied by a “mousy” odor due to metabolic products produced by the bacterium. Environmental endurance can be strengthened by adding salt.[3]

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CASE III: V165/17 (JPC 4102987).

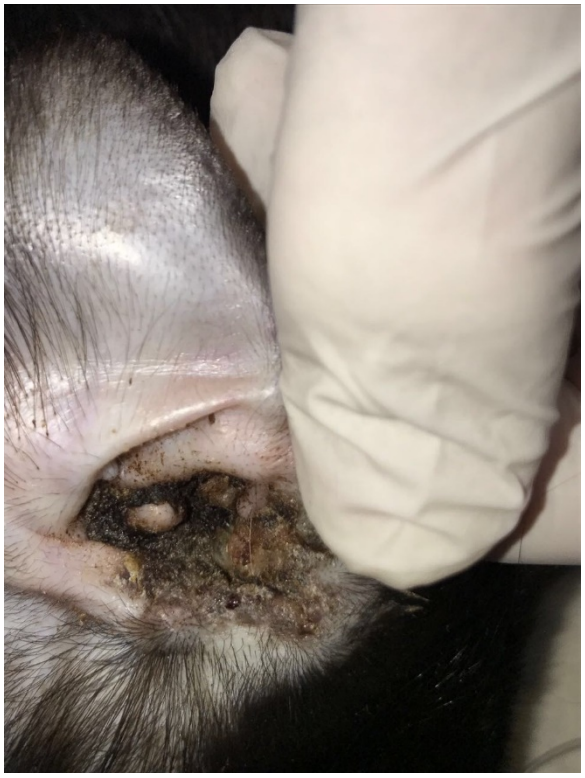
Signalment: 1.5-year-old, male, castrated, domestic shorthair (*Felis catus*), feline.

History: Plaques, erosions and erythema have been present bilaterally for one year on the concave pinna around the entrance to the ear canal. There has been no response to various treatments.

Gross Pathology: The inner aspect of the pinna is thickened by plaques covered by thick dark brown-black exudate which occludes the entrance to the ear canal.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.):
None provided.

Microscopic Description:
The skin is covered by a thick crust composed of keratin and proteinaceous debris, large collections of lytic granulocytes (neutrophils and eosinophils), RBC and scattered bacterial colonies. There is severe diffuse hyperplasia of the epidermis and outer root sheath with widespread spongiosis. Dead keratinocytes with shrunken, angular, hypereosinophilic cytoplasm and pyknotic nuclei are present predominantly in the hyperplastic outer root sheath. They occur either as individual cells or form small groups and are often found in the upper layers of the epidermis or outer root sheath. There is prominent luminal folliculitis characterized by pronounced infundibular dilation, parakeratotic hyperkeratosis and formation of coalescing eosinophilic and neutrophilic pustules. The debris in the follicular lumen is continuous with the superficial crust. Keratinocytes with pale cytoplasm form an irregular layer below



Pinna and vertical ear canal, cat: Plaques of a thick dark exudate largely occlude the vertical ear canal and spread outward over the inner surface of the pinna. (Photo courtesy of: Department of Veterinary Resources, Weizmann Institute of Science, Rehovot 76100, Israel, http://www.weizmann.ac.il/vet/)

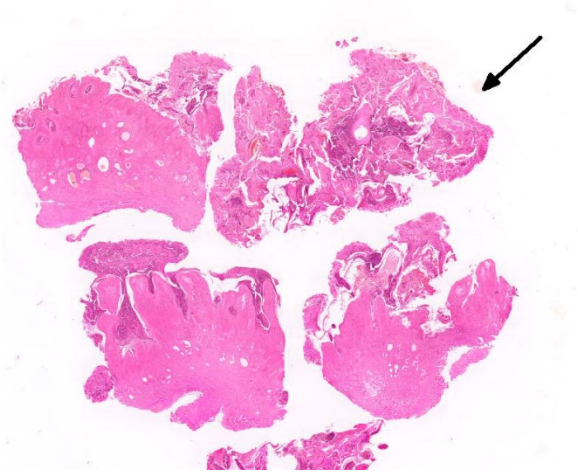
the disturbed stratum corneum of the most severely dilated hair follicle in this sample. A low number of eosinophils and probable lymphocytes migrate through the hyperplastic epidermis and outer root sheath. In the superficial and perifollicular dermis there is moderate to severe multifocal to coalescing infiltration of eosinophils, mast cells, neutrophils, a few lymphocytes, plasma cells and occasional macrophages with intracytoplasmic light brown granular pigment consistent with secretory material.

Contributor's Morphologic Diagnosis:
Severe eosinophilic and mastocytic proliferative, hyperkeratotic and necrotizing dermatitis and folliculitis

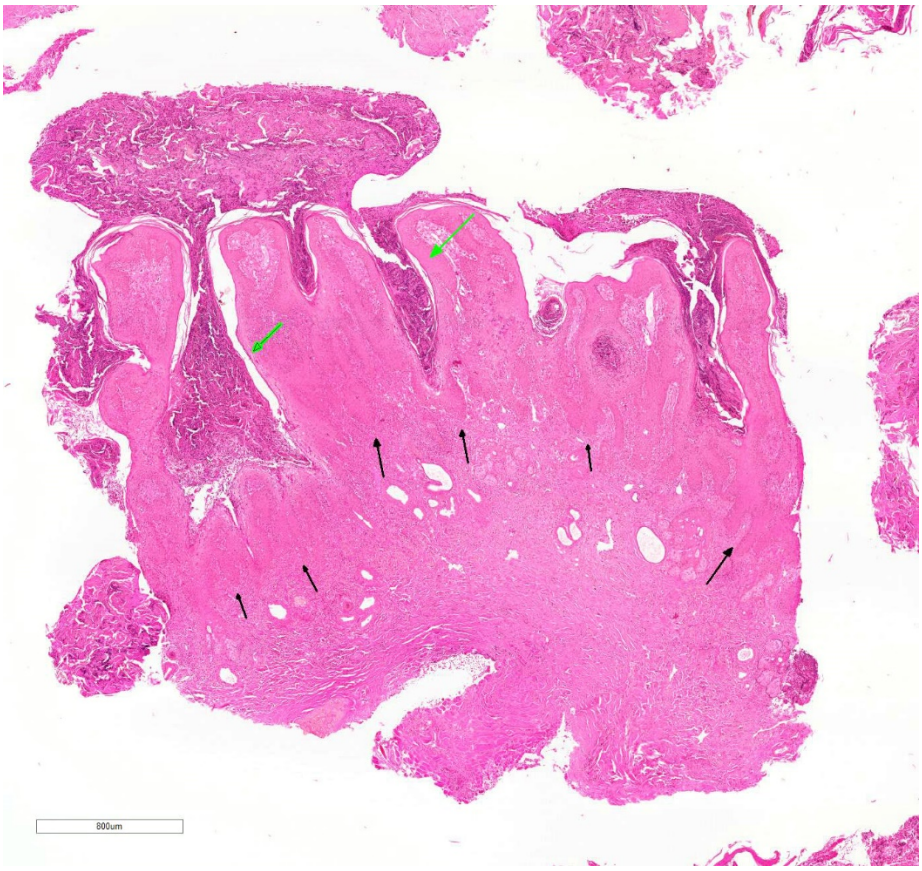
Contributor's Comment: This rare and distinctive skin disease of cats was first reported as "Proliferative necrotizing otitis of kittens" in the 2nd edition of Gross et. al..[1] It was initially reported to affect cats <1 year-old, but a later publication describes cases also in adult cats.[1,2]

Clinically, the disease presents as bilateral, sharply demarcated, thick brown crusts usually on the inner surface of the proximal pinna and around the entrance to the ear canal.[1,2,3] In some cases the vertical ear canal is involved.[2,3] The proliferative tissue is friable leading to erosion, ulceration and in some case occlusion of the ear canal.[1,2] The condition may regress spontaneously (by 1 to 2 years-old)[1] or persist for a long time (e.g. 4.5 years)[2]. In the initial report, cats were described as indifferent to the lesions or demonstrated evidence of only mild pruritus when ulceration is present.[1] More significant pruritus was noted in the cats of the 2nd report which had concurrent otitis due to bacterial or yeast infection.[2]

Gross et al. classify this condition as a necrotizing disease of the epidermis.



Vertical ear canal, cat. Multiple biopsies of ear pinna and overlying crust (black arrow) are submitted for examination. (HE, 7X)

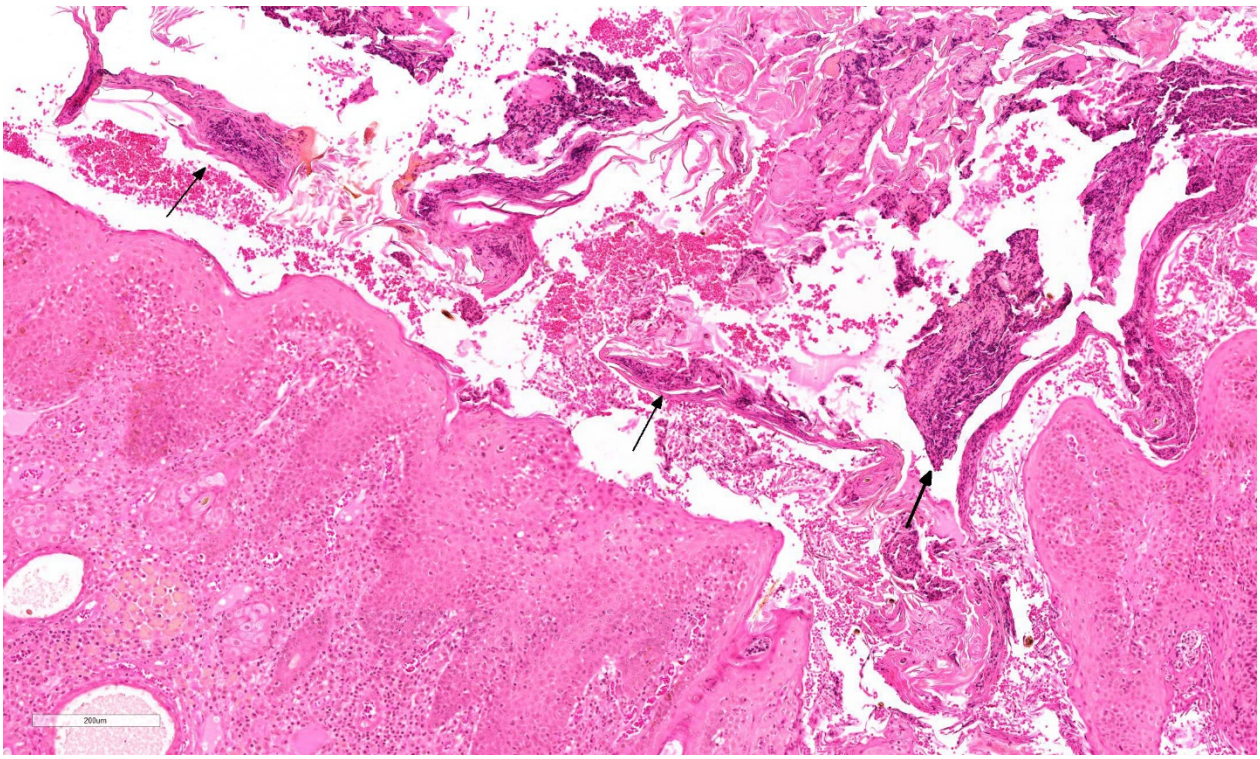


Vertical ear canal, cat. There is diffuse marked epithelial hyperplasia. The epidermis is covered by a thick serocellular crust which extends down into follicles (green arrows). (HE, 28X)

Diseases in this group are characterized by death of keratinocytes either by necrosis or apoptosis. Since, the morphologic features of dead keratinocytes do not allow distinction between the two processes, the convention is that apoptosis is considered to be the underlying mechanism when death affects individual keratinocytes (e.g. erythema multiforme) and necrosis when death affects many confluent cells (e.g. toxic epidermal necrolysis). To complicate matters, dyskeratotic cells are also indistinguishable from dead keratinocytes.[1] According to Mauldin et al. dead keratinocytes are limited to the outer root sheath and are not present in the interfollicular epidermis. The embedding of our samples is unfortunately oblique, but to the degree that we were able to pursue this point we tend to agree with Mauldin et. al. possibly with rare minor exceptions.

The cause of this condition is unknown. The possibility of viral infection was investigated by PCR and IHC but to date no evidence of viral involvement has been found.[1,2] The two main references for this condition describe keratinocytes with abundant pale eosinophilic cytoplasm[1,2], a finding which is present to a limited degree in the submitted sample (around the most dilated hair follicle). However, we did not identify enlarged nuclei with marginated chromatin without inclusion bodies.[2]

Individual keratinocyte death, presumably by apoptosis, suggests an immunologic basis[1], and this is supported by the good response to treatment with Tacrolimus® (FK -506), an inhibitor of T-cell-mediated cytokines.[2,5] Immunohistochemical analysis done on one case showed a close association between CD3+T cells and caspase-3-stained keratinocytes, which suggests that



Vertical ear canal, cat. The serocellular crust contains numerous pustules (black arrows). The underlying epithelium is markedly hyperplastic and there is a profound neutrophilic infiltrate of the superficial dermis. (HE, 111X)

keratinocyte apoptosis is induced by epidermal infiltrating T cells, but the reason this occurs is unknown.[5]

JPC Diagnosis: Haired skin (external ear canal): Otitis externa, proliferative and hyperkeratotic with luminal folliculitis and keratinocyte apoptosis, domestic shorthair (*Felis catus*), feline.

Conference Comment: Proliferative and necrotizing otitis of kittens is a rare disorder that was first described in a 2007 academic journal by the moderator, Dr. Elizabeth Mauldin[2], which characteristically presents as well-demarcated, erythematous plaques with adherent keratinaceous debris on the inner aspect of the pinna extending into the pre-auricular regions of the face. Although the gross appearance is unique and largely diagnostic, lesions can be easily confirmed via biopsy. Microscopically, the lesions are characterized by a markedly hyperplastic epidermis with brightly eosinophilic, shrunken keratinocytes (apoptotic or dyskeratotic) scattered subjacent to areas of prominent parakeratotic hyperkeratosis that contains layers of viable and degenerate neutrophils. Adjacent hair follicles may also be involved and are often plugged with keratinaceous debris and inflammatory cells.[1,4]

Histologically, this lesion is similar to hyperkeratotic erythema multiforme (EM), with several distinct differences: (1) affected keratinocytes are not routinely surrounded by lymphocytes (ie. satellitosis) and are only present in areas with prominent hyperkeratosis (suggesting that they may be dyskeratotic rather than apoptotic); and (2) there is minimal to mild epithelial hyperplasia and neutrophilic crusting, as

evident with proliferative and necrotizing otitis. .[1,4]

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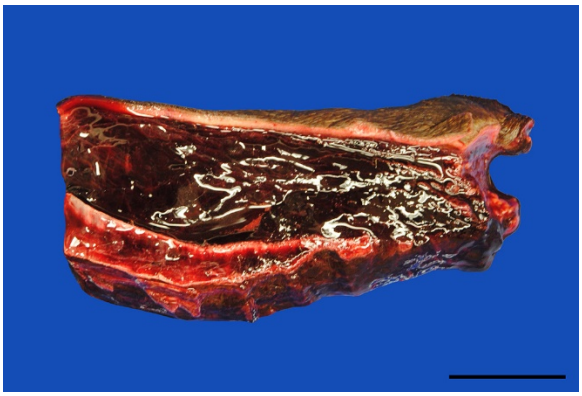
CASE IV: S 504/16 (JPC 4102124).

Signalment: 6-year-old, female, Warmblood (*Equus ferus caballus*), equine.

History: Severe, subcutaneous edema affected all four limbs, the ventral abdomen, and the head. Multifocal petechial and ecchymotic hemorrhages were visible on all mucous membranes including the mouth and the vagina. The rectal temperature was 39.7°C (reference interval 37,0 – 38,0°C) and white blood cell count was elevated (15.7 x 109/l, reference value 5 – 10 x 109/l). The horse was treated with antibiotics, flunixin (nonsteroidal anti-inflammatory drug) and high dosages of dexamethasone. The horse rapidly deteriorated and was humanely euthanized upon the owner's request.



Haired skin and subcutis, horse. The subcutis is markedly expanded by edema and hemorrhage. (Photo courtesy of: Department of Veterinary Pathology, Freie Universitaet Berlin, http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html)

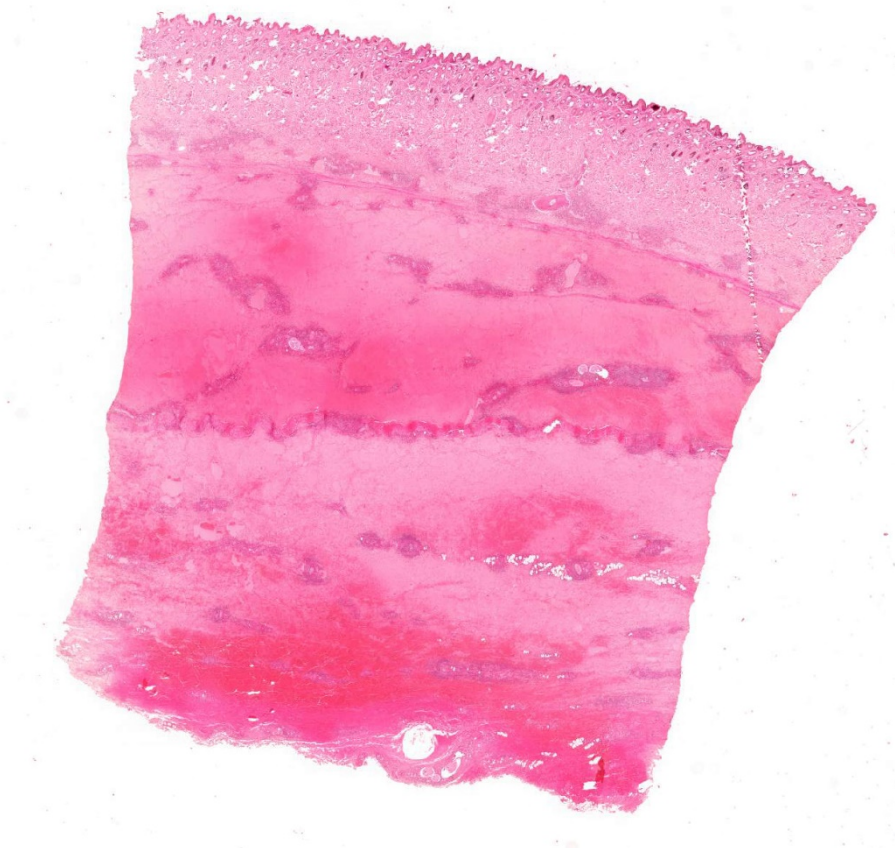
Gross Pathology: In addition to the mucous membranes, petechial to ecchymotic hemorrhages were striking in the parietal and visceral pleura, the lung, myo- and pericardium as well as the mediastinum. Small proportions of pus drained from the cut surface of the right retropharyngeal lymph node. The subcutis of the ventral body parts including the legs, ventral abdomen and scrotum was severely thickened up to 5 cm by edema.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.):
Streptococcus equi ssp. equi was isolated from the right retropharyngeal lymph node.

Microscopic Description:
Skin: The subcutis is severely thickened by a mainly homogenous and partially fibrillary pale eosinophilic material, clear space (edema) and extravascular erythrocytes (acute hemorrhages). Surrounding blood vessels, high numbers of leukocytes are present, predominantly degenerate

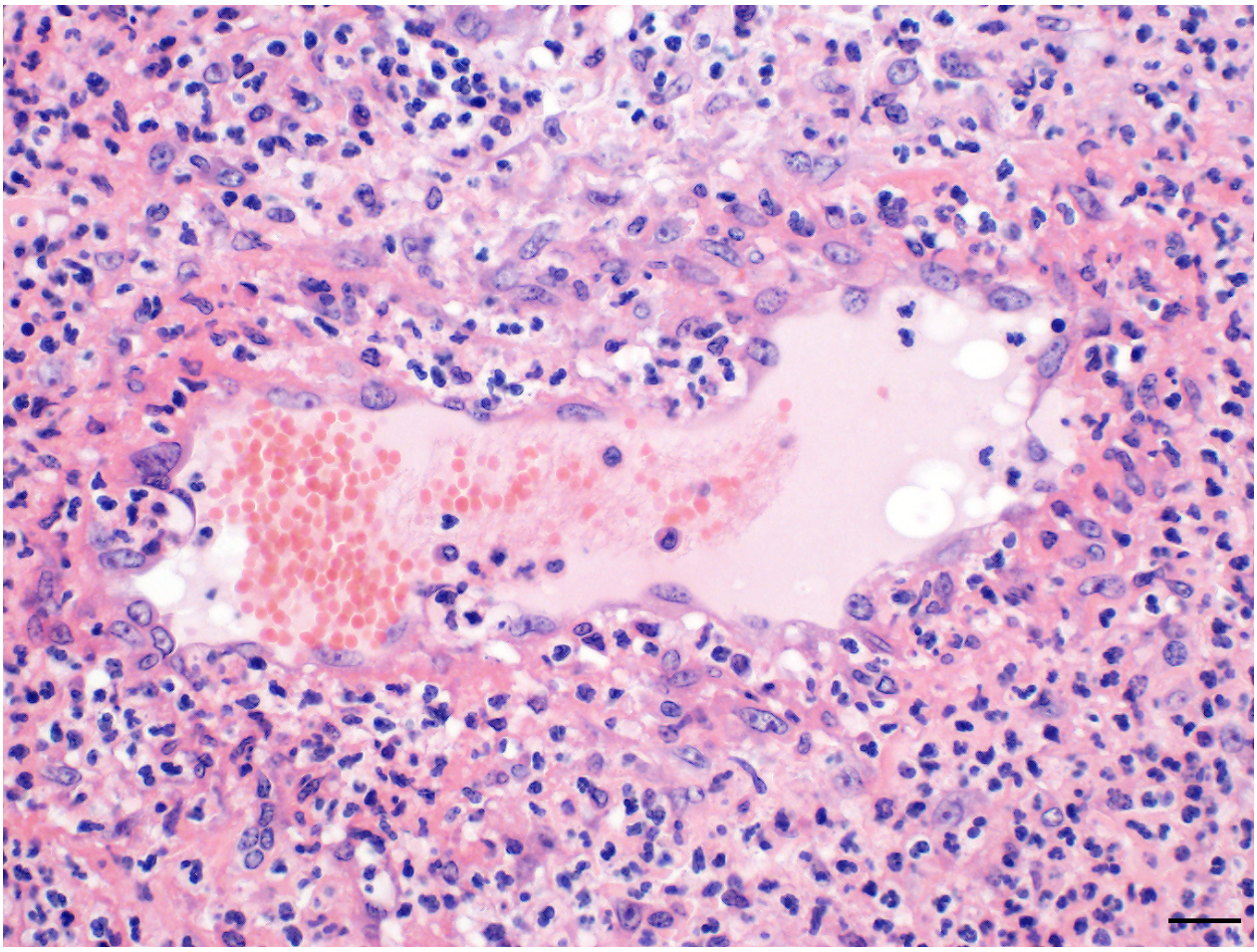
neutrophils and fewer numbers of macrophages. Almost all blood vessels are multifocally disrupted and expanded by moderate deposition of a loosely arranged eosinophilic homogenous to fibrillar material (fibrin) admixed with few degenerate neutrophils and cellular and karyorrhectic debris (fibrinoid necrosis). The endothelial lining is partially lost or endothelium cell nuclei are prominent and oval in shape (endothelial activation). The fibrinoid necrosis of the blood vessels and the perivascular infiltration expand into the surrounding dermis. The epidermis is normal.

Contributor's Morphologic Diagnosis:
Skin, subcutis: Vasculitis, severe, acute, diffuse, leukocytoclastic with severe edema, multifocal hemorrhage and dermal necrosis.



Haired skin and subcutis, horse. Subgross examination reveals the extent of the hemorrhage and edema of the subcutis. There is a markedly cellular infiltrate surrounding all vessels. (HE, 5X)

Contributor's Comment: The syndrome purpura hemorrhagica, formerly known as *morbus maculosus equorum*, is consistent with a generalized leukocytoclastic vasculitis almost always including the skin.[14] It is clinically characterized by subcutaneous edema of ventral body parts and petechial or ecchymotic hemorrhages in the visible mucous membranes, i.e mouth and vagina. Underlying hemorrhagic diathesis is caused by endothelial and / or vascular damage in context of a generalized vasculitis. The latter is considered to result from a type III hypersensitivity with deposition of antigen-antibody immune-complexes in blood vessel walls. Activation of complement components and a subsequent recruitment and activation of leukocytes is responsible for the damage of the blood vessels. Considering the pathogenesis, the syndrome is not primarily caused by a specific infectious agent and hence not transmissible. However, in most cases, a previous infection provokes the hypersensitivity reaction. Most commonly, as in the current case, a preceding infection with *Streptococcus equi* spp. *equi* (Strangles) can be referred as the initiating cause of purpura hemorrhagica. It has been detected in approximately 5.4 to 6.5% of the infected horses, typically 2 to 4 weeks post infection.[4,14] Although the pathogenesis of *Streptococcus equi* spp.



Haired skin and subcutis, horse. Vessel walls are expanded by numerous neutrophils, cellular debris, and fibrin (vasculitis) and numerous viable neutrophils have migrated into the surrounding tissue. (HE, 400X) (Photo courtesy of: Department of Veterinary Pathology, Freie Universitaet Berlin, http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html)

equi associated purpura hemorrhagica is not fully understood, high serum concentrations of IgA antibodies and low serum concentrations of IgG antibodies seem to favor the development of immune complexes on the basis of the M-like protein of *Streptococcus equi* (SeM).[14] Vaccination with SeM may trigger purpura hemorrhagica.[6] Furthermore, additional infectious agents are discussed as possible cause of this syndrome including viruses (equine influenza virus, equine herpes viruses, equine arteritis virus) and bacteria (*Corynebacterium pseudo-tuberculosis*, *Streptococcus equi* spp. *zooepidemicus*, *Rhodococcus equi*).[2,11,16]

Purpura hemorrhagica, i.e, a leukocytoclastic vasculitis, may be diagnosed antemortem by full-thickness punch skin biopsies.[11,16] However, biopsies should be taken prior to treatment with corticosteroids as these may tremendously reduce the severity of the vasculitis and even suppress any histological evidence whatsoever.[11]

The prognosis of purpura hemorrhagica is difficult to predict, however may be favorable with an immediate and aggressive therapy including immunosuppression with corticosteroids.[6] Complications like laryngeal edema (dyspnea), dermal necrosis,

thrombophlebitis, glomerulonephritis, and infarctions of skeletal musculature, lung, skin and the intestinal tract as well as colic due to severe edema, gastric rupture, intestinal intussusception, torsion and prolapse recti, may occur.[5,8,9,13,14]

JPC Diagnosis: Haired skin and dermis: Vasculitis, necrotizing, diffuse, severe with marked hemorrhage, edema, and moderate neutrophilic dermatitis and cellulitis, Warmblood (*Equus ferus caballus*), equine.

Conference Comment: Purpura hemorrhagica (from the Latin meaning "purple") is a type of immune-mediated vasculitis characterized grossly as red or purple areas of hemorrhage of the skin and mucus membranes with subsequent edema. Microscopically, affected vessels are surrounded by neutrophils that cause destruction of vascular walls (leuko-cytoclastic vasculitis), perivascular edema, hemorrhage, and fibrin. Commonly associated with previous infection with *Streptococcus equi* (usually involving the respiratory tract with abscessation), *Corynebacteirum pseudotuberculosis*, or vaccination with *S. equi* M protein (SeM); purpura hemorrhagica is classified as a type III hypersensitivity reaction and is caused by immune complexes (antigen and immuno-globulins) that deposit on the walls of small blood vessels (resulting in vasculitis) or renal glomerular capillaries and vessels (resulting in glomerulonephritis).[6,7,12]

Clinically, edema is well-demarcated and most often noticed in the distal limbs, followed by the ventrum and head with fever, tachycardia, tachypnea, anorexia, and depression. Clinical signs vary based on the organ system involved and can include all of the following: colic, lameness, neurologic signs, renal dysfunction, small intestinal intussusceptions, and muscular infarcts. Clinical pathology abnormalities include: leukopenia or leukocytosis, anemia, thrombocytopenia, hypergamma-globulinemia, increased acute phase proteins, and increased muscle enzyme activities. Diagnosis is based on a combination of history, clinical signs, antibody titers (very high Se-M specific titers), and skin biopsies (to confirm leukocytoclastic vasculitis). Treatment is based on supportive care, immune suppression, and removal of the underlying cause.[6,12]

Differentials for purpura hemorrhagica include endotheliotropic viruses such as: equine viral arteritis, African horse sickness (*Orbivirus*), Hendra virus, or equine herpesvirus-1. Equine viral arteritis virus, an *Arterivirus*, is transmitted in contaminated body fluids via venereal or respiratory routes. The virus targets mononuclear and endothelial cells; clinical signs are usually associated with vasculitis. Clinical signs include: fever, leukopenia, depression, periorbital and supraorbital edema, conjunctivitis, lacrimation, petechiae, respiratory signs, colic or diarrhea, and abortion.[6] African horse sickness (AHS) is a foreign animal disease (never currently been reported in the United States) that results in edema, predominately of the nuchal ligament and supraorbital fossa, pulmonary edema, and myocardial failure. Equine *Orbivirus* is transmitted by *Culicoides imicola* and is endotheliotropic (related to the causative agent of bluetongue). In Africa, all equids are susceptible, with horses being the most susceptible, followed by mules, donkeys, and zebras. Hendra virus causes fatal pneumonia, encephalitis, and rarely subcutaneous edema in horses and humans (zoonotic).[3] Finally, equine herpesvirus-1, though most often associated with the central nervous system, is also an endotheliotropic virus and with chronicity rarely causes petechial

hemorrhage and edema in cutaneous and subcutaneous tissues.[1]

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