Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2012-2013

Conference 7

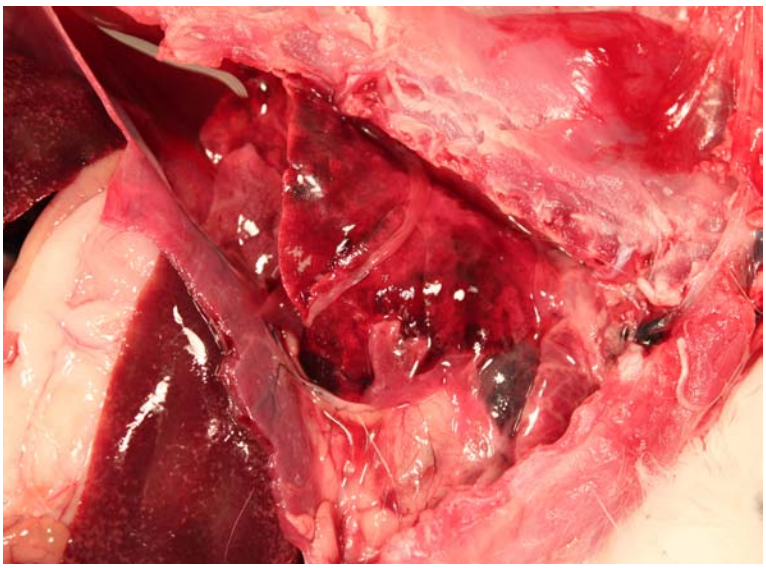
7 November 2012

CASE I: 12-670 (JPC 4019866).

Signalment: Young adult, intact female New Zealand White rabbit (*Oryctolagus cuniculus*).

History: Found dead in the morning 3 days after arrival at facility. Feces and urine were present in the cage pan; food provided the day before had not been eaten. A jugular catheter had been placed by the vendor 1 week prior to arrival. There had been no subsequent experimental manipulation.

Gross Pathology: The fur around the neck and cranial thorax is shaved. Within the right jugular vein is an indwelling catheter that tunnels subcutaneously to be secured to the overlying skin of the dorsal right neck. Adjacent to the catheter, midway along the neck, the wall of the right jugular vein is thickened with a yellow/white 2 x 2 x 3 mm, firm focal swelling.



1-1. Thoracic cavity, rabbit: The pleural cavity contains approximately 10mL of cloudy red-orange fluid. Dozens of 1-3mm thick strands and large coalescing mats of fibrin bilaterally cover the cranial lung lobes. Bilaterally, the cranioventral 15% of the lungs is dark red and consolidated. The remainder of the lung is mottled dark red. (Photograph courtesy of Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10065)

The pericardium of the heart is diffusely thick (up to 3mm wide), discolored red-tan, and rough. Approximately 1mL of yellow-red, opaque pericardial fluid is present. The epicardial surface of the heart is rough and covered with multifocal strands of gelatinous to stringy, tan material (fibrin). The endocardial surface of the heart and heart valves are grossly within normal limits.

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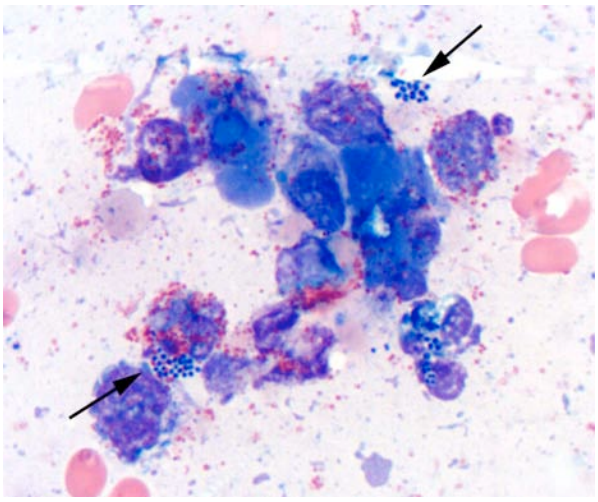
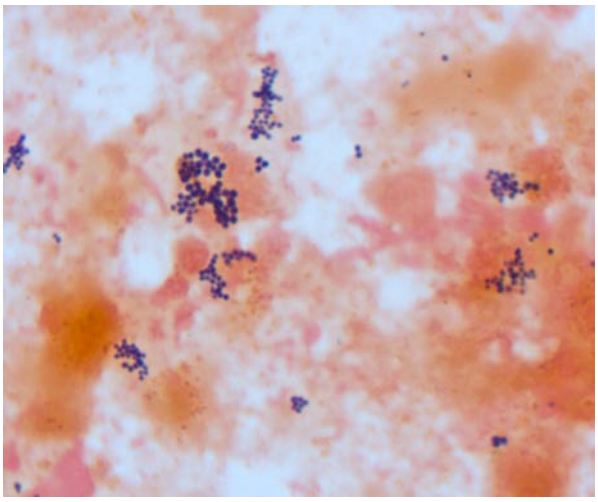
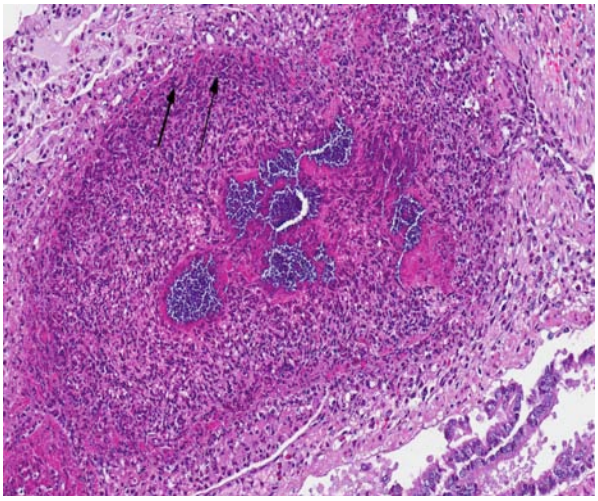


1-2. Pericardial fluid: Cytology of pericardial fluid revealed abundant degenerate heterophils with myriad intra- and extra-cellular Gram positive cocci in clusters. (Photograph courtesy of Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10065)



1-3. Pericardial fluid, Gram stain: Heterophils contain numerous intracellular, gram positive cocci (arrow). (Photograph courtesy of Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10065)



1-4. Lung, rabbit: Multifocally, arteriolar walls are necrotic and contain large numbers of heterophils admixed with fibrin, hemorrhage, and cellular debris (fibrinoid necrosis) (arrows). Occlusive thrombi within the arteriolar lumen is composed of fibrin, degenerate heterophils, and abundant cellular debris and are centered upon large colonies of cocci. (HE 210X) (Photograph courtesy of Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10065)

Multifocally throughout the liver are hundreds of coalescing 1-2mm pale tan foci.

Laboratory Results: Cytology of pericardial fluid revealed abundant degenerate heterophils with myriad intra- and extra-cellular Gram-positive cocci in clusters. Microbial culture of the pericardial fluid, pleural fluid, lung, and liver grew *Staphylococcus aureus*.

Histopathologic Description: Multifocally within the lungs, many small, medium and large pulmonary vessels have a lumen that is occluded by large numbers of degenerate heterophils and moderate amounts of fibrin and cell debris, often with centrally located colonies of large coccoid bacteria. Transmurally, the vascular wall is hypereosinophilic with loss of cellular detail (fibrinoid necrosis), and moderate numbers of heterophils extend into the perivascular tissue and adjacent alveolar spaces. Within some sections of lung there are locally extensive areas of coagulative necrosis and hemorrhage that are often adjacent to a thrombosed vessel. Degenerate heterophils are often present at the margin of necrotic and viable tissue with proliferation of coccoid bacterial colonies within areas of necrosis. Moderate numbers of heterophils and few megakaryocytes are present multifocally within the interstitium. Alveolar spaces multifocally contain large amounts of eosinophilic foamy material and mild hemorrhage, and the alveolar capillaries are markedly congested. Multifocally (not present on every slide), the pleural surface of the lung is necrotic and expanded by moderate amounts of fibrin and degenerate heterophilic cellular debris.

Contributor's Morphologic Diagnosis: Lung: Marked, acute, multifocal, heterophilic and necrotizing pulmonary thrombosis, vascular fibrinoid necrosis and heterophilic, necrotizing bronchointerstitial pleuropneumonia with intralesional coccoid bacteria and marked, multifocal pulmonary congestion and edema.

Contributor's Comment: This rabbit had an intravenous catheter inserted into the right jugular vein by the vendor, approximately one-week prior to arrival at the research facility. The unexpected death of this otherwise unmanipulated rabbit was attributed to catheter-related septicemia. *Staphylococcus aureus* was isolated from the lung, liver, pericardial fluid, and pleural fluid. Large coccoid bacteria were also

visualized histologically within the large fibrinosuppurative thrombus in the right jugular vein, near the point of catheter entry. It is hypothesized that infection was initiated at either the catheter exit point in the skin or from an infection of the catheter hub, which tracked down the catheter to the lumen of the vein. Embolism of the thrombus within the jugular vein would result in lodgment of the emboli in the next vascular bed, in this case, the lungs. This is consistent with the vasculocentric distribution of the fibrinosuppurative and necrotizing embolic lesions in lungs of this rabbit.

Vascular catheters are commonly used indwelling medical devices in both animals and humans. In a research setting, they are frequently used in laboratory animals to provide convenient vascular access for repeated injections with less restraint and handling stress on the animal. However, catheterization can be associated with many complications, the most serious being catheter-related septicemia.[1] Although catheter-related complications in humans are widely reported, there are very few reports of cases in animals.

Microbes that colonize catheter hubs and the skin surrounding the insertion site are the most common source of catheter-related infections.[2] Migration of skin organisms into the cutaneous catheter tract with subsequent colonization of the catheter tip is the most common route in humans.[2] Infection of the catheter hub, hematogenous seeding from a distant site of infection, or contaminated infusate are other sources of catheter-related infection.[1,2,3] Bacteria, including *Staphylococcus epidermidis*, *S. aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterococcus faecalis*, and the *Candida* spp. of fungi are commonly involved in human catheter-related infections.[1,2]

Studies have shown that virtually all indwelling central venous catheters in people are colonized by microorganisms embedded within a biofilm matrix.[2] The biofilm consists of both host and microbe-associated components. As the host reacts to the catheter, a thrombin sleeve rich in fibrin and fibronectin coats the catheter. Microbes may also produce an extracellular polysaccharide 'slime'. Together, these form the biofilm which enhances adherence of organisms and also protects organisms from phagocytosis, antibiotics and antibodies.[1,2]

The presence of a catheter within a vessel lumen can result in endothelial injury as well as alterations in normal blood flow around the catheter, both of which predispose to thrombosis.[4] There are four possible sequelae to thrombus formation – propagation, embolization, dissolution, or organization and recanalization.[4] In this rabbit, multiple emboli showered the pulmonary vessels of the lungs resulting in acute death.

JPC Diagnosis: Lung: Pneumonia, heterophilic and necrotizing, diffuse, severe, with necrotizing vasculitis, thrombosis, and large colonies of intraalveolar and intravascular cocci.

Conference Comment: Conference participants discussed the aspects of intravenous catheters that contribute to thrombosis, as accurately described by the contributor. Three primary abnormalities, referred to as "Virchow's triad", lead to thrombus formation: 1) endothelial injury, 2) alterations in blood flow (stasis or turbulence), and 3) hypercoagulability of blood.[4] Of these, endothelial injury is the most important factor, and damaged endothelium can affect the other aspects of the triad; specifically, it can lead to abnormal blood flow and increased coagulability. Under normal conditions, intact endothelial cells prevent thrombosis by a combination of antiplatelet, anticoagulant and profibrinolytic effects. Antiplatelet properties of endothelial cells include the production of prostacyclin and nitric oxide, which inhibit platelet adhesion and promote vasodilation. Endothelial cells also produce adenosine diphosphatase which degrades ADP, an effective activator of platelet aggregation. Membrane-associated heparin-like molecules, thrombomodulin, and tissue factor pathway inhibitor mediate the anticoagulant properties of normal endothelium. Heparin-like molecules are cofactors in the activation of antithrombin III, which in turn enhances the inactivation of thrombin and other serine protease coagulation factors (factors IXa, Xa, Xia, and XIIa). Thrombomodulin converts thrombin to an anticoagulant which activates protein C, resulting in the inactivation of clotting factors Va and VIIIa. Tissue factor pathway inhibitor further impedes the coagulation cascade by inhibiting tissue factor-VIIa and factor Xa. Additionally, normal endothelial cells produce tissue type plasminogen activator (t-PA). t-PA cleaves plasminogen to form plasmin; plasmin then cleaves fibrin to degrade thrombi.

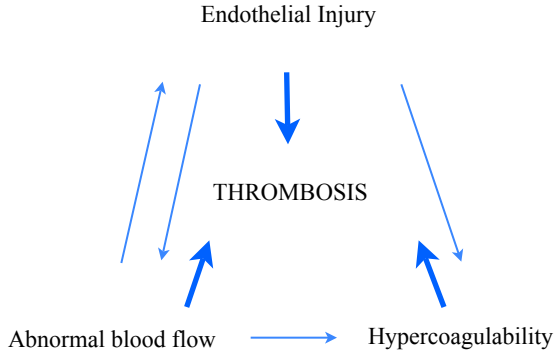
In contrast to these antithrombotic properties of normal endothelium, injured or activated endothelium induces thrombosis by inducing platelet binding and aggregation, activating the extrinsic coagulation cascade and enhancing the activation of clotting factors IX and Xa. Endothelial cells can by activated by trauma, infectious agents, hemodynamic forces, circulating plasma mediators, and cytokines. Endothelial injury results in exposure of the underlying extracullular matrix and subsequent adhesion of platelets to it via interactions with von Willebrand factor (vWF). vWF, which is secreted by endothelial cells, acts as a bridge between the ECM and the glycoprotein Ib receptor on platelets, allowing the formation of the primary hemostatic plug. Endothelial cells also produce tissue factor (factor III, thromboplastin), the primary activator of the extrinsic pathway of the coagulation cascade that results in

secondary hemostasis. Finally, endothelial cells secrete antifibrinolytic substances such as inhibitors of plasminogen activator s (PAIs) which restrict fibrinolysis.[4]

The second factor in Virchow's triad, abnormal blood flow (turbulence or stasis), is characterized by a disruption in the normal laminar flow of blood in which cellular elements flow centrally in the vessel, separated from the endothelium by a layer of plasma. Disturbance in this laminar flow promotes endothelial activation, enhances coagulation, brings platelets into contact with the endothelium, and prevents washout and dilution of activated clotting factors, all of which contributes to thrombosis.[4]

The third component of Virchow's triad, hypercoagulability, can be heightened by inflammation, stress, surgery, neoplasia, pregnancy and renal disease (i.e. the loss of antithrombin III in the nephrotic syndrome). Inflammation is the most common cause of hypercoagulability, resulting in a prothrombotic state due to increased amounts of tissue factor, increased platelet reactivity, increased fibrinogen, and decreased thrombomodulin.[4,5] Stress and tissue necrosis (trauma, surgery) can cause transient increases in fibrinognen. Lastly, conditions that increase platelet activation (e.g. heartworm disease, nephrotic syndrome, and neoplasia) can also contribute to increased blood hypercoagulability and thus predispose an individual to thrombus formation.[5]

Virchow's Triad in Thrombosis*:



**adapted from Robbins and Cotran Pathologic Basis of Disease, 8th ed.*

Contributing Institution: Memorial Sloan-Kettering Cancer Center
1275 York Ave
New York, NY 10065

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CASE II: 51529 (JPC 3164931).

Signalment: Adult, male, naked mole rat (*Heterocephalus glaber*).

History: This naked mole rat was one of several that developed a slow-growing subcutaneous mass at a rabies vaccine injection site. Rabies vaccines were given as part of international pre-shipment requirements. Due to the size of the masses and the presence of more diffuse, crusted skin lesions over much of the body, two of the naked mole rats were euthanized. Other affected mole rats with only subcutaneous masses were treated with surgical excision of the masses. All tissues were submitted for histologic examination.

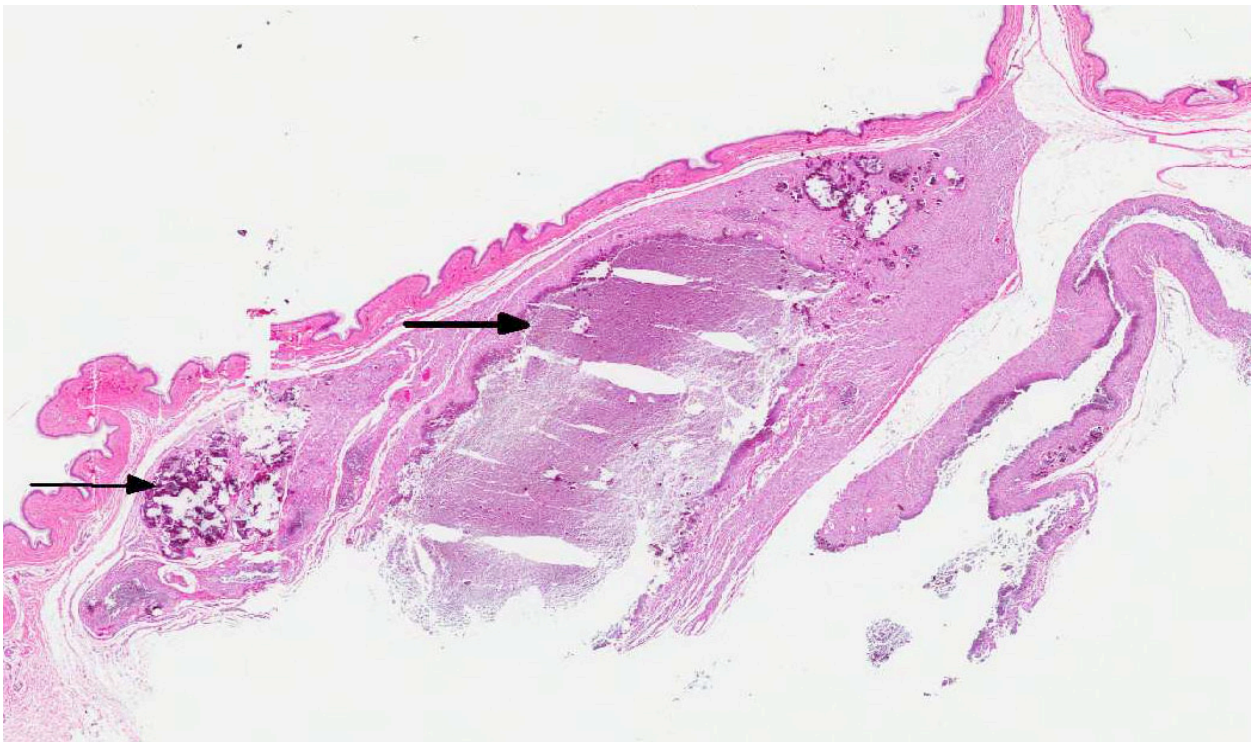
Gross Pathologic Findings: Gross and histologic findings are similar in both euthanized animals. At the base of the neck, within the right, dorsal cervical subcutis is a 2.0 x 1.5 x 1.5 cm, soft mass filled with thick white fluid. On a Wright-Giemsa stained smear, the fluid consists of acellular colorless to pale blue, refractile granular material that is birefringent when viewed with polarized light. The skin over approximately 90% of the ventral thoracic and abdominal body wall is diffusely dark yellow, thickened and rough. Body condition is good with moderate adipose stores and adequate skeletal muscling.

Histopathologic Description: Within multiple sections of haired skin, the integument contains moderate to large amounts of deeply basophilic, fragmented material that stains positive for mineral with a von Kossa stain. In the deep dermis, there are large lakes of mineral surrounded by a variably thick rim of macrophages and multinucleated giant cells (granuloma). In the surrounding dermis there are small numbers of neutrophils and peripheral follicular accumulations of lymphocytes. In the superficial dermis, mineral and granulomatous inflammation is more diffuse and there is mineralization of individual collagen fibers. The overlying epidermis is multifocally mineralized, necrotic, and/or ulcerated. There is mild orthokeratotic hyperkeratosis. A Ziehl-Neelsen acid-fast stain is negative for bacteria.

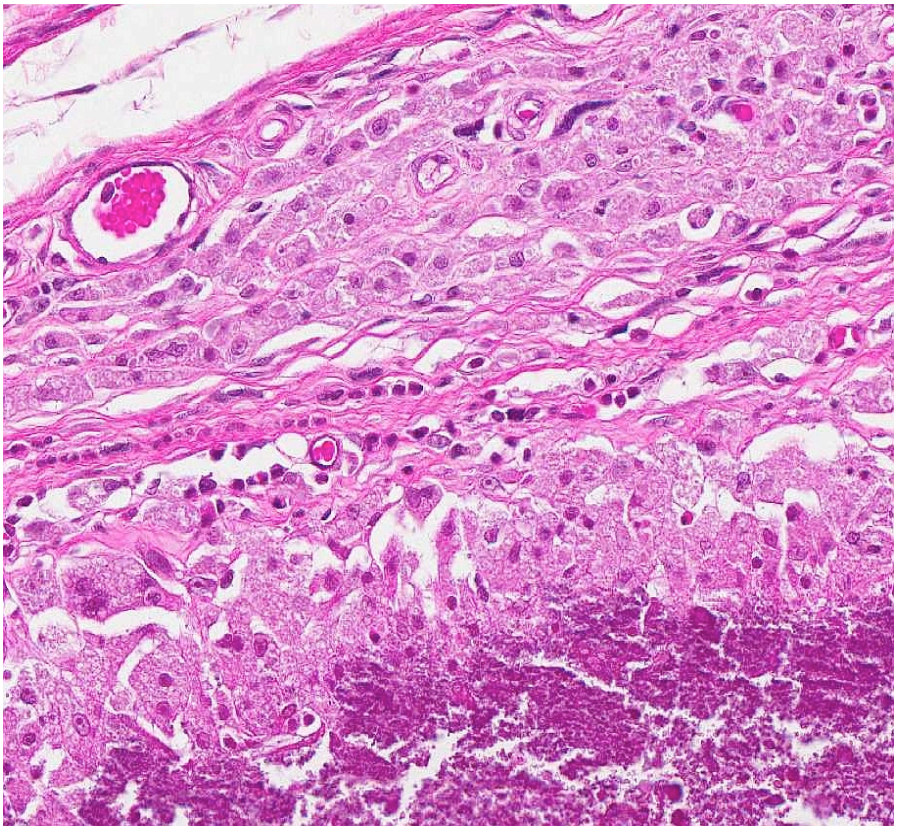
In each of the excisional biopsy samples there is a large dermal granuloma with central mineral as described in the preceding paragraph.

Contributor's Morphologic Diagnoses: Haired skin, neck: Multiple dermal granulomas with intralesional mineral (calcinosis circumscripta).

Haired skin, ventral body wall: Moderate to severe, multifocal dermal mineralization (calcinosis cutis) with granulomatous dermatitis, epidermal necrosis, ulceration, mild orthokeratotic hyperkeratosis and serocellular surface crusts.



2-1. Skin, naked mole rat: Multifocally, the deep dermis and subcutis are expanded by multiple granulomas centered on granular and crystalline mineral (arrows). (HE 11X)



2-2. Skin, naked mole rat: Within the granulomas, large numbers of polygonal epithelioid macrophages border lakes of granular mineral. (HE 228X)

Contributor's Comment: Deposition of calcium salts in soft tissues has a variety of underlying causes which are broadly classified as dystrophic or metastatic based on the mechanism. Dystrophic mineralization is the result of local tissue damage. Causes may include trauma, infection and neoplastic disease. The mechanism of dystrophic mineralization involves increased intracellular calcium secondary to release of calcium from damaged mitochondria and accumulation of extracellular calcium through vesicular phospholipid-binding of calcium and phosphate. Metastatic calcification, in contrast, is a response to hypercalcemia. Underlying causes include parathyroid hormone release (associated with renal disease or parathyroid tumors), parathyroid-like hormone, damage to bone, vitamin D toxicity and increased calcium intake. All of these mechanisms ultimately raise serum calcium levels leading to precipitation of mineral into otherwise normal soft tissue. Mineralization tends to occur in tissues that lose acid such as the lung, kidney and stomach.[7]

Calcinosis cutis and calcinosis circumscripta are characterized by ectopic cutaneous and subcutaneous deposition of calcium salts and may be part of a metastatic or dystrophic process, though in domestic mammals the lesions are often idiopathic.[10] Calcinosis cutis refers to lesions in which there is more diffuse involvement of collagen and adnexa while the diagnosis of calcinosis circumscripta is reserved for circumscribed mass-like lesions (also called tumoral calcinosis).[5] Interestingly, the lesions in these naked mole rats appear to have features of both. The larger mass at the neck and shoulder is a nodular accumulation of mineral with secondary granulomatous inflammation, consistent with calcinosis circumscripta. Dermal lesions along the body wall are more diffuse and characteristic of calcinosis cutis.

In domestic animals, calcinosis cutis is most often associated with either iatrogenic or Cushing's disease-related hyperglucocorticoidism. In domestic dogs, calcinosis circumscripta has been reported in the footpads in association with renal failure. Lesions also occur in the tongue, possibly secondary to local trauma. Additionally there is an uncommon form of idiopathic cutaneous calcinosis circumscripta that seems to occur mainly in large breed dogs, particularly of the German Shepherd dogs. In these cases, dogs often have normal serum calcium and phosphorus levels, a lack of identifiable nutritional deficiency or excess and lack of known pre-existing trauma to the affected areas.[10]

As is frequently the case in domestic mammals, the cause of the lesions in these naked mole rats is poorly understood. There is a strong temporal and spatial association between rabies vaccination and the tumoral calcinosis, but this may not explain the calcinosis cutis–like lesions along the body wall. Skin trauma in naked mole rats does not typically lead to calcinosis; perhaps mineral deposition in these cases was exacerbated by the injection. The vaccine given (IMRAB® 3, Merial) makes use of an aluminum hydroxide adjuvant to promote macrophage antigen presentation and contains gentamicin as a preservative. Naked mole rats are not typically vaccinated for rabies at this institution so it is unknown if the type of vaccine and/or adjuvant had anything to do with the development of the lesions. In those animals treated via surgical excision, tumoral lesions did not recur.

There did not appear to be calcinosis cutis-like lesions in the biopsied animals, though sampling of skin in places other than at tumoral lesions was not attempted. Injection site calcinosis circumscripta has been previously reported in a dog that had received medroxyprogesterone acetate contraception.[4] No reports were found in which calcinosis circumscripta was associated with vaccination.

Given that multiple members of the colony were affected, a review of commonalities among these animals (such as diet, genetics and clinical history) and possibly serum chemistry analyses may be helpful. Adrenal and pituitary glands in the necropsied cases were grossly and histologically within normal limits, so hyperadrenocorticism was considered less likely. Given their subterranean lifestyle and lack of normal exposure to vitamin D, differences in calcium/phosphorus metabolism could make this animal more susceptible to cutaneous calcinosis. Abnormal skin calcification has been reported previously in naked mole rats. In this study, despite massive dietary vitamin D overload, mineralization was only evident in the skin and in increased tooth density. It was speculated that in naked mole rats, skin and teeth may be used for deposition and subsequent sloughing of excess calcium, perhaps to help prevent metastatic calcification in other organs.[3]

Naked mole rats are a fascinating rodent species of relatively recent interest as research animals. Naked mole rats are native to Africa where they live deep underground in a complex system of burrows. They are a eusocial species, meaning they have a complex social structure whereby many non-reproductive 'workers' care for a single dominant 'queen' and a few other reproductively active individuals.[8] They are extraordinarily long-lived for a rodent species (in excess of 20 years). Naked mole rats appear to lack pain response to certain irritants (ammonia, capsaicin and acids) and have a high tolerance for hypoxic conditions, adaptations that are likely courtesy of the extreme environment in which they live. Neoplastic disease has yet to be reported in this species.[1,2,6] For these reasons, naked mole rats are being utilized in aging, pain and cancer studies.

JPC Diagnosis: 1. Haired skin, neck: Dermal granulomas, calcareous, multiple, coalescing (calcinosis circumscripta), with mild epidermal pigmentary incontinence.
2. Haired skin, ventral body wall: Dermatitis, granulomatous, diffuse, marked, with multifocal dermal mineralization (calcinosis cutis) and fibrosis, moderate acanthosis, orthokeratotic hyperkeratosis and serocellular surface crusts.

Conference Comment: The contributor provided an excellent review of cutaneous calcium deposits. Calcinosis circumscripta occurs in both humans and animals. In humans, calcinosis circumscripta has been associated with pressure points, tendon sheaths, and distal digits.[9] It is more commonly reported in females than males and may be associated with connective tissue disorders such as Reynaud's phenomenon, scleroderma, systemic lupus erythromatosis, telangiectasia, and dermatomyositis. Trauma, insect bites, renal failure, and inherited disorders such as pseudoxanthoma elasticum, Werner's syndrome, and Ehlers-Danlos syndrome are suspected causes of calcinosis circumscripta in humans. A recently described autosomal recessive disorder termed normophosphatemic familial tumoral calcinosis (NFTC) has also been implicated in the formation of calcinosis circumscripta lesions in people. NFTC is associated with an absence of functional SAMD9, a tumor suppressor and anti-inflammatory protein, and histologically appears similar to dystrophic calcinosis.[9]

In addition to the naked mole rats described by the contributor, other animals affected by calcinosis circumscripta include dogs, cats, horses, cows, buffalo, rabbits, turtles, nonhuman primates and a captive antelope. Reports of calcinosis circumscripta in nonhuman primates include two female rhesus macaques and a male common marmoset, in which the lesion was associated with microchip implantation. Additionally, idiopathic calcinosis circumscripta has been reported in two cynamologus macaques and a case of bilateral dystrophic calcinosis circumscripta was recently reported in a six-year-old female cynomolgus macaque with a history of bilateral foot trauma.[9]

There was moderate slide variation; the slide used for the JPC morphologic diagnosis did not exhibit the necrosis and ulceration associated with the calcinosis cutis as described by the contributor.

Contributing Institution: Wildlife Disease Labs
San Diego Zoo Institute for Conservation Research
http://www.sandiegozoo.org/conservation/

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CASE III: 12/253 (JPC 4020024).

Signalment: 4-year-old female, Norwegian red, *Bos taurus*.

History: The cow was submitted to the large animal clinic at the Norwegian School of Veterinary Science due to a large (walnut-sized) tumor-like growth in the cornea of the right eye. A squamous cell carcinoma or a granuloma due to trauma was suspected, and the eye was enucleated and submitted for histopathologic examination.

Gross Pathology: Slightly off the center in the cornea there is a 3x2x2 cm large exophytic pedunculated nodule with a stalk that measures 1.5 cm in diameter. The surface of the lesion is rugged with a grey to yellow discoloration. There is opacity of the surrounding corneal tissue. On the cut surface the lesion appears to be composed of an outward bulging of the cornea with a central core of gelatinous material. Black pigmented tissue (iris) is adhered to the internal surface of the cornea and also protrudes into the stalk of the lesion.

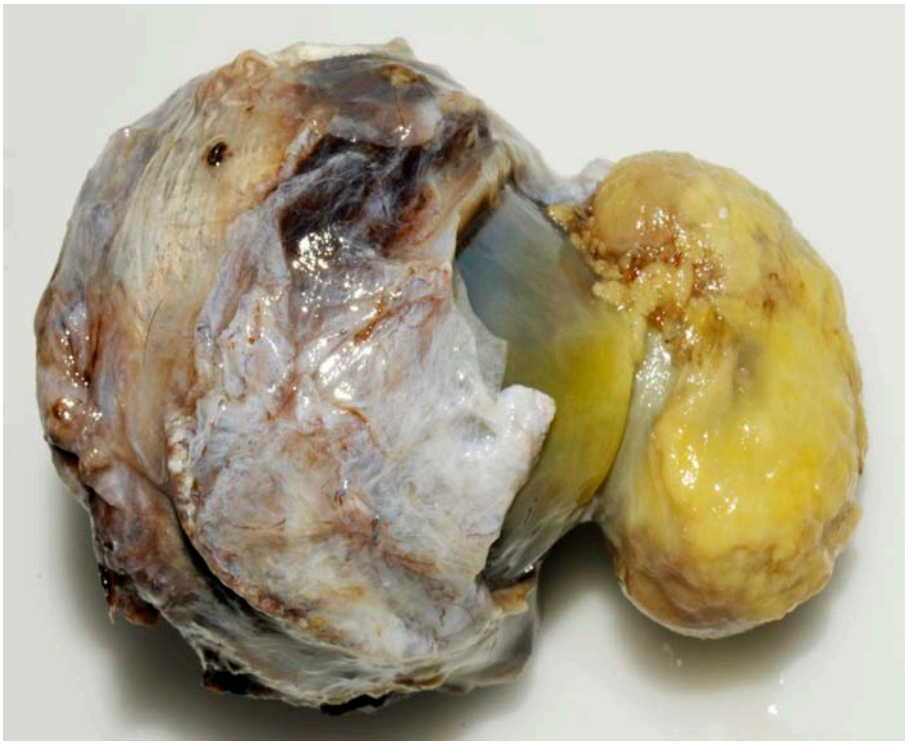
Laboratory Results: The intraocular pressure was decreased in the eye.

Histopathologic Description: In the eye there is a focally extensive anterior bulging of a fibrous membrane, and the bulging area consists of connective tissue with collagen fibers parallel with the surface and vessels that are perpendicular to the surface (granulation tissue). The granulation tissue is continuous with cornea on each side. The outer surface of the bulging area is severely inflamed and infiltrated by numerous leukocytes, mainly necrotic neutrophils.

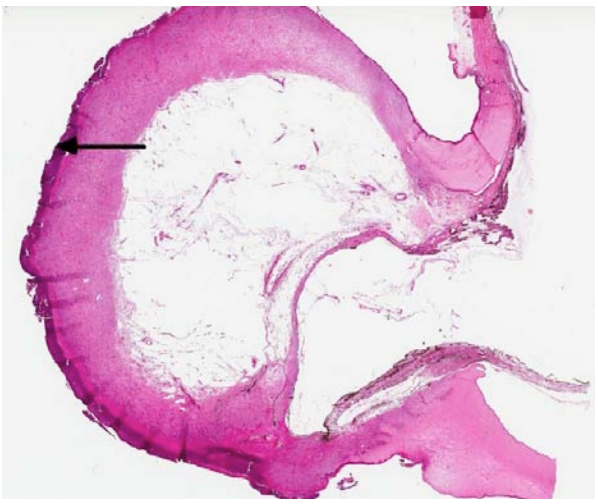
Descemet's membrane is ruptured, and the ends are located at each side of the stalk area, thus the inner surface of the granulation tissue is not lined by Descemet's membrane. The center of the nodule (macroscopic gelatinous area) consists of scattered vessels and spindle shaped cells or round cells with dark brown pigment, and very few lymphocytes and plasma cells. Towards the anterior chamber, the lesion is lined by iridal pigmented tissue that is adhered to the cornea (anterior synechia) and bulging into the defect in the cornea. The pigment in the adhered iris and the central area was Prussian blue negative, and it was bleached by potassium permanganate (melanin).

Contributor's Morphologic Diagnosis: Eye: keratitis, ulcerative and purulent, focally extensive, chronic, severe with anterior synechia and corneal staphyloma.

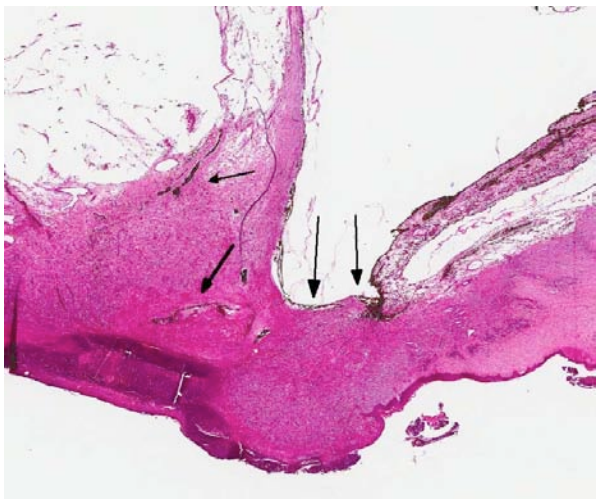
Contributor's Comment: The lesion in this eye was interpreted to have been caused by a traumatic injury to the eye, most likely perforating the cornea, although there was no known history of trauma. Transcorneal gaps may be initially plugged with fibrin and sometimes by prolapsed iris.[3] The outward bulging of the cornea in this specimen has the morphology of granulation tissue, indicating the severity and chronicity of the condition. Although severely inflamed, the inflammation is mainly present in the superficial layer of the granulation tissue that was continuous with the cornea. Infectious ulcerative keratitis, e.g. mycotic of bacterial, may also cause severe corneal lesions. Proteases from microbes or leukocytes may cause stromal liquefaction (keratomalacia) that may reach Descemet's membrane and result in its forward bulging as a descemetocele.[3] In this case, the Descemet's membrane was ruptured with its ends located at each side



3-1. Eye, ox: Arising near the central cornea is a 3x2x2 cm, exophytic, pedunculated nodule. (Photograph courtesy of Norwegian School of Veterinary Science, P.O. Box 8146 Dep, 0033 Oslo, Norway. www.nvh.no)



3-2. Eye, cornea, ox: Diffusely, the cornea is markedly thickened and the anterior edge is extensively ulcerated (arrow). (HE 3X)



3-3. Eye, cornea, ox: Portions of the iris and ciliary body are entrapped within the granulation tissue replacing the cornea (staphyloma/anterior synechia). (HE 80X)

of the protruding iridal tissue. Staphyloma is defined as a protrusion of the sclera or cornea, usually lined by uveal tissue. The lesion can be anterior or posterior and affect both sclera and cornea. Corneal staphyloma is defined as a bulging of the cornea with adherent uveal tissue, as was present in this case.[1] Between the granulation tissue and the protruding iridal tissue there was a large area with scattered blood vessels and pigmented cells. The pigment was negative in Prussian blue staining, however it was bleached in potassium permanganate treated slides, indicating that these cells contain melanin pigment.

JPC Diagnosis: Cornea: Keratitis, ulcerative, chronic, focally extensive severe with staphyloma.

Conference Comment: Corneal injury, such as was suspected in this case, often results in alterations of normal healing that disrupt the normal structure and interfere with the function of corneal tissue.[2] The cornea, which covers the anterior portion of the globe, serves to both protect the intraocular structures and to refract light for vision. Therefore its most important feature is its transparency, which is achieved by its architecture. The cornea is composed of three layers (epithelial, stromal and endothelial layers) and two acellular layers (Bowman's layer and Descemet's membrane, which separate the epithelium from the stroma and the stroma from the endothelium, respectively). These layers have a uniform, consistent arrangement that allows light transmission and refraction. The corneal epithelium is the outermost layer; it is made up of five to seven layers of nonkeratinized, stratified epithelia. The basal epithelial cells of this layer have a prominent nucleus and are mitotically active, as epithelial cells turn over every five to seven days. The basal cells adhere to the basement membrane via a complex t hat also anchors the epithelium to Bowman's layer. Subjacent to the epithelium lies the stroma, which is the thickest layer, accounting for approximately 90% of the cornea. It is composed of highly organized connective tissue (predominantly collagen type I fibrils) arranged in orderly sheets that form lamellae, admixed with proteoglycans that maintain the regular spacing between the lamellae. A meshwork of flat cells (called "keratocytes") is also found between the collagenous lamellae. Keratocytes slowly secrete collagen and ECM components that are needed to maintain the stroma. The endothelium is the innermost layer of the cornea, formed by a single layer of polygonal cells which secrete Descemet's membrane and regulate water content in the stroma via Na+/K+-ATPase pumps. Descemet's membrane is composed mostly of collagen type IV, glycoproteins and fibrin. Dysfunction of the endothelium causes stromal edema and corneal opacity. In addition to these components, the cornea also contains numerous nerve endings (300-400 times more than similarly-sized sections of skin). Most of these nerves are sensory, derived from the ciliary nerves of the trigeminal ganglion ophthalmic branch.[2]

Once the cornea is damaged, the outcome depends on the degree of injury. If only the outer epithelium is damaged, adjacent epithelial cells will quickly migrate to cover the injury. This initial response is immediately followed by a proliferation to regain normal epithelial thickness. Concurrently, upon receiving signals from the damaged epithelium (via cytokines, neuropeptides, growth factors, lipid mediators and chemokines) underlying keratocytes undergo apoptosis and activate adjacent keratocytes. If the stroma is damaged as well, there is a stronger activation of keratocytes, resulting in their transformation into fibroblasts and myofibroblasts,

leading to scar tissue formation and loss of transparency.[2]

Corneal injury will also result in an inflammatory response, generally characterized by infiltration of the stroma by neutrophils and proinflammatory lipid mediators. If this inflammation is not resolved, then corneal fibrosis, pigmentation, and neovascularization occur, ultimately resulting in scarring and disruption of the blood-ocular barrier.[2]

Contributing Institution: Norwegian School of Veterinary Science
www.nvh.no

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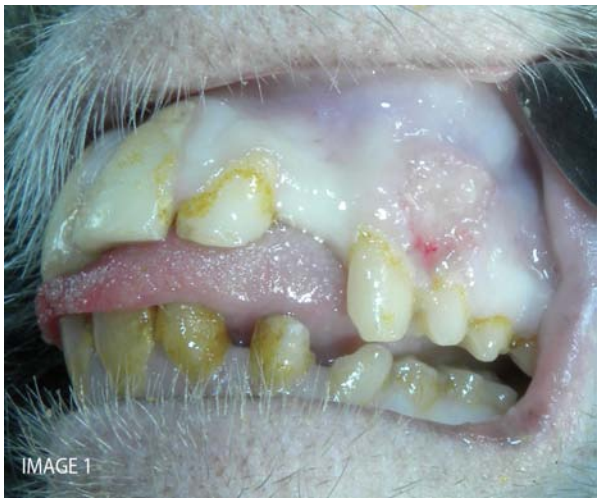
CASE IV: 11A-259 (JPC 4019137).

Signalment: 15-year-old female cynomolgus macaque (*Macaca fascicularis)*.

History: This animal was acquired in 2005 from a domestic source. In-house assays for *Macacine herpesvirus 1* and SRV-2 were negative during quarantine examination. Lethargy was reported on 4-19-11. Gingival erosion and hepatomegaly were noted on physical examination. Pertinent laboratory data appears below. A lingual vesicle was noted on 4-20-11. Microscopic examination of a hepatic biopsy collected via ultrasound guidance on 4-20-11 revealed a necrotizing hepatitis. Despite supportive care, the animal was found expired on 4-23-11.

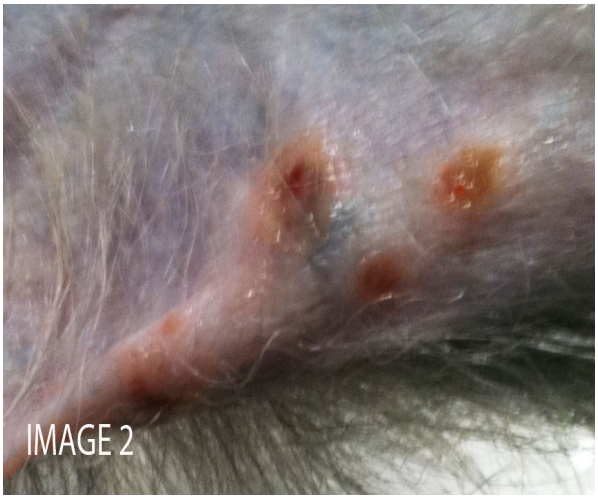


4-1. Gingiva, cynomolgus monkey: At initial presentation, gingival erosions and hepatomegaly were noted. At necropsy, an additional 4cm ulcer was noted over the left maxillary canine tooth. (Photograph courtesy of Oregon National Primate Research Center. http://onprc.ohsu.edu)

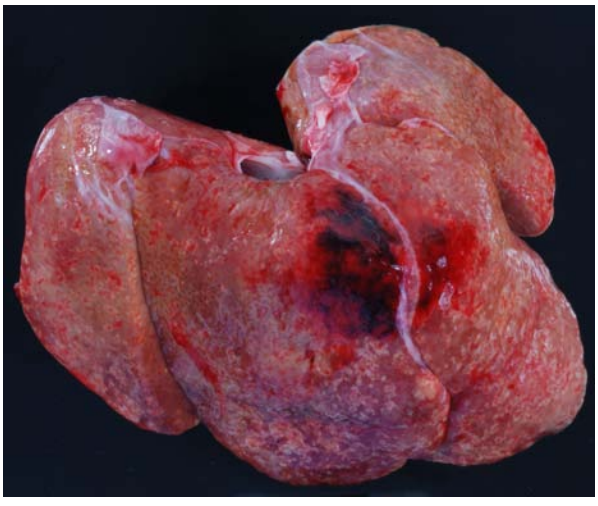
Gross Pathology: Multiple cutaneous crusting to weeping ulcers and erosions were present over the dorsal thorax, the inguinal region, the left upper lip and the posterior aspect of the left crus. There was an ulcer over the left maxillary canine tooth that measured approximately 4 cm in diameter. The tonsils protruded from the crypts and were mildly enlarged and soft with multifocal hemorrhage.

A large gelatinous blood clot was present within the thoracic cavity on the right side. The lung lobes were soft, edematous, and congested. The right side was mildly to moderately affected. The left was mildly affected.

Approximately 150 cc of hemorrhagic fluid was present within the peritoneal cavity. The liver was pale, friable and moderately enlarged with rounded margins. The hepatic capsule was dull and irregular with random, multifocal to coalescing areas of necrosis interspersed among beaded yellow-brown foci arranged in a reticulate fashion. The necrotic foci were either miliary or exhibited target-like lesions with distinct, raised white rims and depressed red centers that measured up to 0.4 cm in greatest dimension. The spleen was pale, enlarged and pulpy with multiple, irregular foci of necrosis. The splenic margins were rounded and the capsule was smooth and taut. The kidneys were streaked and pale and bulge on sectioned surface. There were multifocal hemorrhages throughout the adrenal glands. Multiple cervical hemorrhages were present and the mucosa was overlain by a fibrinopurulent exudate.



4-2. Multiple cutaneous, crusting to weeping ulcers and erosions were present over the dorsal thorax, the inguinal region, the left upper lip and the posterior aspect of the left crus. (Photograph courtesy of Oregon National Primate Research Center. http://onprc.ohsu.edu)



4-3. Liver, cynomolgus monkey: The liver was pale, friable and moderately enlarged with rounded margins. The hepatic capsule was dull and irregular with random, multifocal to coalescing areas of necrosis interspersed among beaded yellow-brown foci arranged in a reticulate fashion. (Photograph courtesy of Oregon National Primate Research Center. http://onprc.ohsu.edu)

Laboratory Results:

***Macacine herpesvirus* 1 Testing**

Sample	Test	Result
Vaginal mucosal swab	Cell culture, PCR*	Positive
Oral mucosal swab	Cell culture, PCR*	Positive
Liver	Cell culture, PCR*	Positive
Spleen	Cell culture, PCR*	Negative
Serum, 6 years prior to death	IgG Antibody ELISA**	Negative
Serum, 5 weeks prior to death	IgG Antibody ELISA & Western blot*	Negative
Serum, 2 days prior to death	IgG Antibody ELISA	Positive
Serum, 2 days prior to death	IgG Antibody Western blot	Negative
Serum, 2 days prior to death	IgM Antibody Western blot	Positive

*National B Virus Resource Laboratory, Georgia State University
**In-house laboratory

Analyte (unit)	Value	Reference interval
ALT (IU/L)	202	0 – 37
ALP (IU/L)	1887	30 – 120
Total protein (g/dl)	4.8	6.0 – 8.0
Albumin (g/dl)	1.3	3.0 – 4.1
RBC ($x10^6/\mu L$)	3.9	5.0 – 6.5
PCV (%)	21.1	35 – 45
Platelets ($x10^3/\mu L$)	112	330 – 650
Leukocytes ($x10^3/\mu L$)	5.0	6.00 – 14.00
Neutrophils ($x10^3/\mu L$)	2.45	2.40 – 11.20
Bands ($x10^3/\mu L$)	0.50	0
Lymphocytes ($x10^3/\mu L$)	2.35	1.50 – 7.00
Monocytes ($x10^3/\mu L$)	0.15	0 – 1.12
Eosinophils ($x10^3/\mu L$)	0	0 – 0.70
Basophils ($x10^3/\mu L$)	0	0 – 0.28

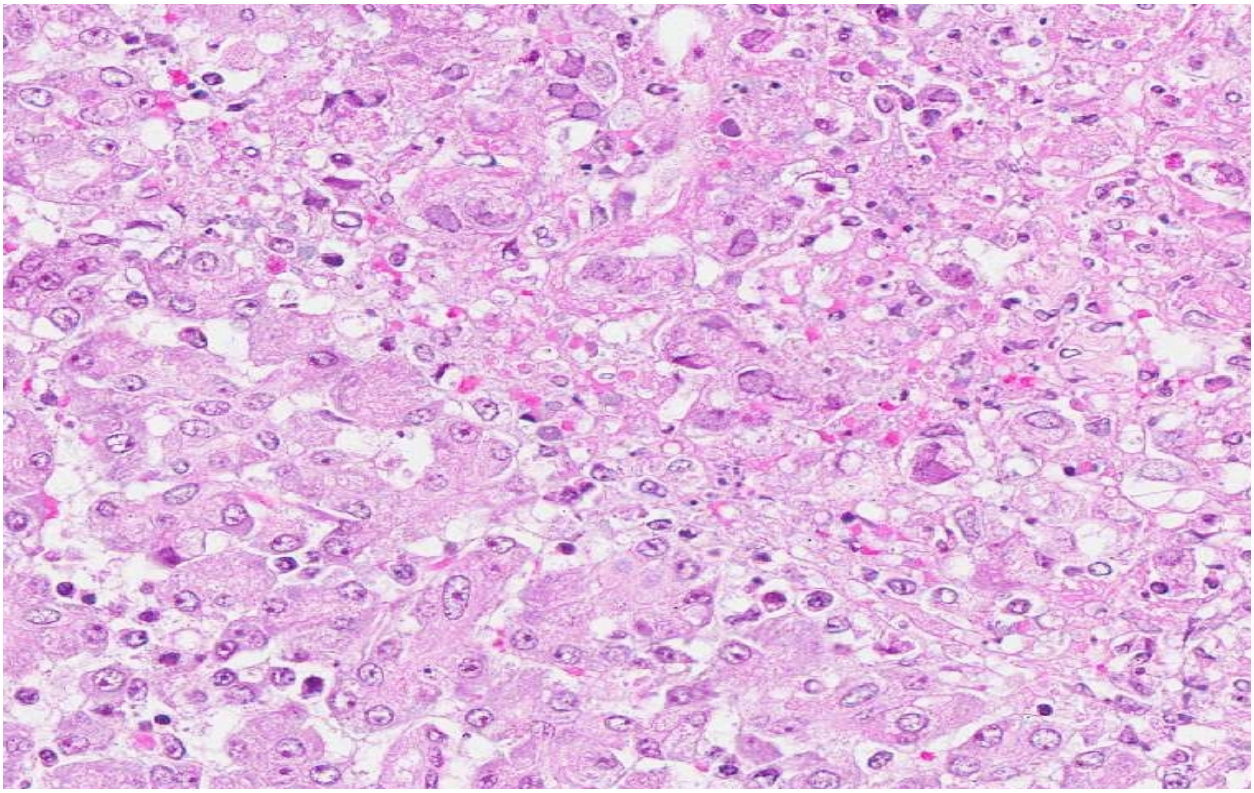
Histopathologic Description: Randomly distributed throughout the pancreatic parenchyma are mild to moderately extensive foci of lytic necrosis characterized by central accumulation of eosinophilic material and cellular and karyorrhectic debris surrounded by degenerate neutrophils and moderate numbers of cells containing amphophilic to eosinophilic intranuclear inclusions with marginated chromatin and occasionally a clear halo. There are numerous syncytia with 3-7 nuclei bearing intranuclear inclusions. Multifocally, the interlobular septa and perivascular spaces are mildly expanded by clear space (edema) and an inflammatory infiltrate composed of neutrophils, lymphocytes, plasma cells, and eosinophils.

Contributor's Morphologic Diagnosis: Pancreas: Pancreatitis, fibrinonecrotic, neutrophilic, with fewer lymphocytes, histiocytes and eosinophils, multifocal, moderate with moderate numbers of amphophilic to eosinophilic intranuclear inclusions and syncytia with intranuclear inclusions.

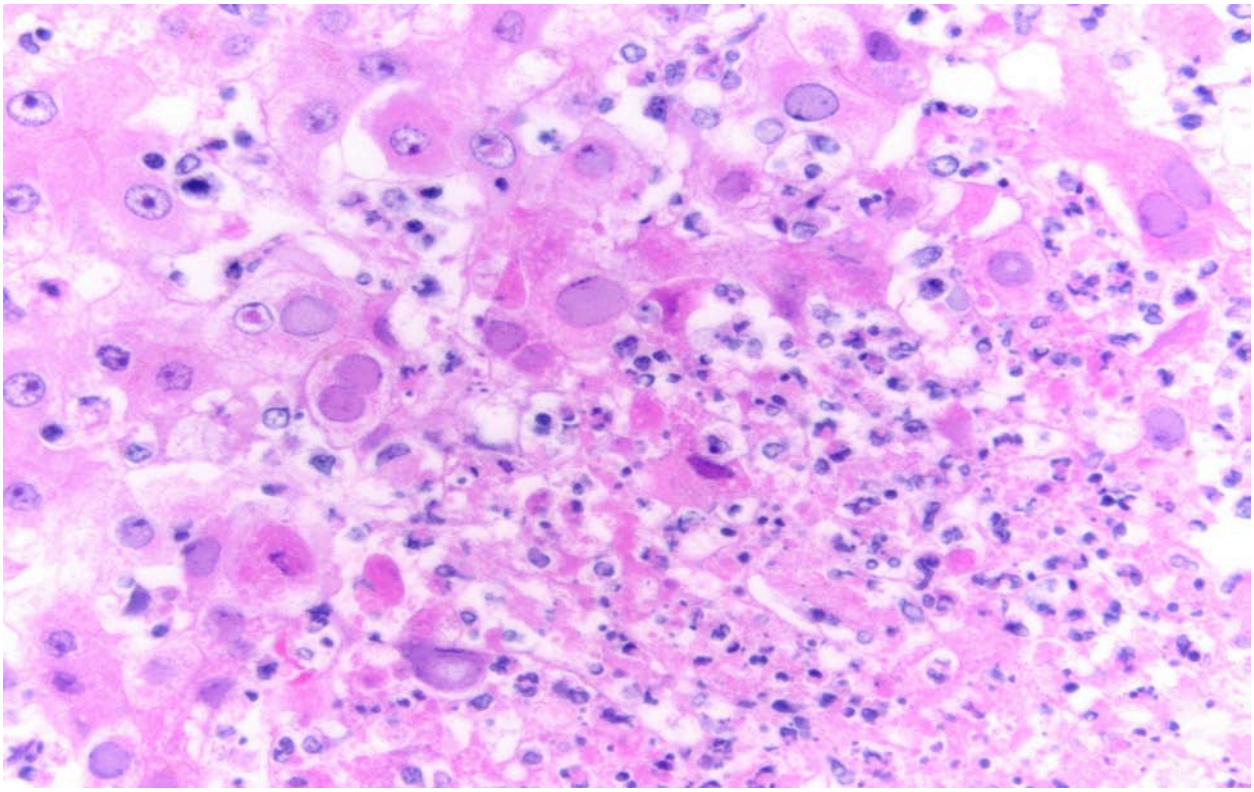
Microscopic findings of tissues not submitted: Neutrophilic fibrinonecrotizing inflammation affected the spleen, adrenal gland, liver, tonsil, cervix, esophagus, haired skin, gingiva, and lip. Most tissues contained intranuclear inclusion bodies and syncytia as described in the pancreas.

Contributor's Comment: This was a laboratory-confirmed case of a fatal, disseminated infection by *Macacine herpesvirus* 1 (McHV1; formerly *Cercopithecine herpesvirus* 1). Primary findings in this case included moderate to severe fibrinonecrotic inflammation of the gingiva, haired skin of the lip, inguinal region, dorsal thorax, left leg, liver, pancreas, adrenal gland, palatine tonsil, and cervix. Rare to high numbers of viral intranuclear inclusions and syncytia were present in most of the affected tissues. Multifocally, necrotizing vasculitis was present and rarely viral inclusions were noted within endothelial cells. The viral infection is thought to have induced disseminated intravascular coagulation.

Swabs of the vaginal and oral mucous membranes and hepatic and splenic tissue samples were submitted to the National B Virus Resource Laboratory at Georgia State University for virus isolation. All samples were positive for McHV1. Serum collected five weeks prior to death was McHV1 IgG antibody negative and a second sample collected two days prior to death had detectable levels of IgG antibody on recombinant ELISA and IgM antibody on western blot. Seroconversion during experimental primary infection results in detectable levels of IgM six days post-infection and IgG 12 days post-infection.[9] IgM levels peak at 12 days, followed by rapid decline. In this case, prior negative tests, positive serum IgM and IgG antibodies, and the acute disease course suggest this was a primary infection.



4-4. Pancreas, cynomolgus monkey: Poorly demarcated areas of necrosis (top left) are scattered throughout the pancreas. Normal acini at bottom left. (HE 228X)



4-5. Liver, cynomolgus monkey: Hepatocytes frequently contain large, amphophilic intranuclear inclusion bodies that peripheralize nuclear chromatin. (Photograph courtesy of Oregon National Primate Research Center. http://onprc.ohsu.edu)

McHV1 is an enveloped double stranded DNA virus, member of the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Simplexvirus.* It is endemic in Asian macaques and has been isolated from rhesus (*M. mulatta*), cynomolgus (*M. fascicularis*), stump-tailed (*M. arctoides*), pigtail (*M. nemestrina*), Japanese (*M. fuscata*), bonnet (*M. radiata*), and Taiwanese (*M. cyclopis*) macaques.[8] It is genetically similar to *Human herpesvirus* 1 and 2, though phylogenetic analysis indicates it is more closely related to non-human primate α-herpesviruses *Cercopithecine herpesvirus* 2 (simian agent 8), and *Papiine herpesvirus* 2 (Herpesvirus papio 2).[6]

Transmission occurs horizontally with increasing seroprevalance coinciding with sexual maturity and typically approaching 80% in captive and wild adult cohorts.[6] Primary infection occurs when orogenital mucosa or open wounds are inoculated with virus-contaminated oral or genital fluid, blood, urine, or feces. McHV1 initially replicates in the epithelium at the site of inoculation and may result in vesicular lesions in the mucosae and skin of the mucocutaneous junctions of the oral cavity, genitals, and conjunctiva, though infection is typically asymptomatic. Virus is transported along axons of sensory neurons to ganglia, and subsequent cessation of replication results in latent infection. Virus reactivation and anterograde axonal transport to epithelium with recrudescence and viral particle shedding can occur in the absence of clinical disease or with similar vesicular lesions. Virus shedding is reportedly more prevalent during the breeding season.

Similar to other members of the *Alphaherpesvirinae*, fatal cross-species transmission can occur. As such, McHV1 is an important zoonotic disease for humans interacting with macaque species. Exposure to infected bodily fluids via mucosa membranes, open skin, and penetrating wounds can result in infection. Infection may produce vesicular lesions at the site of exposure, flu-like symptoms of fatigue, body aches, and fever, and fatal encephalitis has occurred in greater than 70% of documented cases. Excellent reviews detailing prevention and post-exposure treatment are available.[4,6,7]

Spontaneous disseminated McHV1 infection in macaque species that results in death or euthanasia is infrequent, and animals that develop any McHV1 lesions are typically culled rather than treated clinically due to the zoonotic risk to personnel. Primary and latent reactivation resulting in systemic infection has been described in rhesus (*M. mulatta*), cynomolgus (*M. fascicularis*), bonnet (*M. radiata*), and pigtail (*M. nemestrina*) macaques and virus has been isolated from brain, oral cavity, esophagus, liver, adrenal gland, pancreas, and skin.[1,2,3,5,11,12] Latent reactivation is typically associated with immunosuppression by comorbid disease, including dystocia and simian retrovirus type D infection, social stress, and with chronic administration of immunosuppressive drugs.[1,2,3,11,12] In this case, serologic testing suggests this was a naïve animal that contracted McHV1 from another animal in the cohort. Review of the clinical history and experimental treatments did not reveal a potential source of immunosuppression. Testing for simian type D retrovirus was done multiple times over a six-year period with consistently negative results (blood, cell culture; serum, IF membrane antibody).

JPC Diagnosis: Pancreas: Pancreatitis, necrotizing, acute, multifocal and random, with syncytia, eosinophilic intranuclear viral inclusion bodies, mild interstitial edema, and diffuse exocrine atrophy, cynomolgus macaque, (*Macaca fascicularis*), primate.

Conference Comment: The contributor provides an excellent review of *Macacine herpesvirus 1*. Herpesviruses are a large, diverse group of viruses that have been found in almost every species of birds and mammals, as well as in insects, fish, reptiles, amphibians, and mollusks. The order *Herpesvirales* consists of three families: *Herpesviridae* (herpesviruses of mammals, birds, and reptiles), *Alloherpesviridae* (herpesviruses of fish and frogs), and *Malacoherpesvirida* (a single virus found in oysters). The family *Herpesviridae* are further divided into three subfamilies: *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae*. Generally herpesviruses are adapted to their individual hosts, often causing disease only in young, immunocompromised individuals or in alternate host species. Persistent infection with periodic or continuous shedding of virus and recurrence of disease brought on by stress occurs in many herpesviral infections. In addition to McHV1, there are numerous members of *Herpesviridae* that are of importance in veterinary medicine (summarized in the included tables).[10]

Alphaherpesvirinae is subdivided into four genera: *Simplexvirus, Varicelovirus, Mardivirus,* and *Iltovirus*.[10] Most alphaherpesviruses grow rapidly, lyse infected cells and establish latent infections in sensory ganglia.

Members of subfamily *Betaherpesvirinae* are also divided into four genera: *Cytomegalovirus*, *Muromegalovirus*, *Proboscivirus* and *Roseolovirus*. Betaherpesviruses are generally characterized by a slow replication cycle and delayed cell lysis; they often remain latent in secretory glands, the kidneys, and lymphoreticular tissue.[10]

Gammaherpesviruses are generally lymphotropic and reside latent in lymphocytes. Some gammaherpesviruses have been linked to oncogenic transformation of lymphocytes such as Epstein-Barr

virus resulting in Burkitt's lymphoma. Although gammaherpesviruses of ruminants and nonhuman primates generally do not cause significant disease in their immunocompetent natural hosts, some may cause severe lymphproliferative disease in alternate hosts.[10]

Alphaherpesviruses of importance in veterinary medicine[10]:

Bovine Herpesvirus 1	Infectious Bovine Rhinotracheitis and Infectious Pustular Vulvovaginitis Viruses
Bovine Herpesvirus 2	Mammillitis/ Psuedo-Lumpy Skin Disease Virus
Bovine Herpesvirus 5	Bovine Encephalitis Virus
Canid Herpesvirus	Systemic hemorrhagic disease in pups <4 weeks old
Caprine Herpesvirus	Abortion; Infectious pustular vulvovaginitis
Macacine Herpesvirus 1	B Virus disease of macaques (formerly Cercopithecine Herpesvirus 1)
Herpes simplex 1	Severe generalized disease with high mortality in New World primates
Cercopithecine Herpesvirus 9	Simian Varicella Virus
Equid Herpesvirus 1	Equine Abortion Virus
Equid Herpesvirus 3	Equine Coital Exanthema Virus
Equid Herpesvirus 4	Equine Rhinopneumonitis Virus
Felid Herpesvirus 1	Feline Rhinotracheitis Virus
Gallid Herpesvirus 1	Avian Infectious Laryngotracheitis Virus
Gallid Herpesvirus 2	Marek's Disease Virus
Suid Herpesvirus 1	Pseudorabies or Aujeszky's Disease Virus

Betaherpesviruses of importance in veterinary medicine[10]:

Murid Herpesvirus 1	Mouse Cytomegalovirus
Murid Herpesvirus 2	Rat Cytomegalovirus
Caviid Herpesvirus 2	Guinea Pig Cytomegalovirus
Rhesus Cytomegalovirus	*Many Old and New World non-human primates have their own cytomegaloviruses
Elephantid Herpesvirus	Endotheliotropic Elephant Herpesvirus
Suid Herpesvirus 2	Porcine Cytomegalovirus

Gammaherpesviruses of importance in veterinary medicine[10]:

Alcelaphine Herpesvirus 1	Malignant Catarrhal Fever
Ovine Herpesvirus 2	Malignant Catarrhal Fever
Equid Herpesvirus 5	Multinodular Pulmonary Fibrosis
Saimiriine Herpesvirus 2 (Herpesvirus saimiri) and Herpesvirus ateles	T lymphocytotropic virus that causes subclinical latent infections in squirrel monkeys (*H. saimiri*) and spider monkeys (*H. ateles*), but rapid and fatal lymphoproliferative disease in New World monkeys (marmosets, tamarins, owl monkeys)
Retroperitoneal Fibromatosis Herpesvirus and Rhesus Rhadinovirus	Retroperitoneal fibromatosis and B-cell lymphomas in retroviral-infected Rhesus macaques

Unassigned members of Herpesviridae of importance in veterinary medicine[10]:

Anatid Herpesvirus 1	Duck Viral Enteritis Virus; Duck Plague Virus

Contributing Institution: Oregon National Primate Research Center
http://onprc.ohsu.edu

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