Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2014-2015

Conference 7

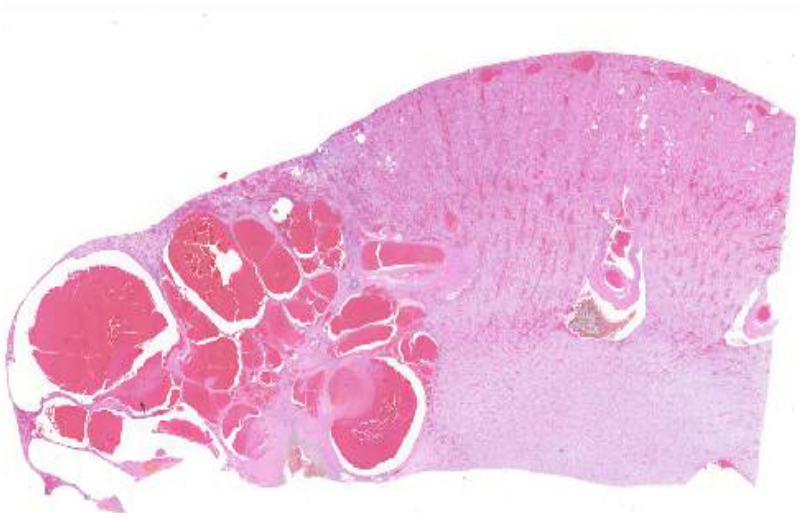
22 October 2014

CASE I: B13-962 (JPC 4049563).

Signalment: 10-year-old spayed female Welsh corgi dog, *Canis familiaris.*

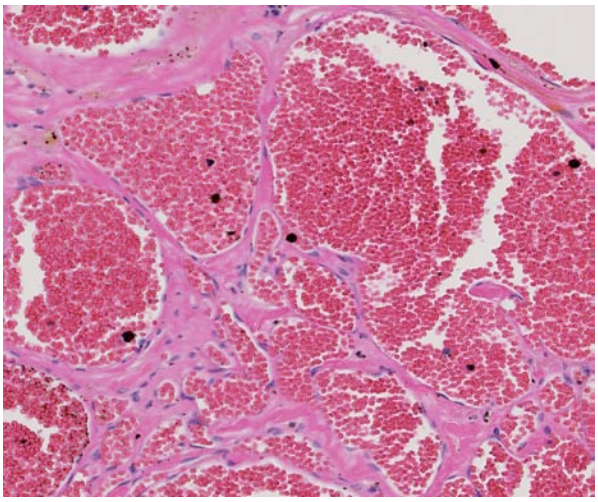
History: The dog presented in 2011 for a 6-week history of hematuria. Urine culture was negative and no signs of uroliths were seen on radiographs. Ultrasound revealed two, 1.7 mm, mineralized foci in the left renal pelvis, but the kidneys were normal in shape and size. No further workup was performed at that time. The dog presented again in July 2013 with continued hematuria. On ultrasound, the left renal cortex had a 2.2 cm, round, heterogeneous mass with multiple, internal, anechoic regions.

Gross Pathology: The renal cortex was extensively disrupted by coalescing, dark red, blood-filled nodules ranging from 0.7 x 0.5 x 0.2 cm to 2.5 x 2 x 2 cm.

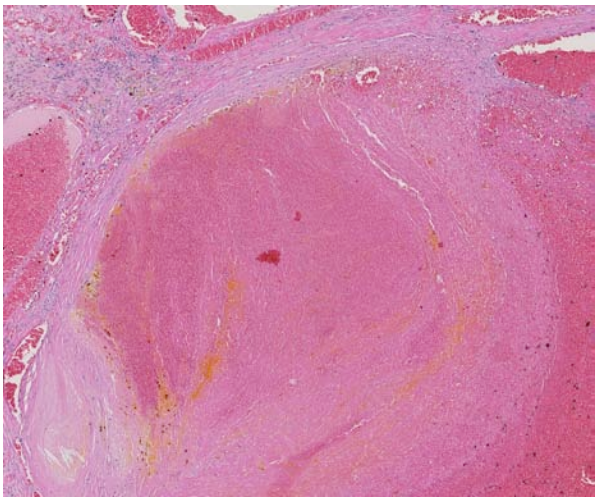


1-1. Kidney, Welsh corgi: The cortex is expanded by large, ectatic, thin-walled vessels which efface renal parenchyma. (HE 6.3X)

Histopathologic Description: Kidney: The renal interstitium is markedly expanded by many, severely dilated, blood-filled vascular spaces lined by mature endothelial cells surrounded by abundant fibrous tissue. Some vascular spaces contain thrombi with fibrin arranged as lines of Zahn separated by red and white blood cells that are occasionally attached to the vascular wall by fibrous tissue. The intervening and adjacent renal parenchyma is markedly atrophic with replacement of many nephrons by fibrosis, many lymphocytes and plasma cells, and variable hemorrhage. Remaining tubules



1-2. Kidney, Welsh corgi: Vessels are thin-walled and separated by a moderate fibrous stroma. (HE 144X)



1-3. Kidney, Welsh corgi: Larger vessels are partially occluded with fibrinous thrombi which contain lines of Zahn. (HE 35X)

and glomeruli in these areas are often atrophic or sclerotic, respectively. Some tubules contain proteinaceous fluid. The renal pelvis contains degenerating erythrocytes and brown, granular pigment. The more distant renal parenchyma is unremarkable apart from congestion.

Contributor's Morphologic Diagnosis: Kidney: Renal telangiectasia.

Contributor's Comment: Renal telangiectasia is a rare, non-neoplastic proliferation of blood vessels.[3] It has been described in the Welsh corgi as a familial disease, and similar lesions have been described in cattle, dogs, cats, mink and ferrets.[2] Affected corgis typically present for hematuria and may have bilateral lesions or additional lesions in other tissues, such as the liver, duodenum, brain, vertebrae, subcutaneous tissue, and spleen.[3,4] In this case, no contralateral or extra-renal lesions were identified by abdominal ultrasound or at surgery. The primary clinical differential in this case was renal hemangiosarcoma. Renal telangiectasia is differentiated histologically from hemangiosarcoma by the fact that the vascular spaces are lined by a bland, mature endothelium without mitotic activity or cellular atypia.[4]

JPC Morphologic Diagnosis: Kidney: Vascular malformation with multifocal thrombosis.

Conference Comment: Conference participants discussed three optional diagnoses for this case: telangiectasia, hemangioma or vascular hamartoma. The familial lesion of renal telangiectasia in Pembroke Welsh corgis as described in the literature characteristically arises bilaterally with frequent occurrences in other organs. The clinical history in this case suggests the lesion is isolated to one kidney; however, the lesion lacks the well-circumscribed appearance of a hemangioma. The signalment and clinical signs do not correlate well with a hamartoma, which implies a congenital proliferation. The diagnosis of vascular malformation reflects these inconsistencies and participants' inability to arrive at a consensus.

The pattern of presentation in corgis interestingly resembles a familial condition in people known as hereditary hemorrhagic telangiectasia (HHT). Also known as Osler-Weber-Rendu disease, this autosomal dominant disorder occurs at a rate of 1 in 5,000 people and manifests as telangiectasia of the oral mucosa and arteriovenous malformations in the lungs, liver and less often brain. Three mutations have been identified in HHT, all with protein products influencing the TGF-β superfamily.[5]

TGF-β is a growth inhibitor of most epithelial cells but a potent stimulator of fibroblasts. Its binding to cell-surface receptors leads to phosphorylation of SMAD transcription factors, specifically Co-SMAD & R-SMAD. When phosphorylated, these factors overcome SMAD-7 inhibition, thereby promoting transcription and activation of fibroblasts. Additionally and likely pertaining to these vascular lesions, TGF-β is strongly implicated in the stabilization of nascent blood vessels, following their sprouting and

migration through the finely orchestrated events coordinated by VEGF and the Notch pathway.[1]

This inheritable condition has serious consequences in people due to their predisposition for hemorrhages and thrombosis. The understanding of disease pathogenesis has also shed new light on the specific molecular interactions of angiogenesis, one of the hallmarks of cancer. The authors speculated on the value of Pembroke Welsh corgis serving as an animal model for vascular malformations over 30 years ago.[3] While knockout mice have been developed which reproduce these lesions, this naturally occurring canine model may offer further valuable insight in piecing together the complex interactions of angiogenesis and its role in vascular malformations and neoplasia.

Contributing Institution: Tufts Cummings School of Veterinary Medicine
Department of Biomedical Sciences
200 Westboro Road
North Grafton, MA 01536
http://vet.tufts.edu/dbs/

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CASE II: 1259066 (JPC 4048671).

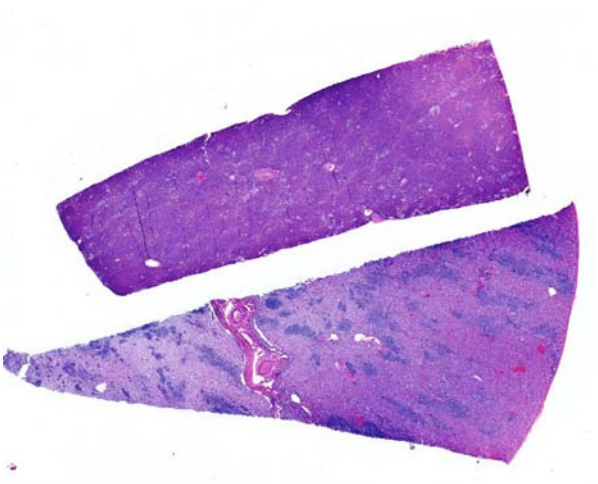
Signalment: 6-year-old spayed female Vizsla dog, *Canis familiaris.*

History: The patient had a 6-month history of minor incontinence followed by a 3-month history of recurrent UTI's, which were treated by the referring veterinarian with estradiol cypionate and antibiotics respectively (consecutive ampicillin, chloramphenicol, cephalexin, and metronidazole). The patient had a 1-month history of ataxia and progressively more frequent vomiting. She presented to Urgent Care Service on 7/27 for worsening neurologic signs, anorexia, lethargy, and PU/PD. Radiographs obtained were inconclusive. The patient was referred for neurology consult, recommended MRI, and remained for inpatient diagnostic testing and treatment for 5 days.

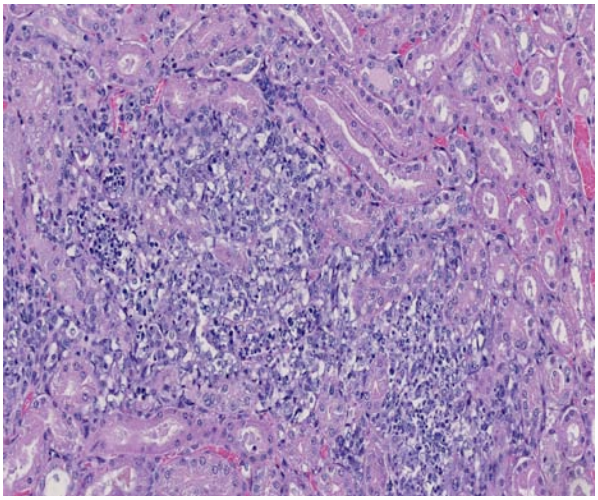
During diagnostic workup, there was mild C5-C6 disc protrusion noted on cervical myelogram. On post-contrast T1 weighted MR images, there was meningeal enhancement spinal cord through C1 and ventral to the brain stem and pons, as well as faint contrast enhancement in the cerebellum. On cerebellomedullary cistern cerebrospinal fluid aspirate cytology there was a severe mixed pleocytosis. *Neospora* and *Toxoplasma* PCR and serology, *Cryptococcus* latex agglutination, and bacterial cultures were all negative. Thoracic radiographs were unremarkable. Serum biochemistry findings were mild hyperglobulinemia and moderately elevated ALT and AST.

Therapeutic interventions included clindamycin, fluconazole, cytoarabine, and prednisolone at an immunosuppressive dose. The tentative clinical diagnosis was meningoencephalitis of unknown origin with concern about fungal disease or GME. The dog was discharged from the hospital 5 days after admission. During the following 2 weeks, the patient re-presented for weight loss, muscle wasting, and progressive ataxia leading to tetraparesis. The patient arrested shortly after presentation to urgent care in lateral recumbency and respiratory distress.

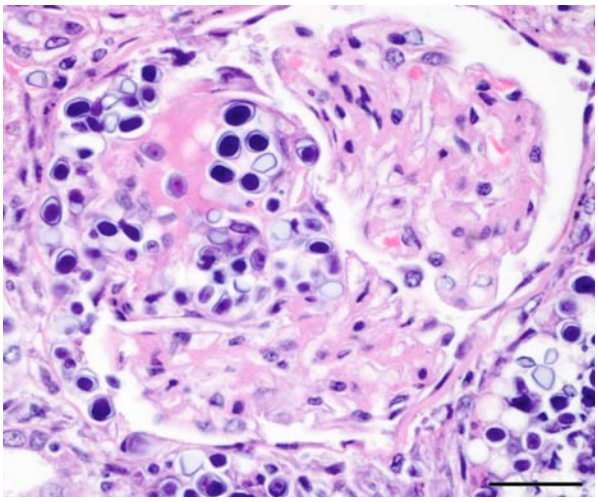
Gross Pathology: At necropsy, the dog was in very poor body condition (BCS = 2/9). Her mucous membranes and non-haired pinnae were markedly pale and eyes were recessed. In the kidneys, there were numerous miliary, pale tan, 1 to 2 mm diameter foci on the capsular surface that often extended in multifocal rays into the medullas. Similar minute nodules and streaks were throughout the myocardium. In the brain and spinal cord, there was moderate purulent subdural exudate (meningoencephalitis). The liver was diffusely friable, and moderately enlarged with rounded edges. The small and large intestines were segmentally reddened and thickened with multifocal irregular mucosal ulcerations. Multiple lymph nodes are moderately expanded by irregular firm to caseated foci. The adrenal cortices were mildly bilaterally thin (atrophy, consistent with steroid administration).



2-1. Kidney, dog: Linear hypercellular foci replace cortical and medullary parenchyma. (HE 7X)



2-2. Kidney, dog: The interstitium is infiltrated and tubules are replaced by large numbers of macrophages, lymphocytes and plasma cells. Algal sporangia are often seen within macrophages (arrows). (HE 228X)



2-3. Kidney, dog: Sporangia are occasionally seen within glomerular mesangium and Bowman's space. (HE 400X) (Photo courtesy of: Colorado State University, Department of Microbiology Immunology and Pathology: http://csu-cvmbs.colostate.edu/academics/mip/Pages/default.aspx)

Laboratory Results: Myocardial impression smears were taken at the time of necropsy. There were numerous round unicellular organisms with refractile cell walls, basophilic cytoplasm, and internal septation.

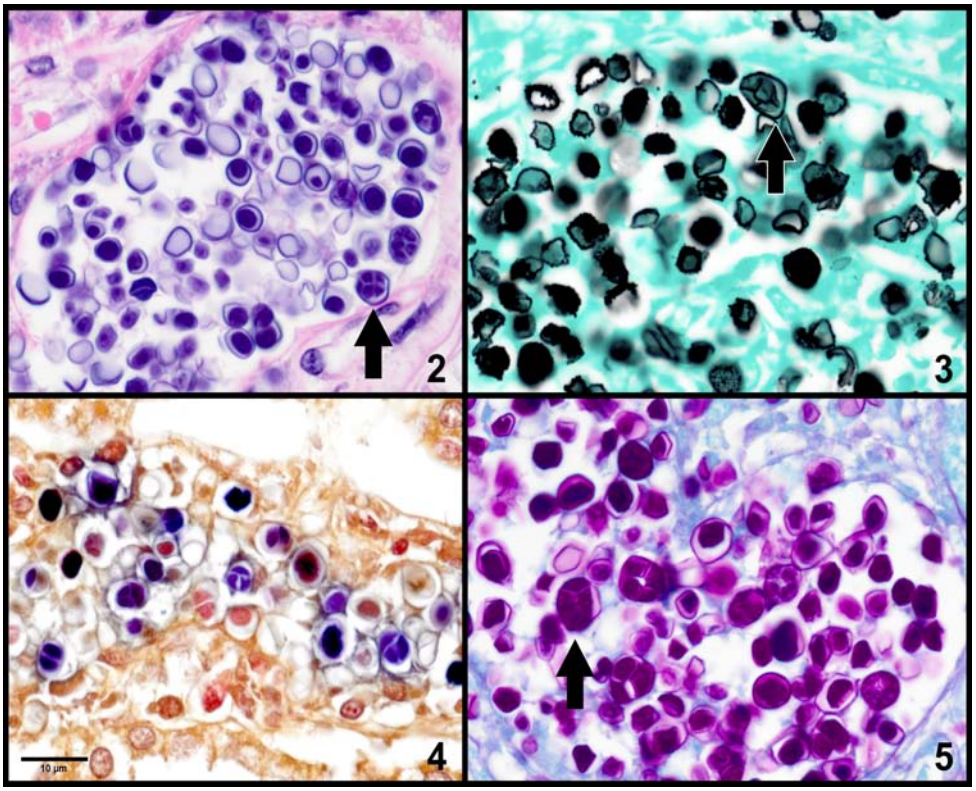
Histopathologic Description: Kidney: Effacing the renal cortex and medulla are numerous large irregular foci of necrotic tubules and obliterated interstitium with moderately to markedly increased numbers of macrophages, plasma cells, fewer lymphocytes, and admixed neutrophils. Foci extend radially along tubules, effacing up to 1 mm wide tracts through the medulla and cortex. There are numerous unicellular, round to ovoid, 6 to 20 micrometer diameter organisms with pale staining to chromophobic refractile double-layer cell walls. The sporangia (theca) contain single round to ovoid, or multiple wedge-shaped, granular basophilic endospores with small dark basophilic nuclei. There are numerous poorly staining to clear empty theca. Inflammatory cells dissect through the moderately compressed renal interstitium surrounding and infiltrating dense clusters of organisms. Multifocal tubules and Bowman's capsules are distended by organisms, mixed inflammatory cells and karyorrhectic debris. Affected glomeruli are variably shrunken and hypereosinophilic with fibrin deposition and pyknotic to karyorrhectic nuclei. Tubules, with attenuated and necrotic epithelium, are segmentally obliterated and progressively lost, both within and adjacent to the lesion. There is moderate multifocal perilesional congestion.

Of additional interest, sections of liver are provided, demonstrating organisms in sinusoids, multifocal hepatocellular necrosis, dystrophic mineralization, hydropic degeneration, and occasional portal tracts effaced by *Prototheca* sporangia and granulomatous inflammation. Hepatic lesions are variable and clusters of organisms are not captured in all sections.

In tissues not included for conference material, multifocal to coalescing perivascular and random granulomatous inflammation with organisms is present throughout the gastrointestinal tract, pancreas, mesentery, lymph nodes, lung, myocardium, and meninges.

Contributor's Morphologic Diagnosis: Kidney: Nephritis, interstitial, granulomatous to lymphohistiocytic, multifocal, chronic, moderate to marked with tubular necrosis and loss, and with myriad intralesional algal organisms (consistent with *Prototheca* sp.).

Contributor's Comment: *Prototheca* spp. are saprophytic, achlorophyllous algae, closely related to *Chlorella* spp., with worldwide distribution.[7] This opportunistic pathogen is a spherical, 3 to 30 micron diameter, unicellular organism with a 0.5 micron thick poorly-staining refractile cell wall. In the sporangium, there are individual round, or up to 20 irregularly shaped, basophilic endospores produced by asexual cytoplasmic cleavage.[4,7,8] *Prototheca* and *Chlorella* are histologically indistinguishable on H&E-stained sections.[4] For both genera, endospores are Gram-positive and sporangia stain positively by Gomori methenamine silver (GMS). Differentiation is possible on fresh specimens and periodic acid-Schiff (PAS)-stained sections.[4,5,11,12] In gross fresh specimens and wet-mounts *Chlorella*-infected tissue and organisms are green, due to chlorophyll. Additionally, on PAS-stained sections, cytoplasmic starch of *Chlorella* spp. is visible as distinct globules that are strongly PAS-positive and diastase sensitive. The PAS-positive, diastase-resistant endospores of *Prototheca* spp. do not exhibit distinct cytoplasmic globules.[5,8] For an excellent example of PAS-positive starch globules in *Chlorella* spp. readers are referred to images published by Haenichen et al.[5] Morphologic differentiation between species of *Prototheca* is documented, but immunolabeling and 18S rRNA sequence analysis are also available.[8]



2-4. Kidney, dog: Upper left: Within sporangia, endospores are in varying stages of division (arrow). (HE 600X). Upper right: Dark GMS-positive staining is present on the surfaces of sporangia and endospores (arrow). Inside endospores GMS staining is often minimal (GMS 600X). Lower left: Characteristic gram-positive staining endospores are admixed with gram-negative theca. (Gram 600X) Lower right: Both sporangia and endospores are diffusely PAS-positive. In contrast to the symmetric 'cartwheel' appearance of morula-like structures of P. wickerhamii, the asymmetric arrangement of endospores (arrow) is a feature of P. zopfii.[7] (PAS 600X) (Photo courtesy of: Colorado State University, Department of Microbiology Immunology and Pathology: http://csu-cvmbs.colostate.edu/academics/mip/Pages/default.aspx)

Ubiquitous in moist environments, *Prototheca* spp. are found in slime flux of trees, a variety of freshwater environments, soil, animal waste lagoons, and in great abundance in sewage.[7,9] Despite their relative ubiquity, disease in mammals is rare. The two known pathogenic species are *Prototheca zopfii* and *P. wickerhamii*. Previously reported mammalian species infected include cattle, dogs, cats, and humans.[4] In cattle, *P. zopfii* is reported to cause severe mastitis, due to ascending infection from environmental contamination.[6] In humans, three clinical forms of protothecosis are recognized: systemic infection, bursitis, and cutaneous lesions most commonly.[8] In cats, only the cutaneous form is reported, and presumed to be due to trauma. In dogs, systemic dissemination is most commonly reported. Organs frequently infected include the intestinal tract, liver, kidney, heart, eyes, and central nervous system.[4]

The specific pathogenesis of disseminated canine protothecosis is not well studied, largely due to the limited number of cases. Infection is thought to occur by ingestion, and penetration of the colonic mucosa, at which point severe hemorrhagic colitis and diarrhea are often the first clinical findings.[4] Much less frequently, neurologic disease is the first reported finding.[7] Systemic infection occurs through hematogenous and lymphatic dissemination to multiple organs, where colonization, necrosis, and granulomatous inflammation are associated with myocardial collapse, acute renal failure, hepatic failure, blindness due to chorioretinitis and uveitis with retinal detachment, as well as varying presentations of neurologic disease.[2,4,7] Protothecosis is associated with therapeutic or pathologic immune system impairment in both dogs and humans.[7,8] With or without immunosuppression, there may be numerous organisms in tissue sections with relatively mild inflammation. Greater numbers of ruptured, empty theca are associated with much more severe pyogranulomatous reactions.[1]

Many aspects of this case are fairly characteristic for systemic canine protothecosis, including reported immunosuppression. However, the diagnosis made at necropsy was unexpected, due to the local arid climate, and rarity of protothecosis in such environments. Travel history was more thoroughly investigated after necropsy findings, and the dog was reportedly in the Southeastern US from late December to early January.

JPC Morphologic Diagnosis: 1. Kidney: Nephritis, granulomatous, multifocal, moderate, with mild glomerulonephritis and numerous endosporulating algae.
2. Liver, hepatocytes: Degeneration and necrosis, multifocal, random, mild, with mineralization.
3. Liver, hepatocytes: Glycogenosis, multifocal, mild.

Conference Comment: The contributor does an exceptional job comparing and contrasting *Prototheca* and *Chlorella* in histologic sections in addition to detailing the clinical presentation of these rarely reported infections. Conference participants were impressed with the tremendous quantity of organisms present within the kidneys and relatively few inflammatory cells leaving some to speculate on the severity of immunosuppression in this case. The most frequently identified causes of immunosuppression in protothecosis infections in people are steroid administration, neoplasia, diabetes mellitus and acquired immunodeficiency syndrome, however, similar associations have not been made in dogs.[10]

Protothecosis is typically observed as disseminated disease in dogs while it is also reported in cows, but as an important cause of mastitis. *P. zopfii* is most often associated with disseminated disease while *P. wickerhamii* usually associated with cutaneous infections.[3] The disseminated presentation in this case and the timeline of travel, development of urinary symptoms and subsequent neurologic signs would make for an interesting diagnostic exercise in detecting an immunosuppressive disorder and acquired *Prototheca* infection and correlating it with development of clinical signs.

Prototheca is one of the few pathogens which uniquely reproduce by endosporulation. The infective unit of these endosporulators is the endospore, which when implanted in tissues, grow into much larger sporangia. As a part of the maturation process of sporangia, endospores are produced and discharged effectively reinitiating the cycle. The other endosporulators of veterinary significance include: *Rhinosporidium seeberi, Chlorella* sp., *Coccidioides immitis,* and *Batrachochytrium dendrobatidis*.

In this case, the hepatocellular glycogenosis was attricuted to the immunosuppressive doses of corticosteroids noted in the clinical history. The cause for the random hepatocellular necrosis and mineralization was not evident.

Contributing Institution: Colorado State University
Department of Microbiology Immunology and Pathology
http://csu-cvmbs.colostate.edu/academics/mip/Pages/default.aspx

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CASE III: 14-10 (JPC 4048510).

Signalment: 3-month-old female Yorkshire Cross pig, *Sus scrofa domesticus.*

History: This piglet was received at 1 month old and began receiving weekly carbon-14 injections via the ear vein with ketamine/xylazine sedation approximately one week after arrival. She was found down and extremely weak two months after arrival while other animals in same group had no clinical symptoms. The investigator elected euthanasia and a gross necropsy was performed.

Gross Pathology: On postmortem examination, connective tissues throughout the body are edematous and bright yellow. The liver has multifocal to coalescing pinpoint to 3 mm dark red foci, is enlarged, and flabby.

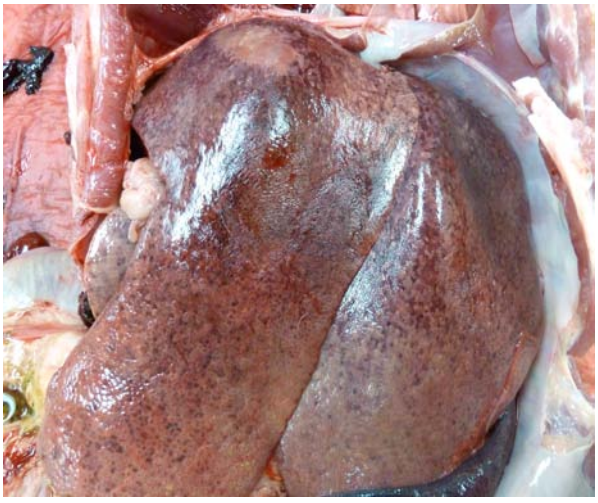
Laboratory Results: Clinical chemistry reveals severely icteric serum, high potassium, ALT, ALP, and amylase. The CBC shows a stress leukogram. Postmortem testing of hepatic selenium concentrations reveal levels as inadequate.

Histopathologic Description: Liver: Within all lobules examined, there is massive centrilobular to diffuse hepatocellular necrosis and hemorrhage. Hepatocytes in these areas are often completely lost and replaced by hemorrhage. In other area, hepatocytes are hypereosinophilic and individualized, with pyknotic or absent nuclei and are often surrounded by cellular debris, neutrophils, and fibrin. Portal areas contain mildly increased numbers of lymphocytes, reactive fibroblasts, and neutrophils, with rare pyknotic cells.

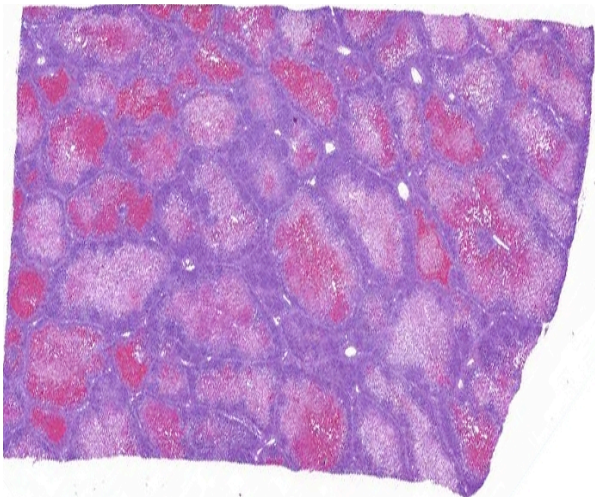
Skeletal muscle: Diffusely throughout the tissue, there is random individual myocyte degeneration characterized by sarcoplasmic hypereosinophilia and swelling, loss of cross-striations, fragmentation, pyknosis, karyorrhexis and karyolysis.

Contributor's Morphologic Diagnosis: 1. Liver: Necrosis, acute, centrilobular to massive, hemorrhagic, Yorkshire cross, pig. 2. Skeletal muscle: Degeneration and necrosis, acute, multifocal, mild to moderate, Yorkshire cross, pig.

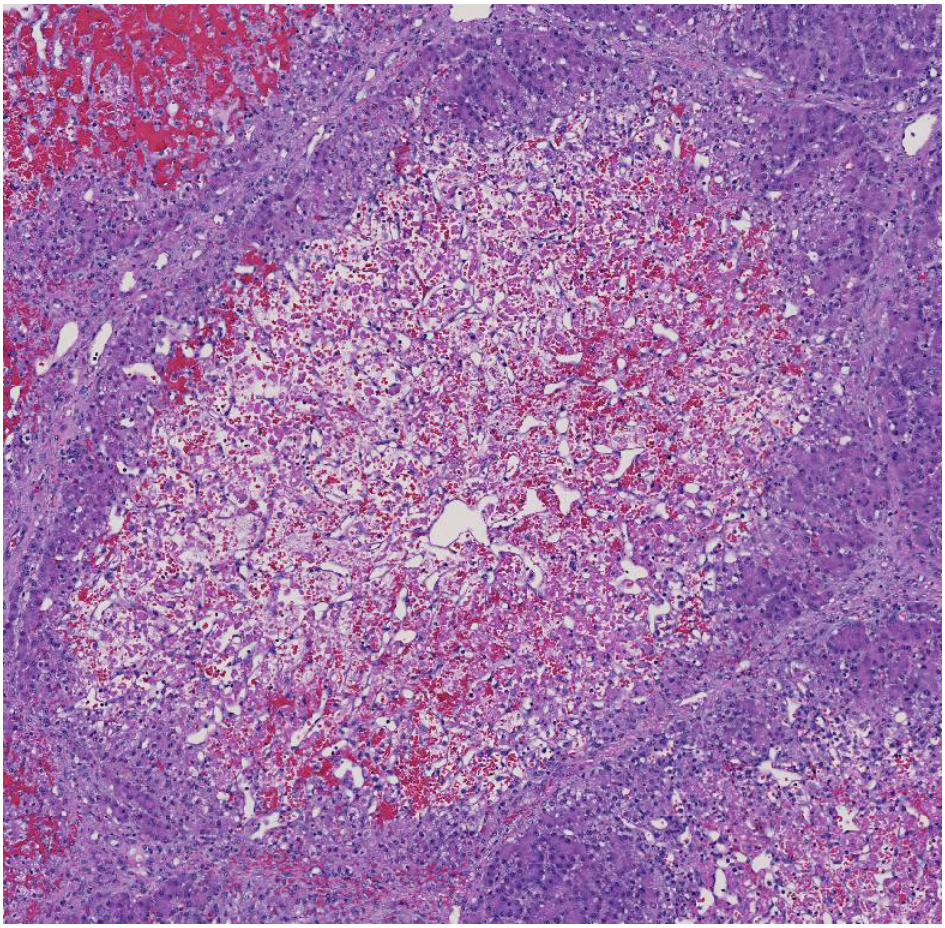
Contributor's Comment: Additional histopathologic findings included moderate multifocal fat necrosis of the mesentery, and minimal multifocal myocardial necrosis. Vitamin E/selenium deficiency was highly suspected due to the gross and histopathologic findings. Postmortem hepatic selenium concentrations were inadequate. Blood samples from the remaining three pigs in the cohort were also tested. A blood sample from one pig was marginal and the other two pigs were adequate. Vitamin E and selenium was supplemented and no clinical symptoms ensued in any of the remaining animals. The pigs had been fed a commercial pig diet and it is still unclear how this deficiency occurred. Unfortunately, there was no remaining feed from the suspect lot available for testing. There were no other reports of problems with other lots of this



3-1. Liver, piglet: The liver has multifocal to coalescing pinpoint to 3 mm dark red foci, is enlarged, and flabby. (Photo courtesy of: Division of Laboratory Animal Resources, University of Pittsburgh, http://www.dlar.pitt.edu/, http://pitt.edu/)



3-2. Liver, piglet: At subgross magnification, centrilobular and midzonal portions of each lobule exhibits necrosis and hemorrhage. (HE 0.63X)



3-3. Liver, piglet: There is diffuse centrilobular and midzonal coagulative necrosis with maintenance of sinusoidal architecture and multifocal hemorrhage. Periportal hepatocytes are spared. (HE 144X)

feed to the vendor. One possibility is that this adult diet had inadequate concentrations of vitamin E and/or selenium for such a young piglet.

In the pig, the most obvious effect of Vitamin E/ Selenium deficiency is sudden death, typically in young, fast growing weaners. Two principle presentations seen at post mortem examination are mulberry heart disease (MHD) and hepatosis dietetia (HD). Selenium is an integral part of the membrane enzyme glutathione peroxidase, reducing toxic lipid peroxidases to hydroxyl acids.[5] Vitamin E is an antioxidant and acts synergistically with selenium to protect membranes from high concentrations of lipoperoxidases.[5]

HD is characterized by hemorrhagic centrilobular to massive hepatic necrosis. Animals that survive the acute disease can develop lesions of parenchymal collapse and post necrotic scarring.[1,6] The most severe injuries are often present dorsally in the diaphragmatic liver lobes.[6] The pathogenesis of HD is not completely understood, but it is thought to be caused by a deficiency in vitamin E and/or selenium.[1] Affected animals respond to vitamin E or selenium supplementation, although, interestingly, it is difficult to experimentally induce this condition by feeding diets deficient in vitamin E and selenium.[1] It is thought that oxidative injury leads to hepatocyte necrosis since vitamin E and selenium-containing enzymes are antagonists of free radical formation and are therefore important in maintaining the stability and integrity of cellular membranes.[1,6] Experimental observations have revealed the need for concurrent deficiencies of sulfur-containing amino acids, tocopherols, and trace amounts of selenium for hepatic necrosis to develop.[6] Yellow staining of the adipose tissue is a common gross finding[6] as was seen in this case. The gallbladder may be edematous.[5] In relapsing cases, animals may be jaundice and hemorrhagic diathesis may occur with the primary manifestation of hemorrhage into or surrounding the joints.[6]

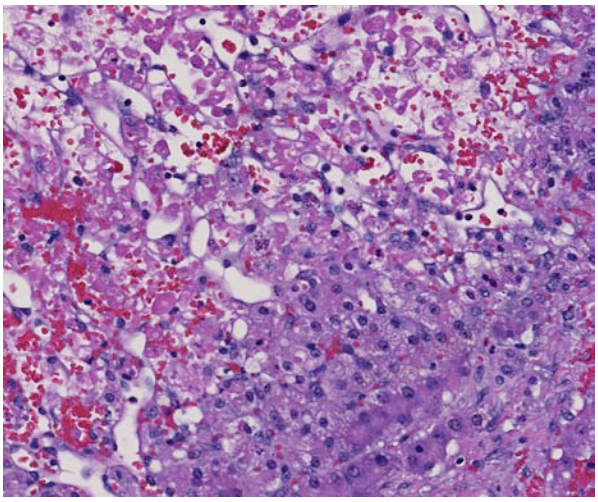
MHD (dietary microangiopathy of pigs) is characterized by fibrinoid necrosis and thrombosis of small vessels resulting in microhemorrhages.[8] The resulting hemorrhages lead to a “mulberry-like” discoloration of the epicardial surface, particularly of the right atrium.[5,8] Hydropericardium is also a common finding. There is often widespread fibrinoid necrosis of small arteries and arterioles with endothelial damage and fibrin thrombi, particularly in the

capillaries of the myocardium.[5,8] Typically, the following microscopic lesions are observed in a heart affected by MHD: interstitial hemorrhage associated with swollen cardiac myofibers that have lost cross striations, are hypereosinophilic, and have pyknotic nuclei.[4] In this entity, selenium concentrations within the liver and heart are often within normal limits.[5] Supplementation of selenium appears to decrease the incidence of HD, but not MHD. Therefore, vitamin E is considered to play a central role in the development of MHD.[5] An additional lesion that can be found in animals surviving greater than 24 hours is bilaterally symmetric softening of the cerebral white matter.[5]

Aside from the disease entities in pigs, vitamin E/selenium deficiency results in a broad spectrum of diseases in a variety of animal species and humans, including but not limited to: myopathy, steatitis, liver necrosis, and encephalomalacia because of increased oxidative stress on cells.[2]

JPC Morphologic Diagnosis: 1. Liver, hepatocytes: Necrosis, centrilobular and midzonal, diffuse, severe, with hemorrhage and periportal hepatocellular lipidosis.
2. Skeletal muscle: Degeneration and necrosis, multifocal, marked.

Conference Comment: Massive hepatic necrosis implies necrosis of the entire hepatic acinus – centrilobular, midzonal, and periportal hepatocytes. Selenium and/or vitamin E deficiency is classically associated with massive hepatic necrosis, a disease in pigs known as "hepatosis dietetica". Conference participants discussed the finding of intact, often regenerating periportal hepatocytes in this case and how it relates to the pathogenesis of massive necrosis. It was hypothesized this lesion may represent an early state of disease prior to full expression of massive necrosis. In addition to vitamin E and selenium deficiencies, other differentials for hepatic necrosis worthy of consideration include blue-green algae, *Amanita* (mushrooms), *Cestrum diurnum, Xanthium* sp. (cocklebur), iron dextran, aflatoxin, fumonisin B1, sporidesmin and pyrrolizidine alkaloids.[5] In horses, serum sickness is characterized by a near diffuse hepatic necrosis with only a narrow rim of usually vacuolated hepatocytes surrounding periportal regions.[1]



3-4. Liver, piglet: The junction of midzonal and periportal hepatocytes contain demonstrates coagulative necrosis of midzonal hepatocytes (upper left) and mild lipid accumulation (degeneration) of periportal hepatocytes (lower right). (HE 320X)

When hepatic necrosis is observed in conjunction with rhabdomyocyte necrosis, vitamin E and/or selenium deficiency should be considered most likely. Selenium deficiency does not only occur in managed feed situations, but also in pasture-raised livestock. Selenium is normally present in soil and taken up by growing plants, however, in areas such as the Pacific Northwest, the soil is naturally deficient. Poor quality forages are also deficient in vitamin E, thus both must be supplemented in some situations.[7] Deficiencies of either result in the body's reduced capacity to scavenge free radicals.

Free radicals are molecules with unpaired electrons rendering them highly reactive as they look to unload or oxidize that electron. They are generated as a normal product of mitochondrial respiration, absorption of radiant energy, enzymatic metabolism of drugs or chemicals, transition metals (Fenton reaction), nitric oxide production and by activated leukocytes during inflammation. Free radicals are very effective at killing cells, both those of pathogens and the normal host. Three reactions most relevant to free radical-induced cellular injury are lipid peroxidation of membranes, oxidation of proteins and DNA damage.[3] Antioxidants serve to protect the host by counteracting this oxidation. The importance of antioxidants in maintaining equilibrium is exemplified by the multitude of lesions associated with vitamin E and selenium deficiencies and nicely illustrated in this case.

Contributing Institution: Division of Laboratory Animal Resources
University of Pittsburgh
http://www.dlar.pitt.edu/
http://pitt.edu/

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CASE IV: O 339/11 (JPC 4019381).

Signalment: 1-day-old male cross breed piglet, *Sus scrofa.*

History: The piglet was born weak with skin lesions. It died after a day. The rest of the litter was healthy and without skin lesions.

Gross Pathology: The skin on the back was dry with cracks creating a tiger-like pattern. Severe hyperkeratosis, hyperemia and cracks were seen on abdomen, legs, ears and nose. The hooves were unaffected. Very sparse amount of coagulated milk was found in the stomach. Internal organs were without pathological findings.

Laboratory Results: None performed.

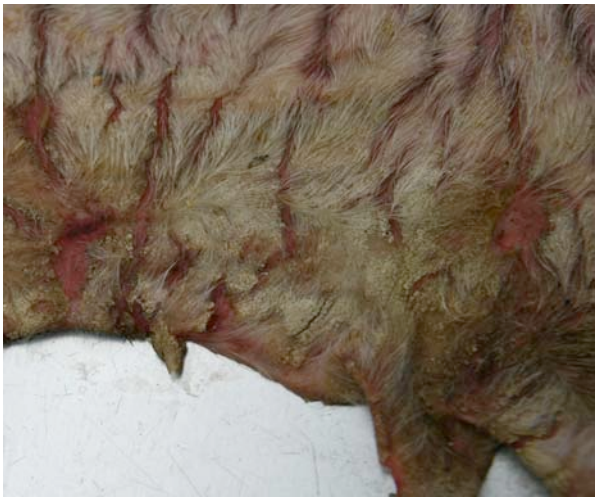
Histopathologic Description: Haired skin: The epidermis shows severe hyperplasia with compact lamellar orthokeratosis and parakeratosis with multifocal infiltration of bacteria (mixed population) and foreign material. Most of the slides show suprabasilar cleft formations. There is moderate to severe vacuolation of keratinocytes in stratum spinosum and stratum granulosum. Multifocally, there is loss of stratum granulosum. Hair follicles are unaffected and no inflammation in dermis is found.

Contributor's Morphologic Diagnosis: Haired skin, epidermal hyperplasia with marked ortho- and parakeratosis, vacuolation of keratinocytes in stratum spinosum and stratum granulosum and suprabasilar clefting, consistent with ichthyosis.

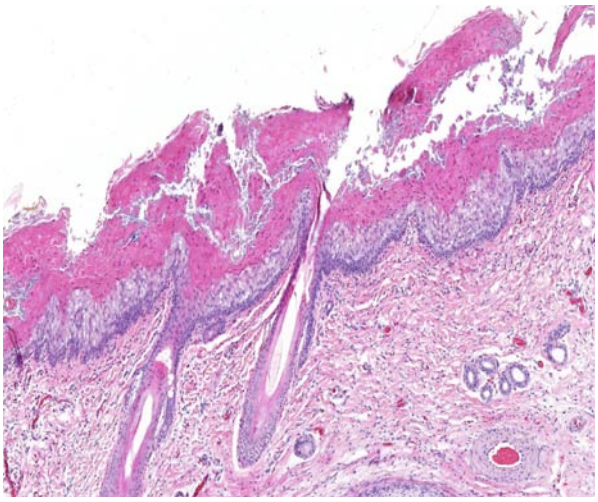
Contributor's Comment: A few cases of ichthyosis have been reported in cattle, dogs, pigs, chickens, laboratory mice and llama.[4] Most of the cases are from cattle.[1-3,7,9] In humans several different kinds of ichthyosis and syndromes including ichthyosis are reported.[8] In the veterinary literature five different types of human ichthyosis are described: ichthyosis vulgaris, x-linked ichthyosis, epidermolytic hyperkeratosis, lamellar ichthyosis and harlequin ichthyosis.[4] The main findings in the different human types are as follows:

- Ichthyosis vulgaris: Orthokeratotic hyperkeratosis and a decreased or absent granular layer.
- X-linked ichthyosis: Orthokeratotic hyperkeratosis with normal or hyperplastic granular layer.
- Epidermolytic hyperkeratosis: Orthokeratotic and parakeratotic hyperkeratosis, vacuolation of keratinocytes in the upper stratum spinosum and stratum granulosum and a markedly thickened granular layer.

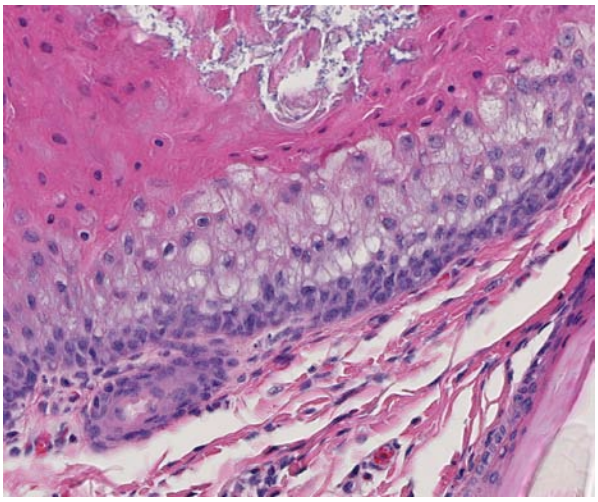
4-1. Haired skin, 1-day-old piglet: Diffusely, the skin was dry with cracks creating a tiger-like pattern. (Photo courtesy of: Department of BVF, Division of Pathology, Pharmacology & Toxicology, SLU (Swedish University of Agricultural Sciences), Box 7028, SE-750 07 Uppsala, Sweden. http://www.bvf.slu.se)



4-2. Haired skin, 1-day-old piglet: Closeup of the skin seen in 4-1. Severe hyperkeratosis is present on closer examination, with fissuring, clefting, and peeling back of the cornified scale. (Photo courtesy of: Department of BVF, Division of Pathology, Pharmacology & Toxicology, SLU (Swedish University of Agricultural Sciences), Box 7028, SE-750 07 Uppsala, Sweden. http://www.bvf.slu.se)



4-3. Haired skin, 1-day-old piglet: The epidermis is covered by a parakeratotic cornified scale up to 400 µm thick, which does not extend into hair follicles. (HE 63X)



4-4. Haired skin, 1-day-old piglet: The granular layer of the epidermis is absent. There is moderate intracellular swelling of the cells of the stratum spinosum and mild hyperplasia and disorganization of the basal layer. (HE 253X)

- Lamellar ichthyosis: Compact orthokeratotic hyperkeratosis and mild acanthosis.
- Harlequin ichthyosis: Thick compact stratum corneum with follicular hyperkeratosis and variable appearance of the stratum granulosum.

In cattle, two forms of ichthyosis are described: ichthyosis fetalis and ichthyosis congenita.[4] In ichthyosis fetalis morphological findings are similar to harlequin ichthyosis. Ichthyosis congenita has a prominent laminated orthokeratotic hyperkeratosis of the epidermis and superficial portion of hair follicles. In dogs, nonepidermolytic and epidermolytic classifications are used.[5] It has been concluded that well characterized cases of ichthyosis in animals do not correlate well with the human classification system.[4]

In this case, the lesions are somewhat similar to epidermolytic hyperkeratosis with orthokeratotic and parakeratotic hyperkeratosis and vacuolation of keratinocytes in the upper stratum spinosum and stratum granulosum, but lack the markedly thickened granular layer that is present in epidermolytic hyperkeratosis. Also lesions consistent with ichthyosis vulgaris (orthokeratotic hyperkeratosis and decreased or absent granular layer) are present. Furthermore, similarities with ichthyosis congenita are seen; however, there is no involvement of the hair follicles in the present case.

Due to the different morphologic findings in the present case and the current human classification systems our conclusion is that the disease is best regarded as ichthyosis with no further classification.

JPC Morphologic Diagnosis: Skin: Hyperkeratosis, lamellar, parakeratotic, diffuse, marked, with acanthosis and spongiosis.

Conference Comment: As is customary at the JPC, conference participants are deprived of clinical history and signalment with WSC cases to maintain relevance for board preparation. Thus, without knowing the age of this pig, participants discussed their differentials for parakeratotic hyperkeratosis in this case and in other species.

The contributor outlines ichthyosis and its variable presentation among domestic animals as the cause of hyperkeratosis in this case. Another condition associated with hyperkeratosis in swine is zinc-responsive dermatosis, which occurs in 2-4 month-old piglets and is a secondary zinc deficiency due to the presence of phytic acid in plant protein rations that affects its availability. Zinc-responsive dermatoses are more commonly identified in dogs as one of three distinct varieties: reduced absorption in Arctic breeds, generic dog food, and lethal acrodermatitis of Bull Terriers. Thallium toxicity is another historical cause in many species and vitamin A-

responsive dermatoses have been described in Cocker Spaniels as causing hyperkeratosis. Superficial necrolytic dermatitis in dogs and people characteristically exhibits parakeratosis along with laminar epidermal edema and basilar hyperplasia. This is also called hepatocutaneous syndrome due to its correlation with liver dysfunction and subsequent deranged glucose and amino acid metabolism inducing hypoaminoacidemia. Canine morbillivirus and pemphigus foliaceous both induce hyperkeratosis of the footpads in dogs.[4]

Congenital ichthyosis occurs in domestic animals as a heterogenous syndrome of autosomal recessive inheritance. Collectively, they are called autosomal recessive congenital ichthyoses (ARCIs) and their pathophysiology involves abnormal synthesis and/or secretion of lipid within the stratum corneum. Recently, specific genetic mutations have been linked to certain cornification disorders affecting particular breeds of dogs. ARCI in Golden Retrievers is associated with PNPLA1 mutations, in Jack Russell terriers an insertion in TGM1 has been described, and most recently an upstream insertion of NIPAL4 (ICHTHYIN) was identified in American bulldogs.[6]

Contributing Institution: Department of BVF
Division of Pathology
Pharmacology & Toxicology
SLU (Swedish University of Agricultural Sciences)
Box 7028, SE-750 07
Uppsala, Sweden
http://www.bvf.slu.se

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