WEDNESDAY SLIDE CONFERENCE 2016-2017

Conference 11

16 November 2016

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CASE I: Case1 (JPC 4088440).

Signalment: Eight-week-old, female, Wistar-Han rat, (*Rattus norvegicus*).

History: Control rat from a 7-day exploratory toxicity study.

Gross Pathology: In the right and left eyes, diffusely opaque lens noted at necropsy.

Laboratory results: None.

Histopathologic Description: Eye, lens: Multifocally, there is disruption and dissolution of the lenticular fibers often replaced by variably sized, irregular vacuoles which contain spherical to irregularly-shaped globular eosinophilic aggregates (Morgagnian globules). Lens epithelial cells are multifocally swollen with abundant eosinophilic microvacuolated cytoplasm (bladder cells). At one pole, lens epithelial cells become spindyloid and are separated by fine collagen fibers (fibrous metaplasia). The iris is attached to the anterior lens capsule (posterior synechia).

Eye, retina: Diffusely, the retina is disorganized. The outer nuclear layer forms numerous rosettes which surround a central space that contain eosinophilic fibrils (rods and cones). There is thinning of the outer plexiform layer and multifocal blending of the inner and outer nuclear layers. Retinal pigment epithelium is frequently vacuolated admixed with occasional nuclear cell debris and infiltration of macrophages. There is minimal hemorrhage admixed with scattered macrophages, lymphocytes, and neutrophils within the vitreous space.

Contributor's Morphologic Diagnosis: 1. Eye, lens: Cataract, diffuse, moderate to severe, with epithelial hyperplasia, posterior synechiae, and fibrous metaplasia.
2. Eye, retina: Dysplasia.
3. Eye, retina: Degeneration, multifocal, mild.

Globe, rat. At subgross, the iris leaflet at right is moderately thickened and adherent to the anterior face of the lens (posterior synechia), and the retina is moderately atrophic. (HE, 5X).

Contributor's Comment: Cataracts are the most common lenticular disease in aged Sprague-Dawley and Wistar rats. Cataracts are subclassified by the location in the lens: nuclear cataract involving the central area of the lens; cortical cataract involving the lenticular surface; and posterior capsular cataract involving the posterior surface of the lens and often arising under the capsule.[6] The case present herein is an example of the latter classification. Posterior capsular cataracts were described in 32% of aged Wistar rats and overrepresented in females.[10] The pathogenesis of cataract formation involves initial lens swelling due to loss of Na-K-dependent ATPase osmotic pumps resulting in potassium loss and sodium and calcium entry into the lens causing vacuolation and protein aggregation of the lens epithelium (bladder cell formation) with subsequent denaturation and hydrolysis of lens fibers (Morgagnian globules).[6] Cataract is considered a major cause of visual impairment in diabetic patients. The initiating mechanism in diabetic cataract formation is the generation of polyols from glucose by the aldose reductase pathway, resulting in a similar increased osmotic stress as described in spontaneous cataract formation leading to lens fiber swelling and rupture.[6]

Retinal dysplasia is the disorderly proliferation and differentiation of the retina and is characterized by blending and folding of the retinal layers, rosette formation, most commonly the inner and outer nuclear layers, and occasionally degeneration. Retinal dysplasia is an incidental developmental anomaly. These retinal folds and blending of the layers have been described in Wistar rats occasionally showing microphthalmia and cataracts.[6] Retinal dysplasia is spontaneous or inherited and is rarely progressive.[9] A linear form of retinal dysplasia has been reported in Sprague-Dawley rats at 7-10 weeks of age consisting of loss of the outer layers of the retina resulting in confluency of the inner nuclear layer with the choroid.[9] The findings
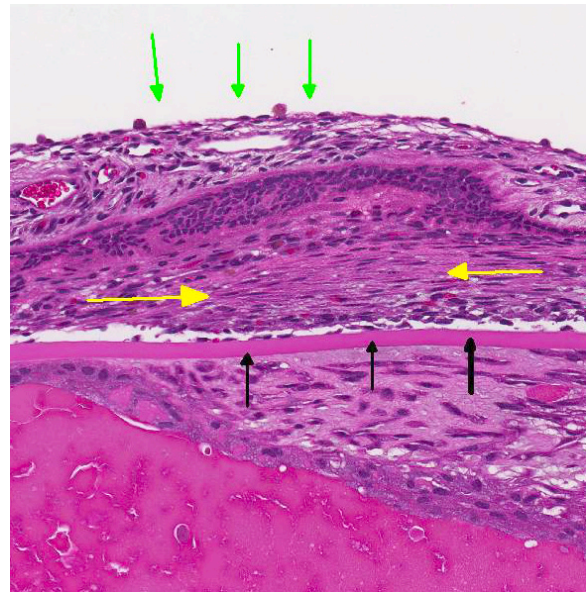

Globe, rat. The iris is covered by a pre- (green arrows) and post-iridal fibrovascular membrane (yellow arrows). The anterior lens capsule is delimited by black arrows. (HE, 80X)

described in this vehicle-treated animal were considered incidental.

JPC Diagnosis: 1. Eye, lens: Cataractous change, subcapsular, diffuse, characterized by Morgagnian globules, bladder cells, and fibrous metaplasia, with posterior synechia, Wistar-Han rat, *Rattus norvegicus*.
2. Eye, retina: Dysplasia.

Conference Comment: The contributor provides a superb example of the histologic changes associated with the formation of cataracts and retinal dysplasia in the rat. Cataracts result from exposure of the lens to a large variety of insults, including ultraviolet light, physical and chemical damage, increased intraocular pressure, numerous toxins, direct trauma, nutrient imbalance, and inflammation.[10] Spontaneous cataracts have also been reported to occur in up to 9.8% of Sprague-Dawley rats and 32% of aged (>2 years) Wistar rats.[2,10] Despite the wide variety of possible causes, the histologic lesions associated with cataractous change are relatively stereotypic across species. The lenticular lesions present in this case include Morgagnian globules, composed of bright eosinophilic globules of
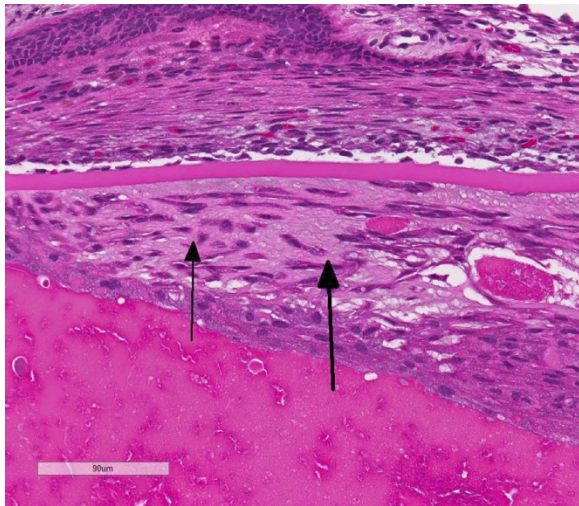

Globe, rat. There is multifocal fibrous metaplasia of subcapsular lenticular epithelium (black arrows). (HE, 228X)

denatured lens protein; bladder cells, which are large foamy nucleated cells that may represent abortive epithelial attempts at new lens fiber formation; lens epithelial hyperplasia; and posterior migration of lens epithelium followed by fibroblastic metaplasia. The latter two changes are associated with chronic cataract formation.[10] Conference participants also noted the large size of the lens resulting in narrowing of the anterior chamber, which is a normal finding in the rat.[3]

This case also generated some spirited discussion among conference participants regarding whether the retinal changes represent a dysplastic or degenerative process. The albino Wistar rat is currently one of the most popular rats used for laboratory research and is exquisitely sensitive to phototoxicity due to the lack of melanin pigment.[3,7] Given the history of bilateral lesions, strain of the rat in this case, and relatively young age of the animal, the conference moderator posited that the cataractous change and retinal lesions could be secondary to phototoxicity. Rats housed in areas of greater light intensity, such as the outer columns and top racks, are more susceptible to developing phototoxic lesions.[7] Additionally, light-induced retinal degenerative changes typically manifest as disorganization and loss of photoreceptor cells in the outer retina, vacuolation of pigmented retinal epithelium, and accumulation of intracytoplasmic lipofuscin pigment, all of which are present in this case.[1]

Conference participants also discussed the possibility that the lesions in this case represent spontaneous and dysplastic change. Albino rodents are well known to have several kinds of spontaneous ocular lesions, including corneal dystrophy (calcium deposition), cataract, and retinal
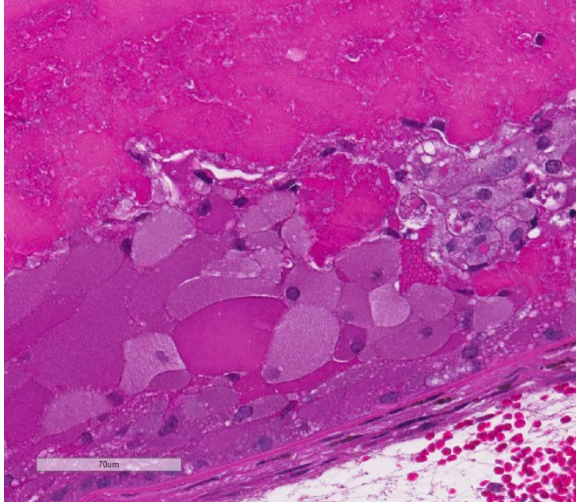
Globe, rat. At the periphery, lens fibers are swollen by large numbers of small vacuoles and often fragmented with liberation of globules of pink protein (Morgagnian cataract). (HE, 324X).

fold/dysplasia.[3] To help elucidate the possible underlying cause(s) of the retinal changes, this case was studied in consultation with the Dr. Leandro Teixeira, a board certified veterinary pathologist and recognized expert with extensive experience in the area of veterinary ocular pathology. Dr. Teixeira agrees with the contributor that the retinal rosettes, retinal folds, retinal atrophy, and blending of the inner and outer layers of the retina are common dysplastic changes in the rat, and are a result of faulty retinal development rather than a degenerative change. Similar dysplastic lesions can be induced by the administration of various toxins and carcinogens, such as cytosine arabinose, cycasin, N-methyl-N-nitrosurea, and trimethylin; however, this animal is reported to be a control rat and exposure to the aforementioned compounds is unlikely. Dysplastic lesions can be unilateral or bilateral, as in this case.[9] The lesions in the retinal pigmented epithelium, such as hypertrophy and vacuolation of pigmented epithelium and accumulation of lipofuscin, are also common mild cellular degenerative changes secondary to retinal dysplasia in the rat.
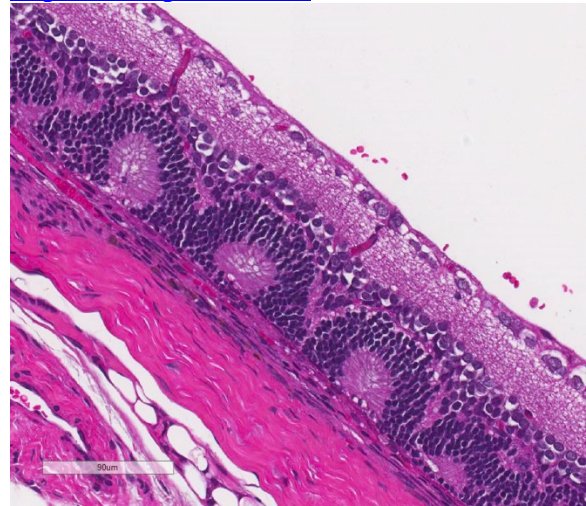
Globe, rat. There are numerous rosettes within the inner nuclear layer, the outer nuclear layer is mildly depleted. (HE, 228)

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CASE II: E3133/15 (JPC 4084739).

Signalment: Twelve-year-old, male, dwarf rabbit, (*Oryctolagus cuniculus*).

History: Both testicles were submitted for histological examination because of increasing testicular size over time.

Gross Pathology: The formalin-fixed testicles measured 9 x 7 x 4 cm and 5 x 3 x 3 cm, respectively. The testicular parenchyma was almost completely replaced bilaterally by multilobulated, solid masses with a greyish cut surface. The mass was located on both sides within the testis and did not extend beyond the tunica albuginea.

Laboratory Results: Immuno-histochemistry was applied using commercially available antibodies. Tumor cells showed a diffuse immunolabelling for Melan-A and a multifocal expression of neuron-specific enolase in about 40-50% of the tumor cells. About 20-30% of the tumor cells displayed an immunolabelling for S-100 protein and single tumor cells expressed vimentin.

Neoplastic cells were negative for cytokeratin, α-smooth muscle actin, glial fibrillary acidic protein, and myelin basic protein.
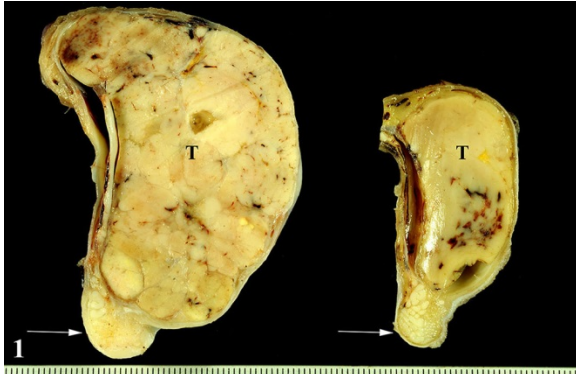
Testes, rabbit. The formalin-fixed testicles measured 9 x 7 x 4 cm and 5 x 3 x 3 cm, respectively. The testicular parenchyma was almost completely replaced bilaterally by a multilobulated, solid mass with a greyish cut surface. The mass was located on both sides within the testis and did not extend beyond the tunica albuginea. (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 17, D-30559 Hannover, Germany,
http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/

Samples for transmission electron microscopy were processed by direct pop-off technique from the HE- stained slide. The cytoplasm of the tumor cells was filled with numerous round, membrane-bound structures measuring about 500 to 1000 nm in diameter. These structures contained several membrane-bound, moderately electron-dense, round structures, measuring about 50 to 150 nm in diameter.

Histopathologic Description: The slide contains parts of the testicle, epididymis and tunica vaginalis. The majority of original testicular tissue is replaced by a multilobular, well demarcated, non-encapsulated, expansive, moderately cell-dense mass extending to the cut border on one side. Neoplastic cells are proliferated in solid fields supported by a small to moderate amount of fibrovascular stroma. The cells measure up to 70 µm in diameter and display a round to polygonal shape with an eccentrically located, 10 µm in diameter large, round to oval nucleus with finely stippled heterochromatin and one distinct, small, basophilic nucleolus. The abundant,

eosinophilic, finely granular cytoplasm is surrounded by indistinct cell borders. Tumor cells show mild anisocytosis and anisokaryosis with a mitotic rate of 0-1 per high power field. In the cytoplasm of the tumor cells, a high number of PAS and PAS-diastase-resistant positive granules are present. These granules were also shown by Luxol Fast Blue staining. Remaining seminiferous tubules are compressed and lined by single Sertoli cells.

Multifocally within the tunica vaginalis there are few inflammatory cells, mainly consisting of plasma cells, lymphocytes, and fewer macrophages. In some slides eosinophilic, homogeneous, acellular material is present within the tunica vaginalis (edema). The epithelium of epididymal tubules is flattened and the diameter of the tubules is severely increased (dilatation). Within the epididymal tubules, no spermatozoa are present.

Contributor's Morphologic Diagnosis: Testicle: Granular cell tumor with severe testicular atrophy, dilatation of epididymal tubules and mild, chronic, multifocal, lymphohistiocytic and plasmacellular infiltration of the tunica vaginalis with


Testis, rabbit. A section of testis is submitted with epididymis at bottom. The testis (top) is replaced by a multilobular neoplasm. (HE, 5X)

edema.

Contributor's Comment: Testicular tumors in rabbits represent a rarely described entity. Mainly adult individuals are affected and interstitial cell tumors are most commonly reported.[6,7,19]

Granular cell tumors occur rarely in domestic and pet animals and have been reported in different species including horses, dogs, cats, guinea pigs, and rats.[2,6] In horses, granular cell tumors represent the most common primary neoplasm of the lung.[2,9] In dogs and rarely in cats, they occur in the oral cavity.[2,11] In rats, a meningeal
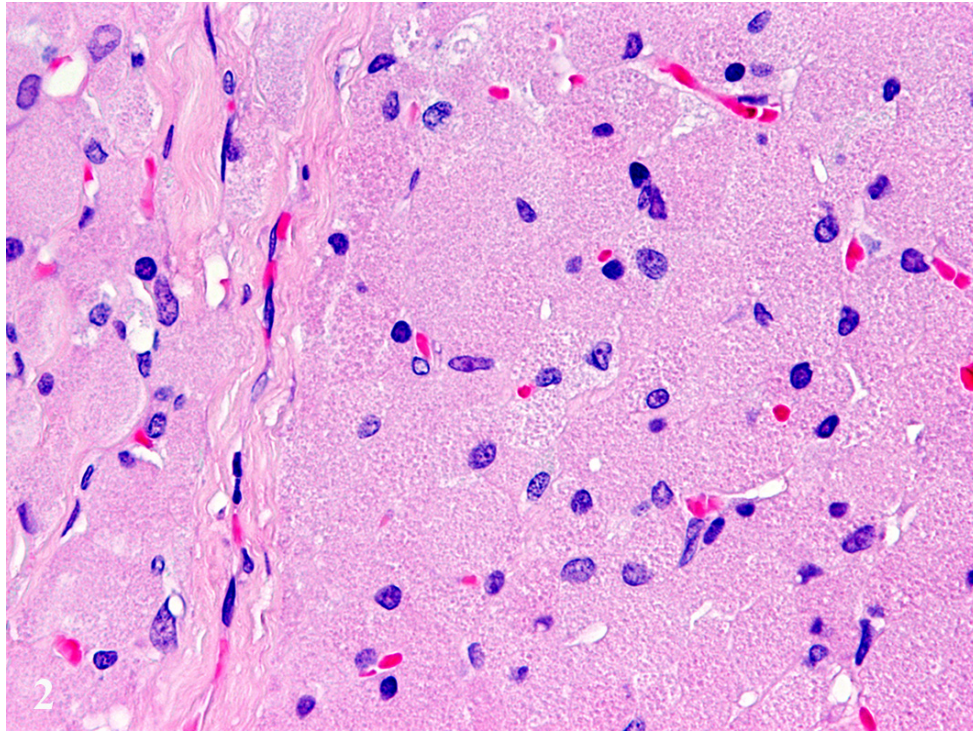
Testis, rabbit. Neoplastic cells range up to 40um in diameter, have, indistinct cell borders, and abundant pink granular cytoplasm. There is mild anisokaryosis. (HE, 400X)

localization has been described.[18] A variant of meningiomas, termed granular cell meningioma, has been reported in dogs located at the cerebral convexity, neurohypophysis, and spinal nerve roots.[1]

Genital involvement of granular cell tumors has been reported in cats affecting the vulva.[5,6] Within the right atrium of a dog a granular cell tumor, also termed myo-blastoma, has been described exhibiting typical cytoplasmic granules.[13] In guinea pigs, cutaneous granular cell tumors have been reported.[17]

The histogenetic origin of granular cell tumors remains unknown, but they are thought to derive from Schwann cells or related cells due to their histological, immunohistological and electron micro-scopic characteristics.[7,11] The immuno-reactivity of granular cells to antibodies specific for S-100 protein, Melan-A, and neuron-specific enolase supports the potential neuro-ectodermal origin.[6,7]

Furthermore, ultrastructurally numerous round cytoplasmic structures are found surrounded by a membrane with a diameter of about 500 to 1000 nm containing round electron-dense structures, measuring about 50 to 150 nm in diameter. These findings are similar to reports about the ultrastructure of equine pulmonary granular cell tumors.[12,15] The membrane-bound structures tend to contain fragments of mitochondria or other organelles and are interpreted as secondary lysosomes.

The majority of granular cell tumors are reported to be benign, except one case in a cat, showing recurrence after excision and a high degree of pleomorphism with a high mitotic rate.[11] Granular cell tumors in cats generally tend to be more anaplastic.[11]
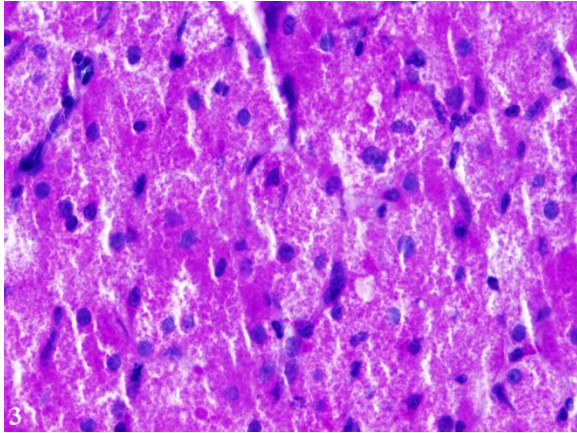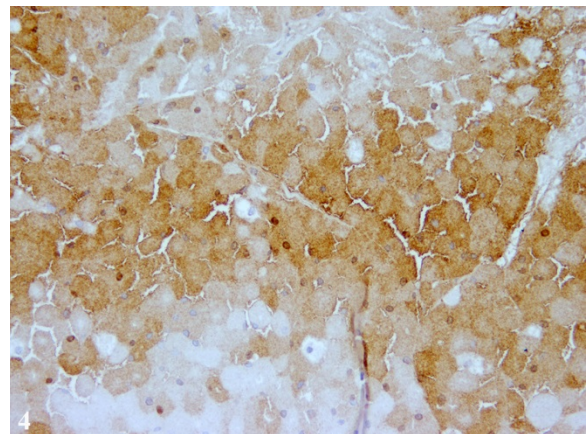

Testis, rabbit. Intracytoplasmic granules of neoplastic cells are strongly positive with a periodic acid-Schiff stain. (PAS, 400X)

As a differential diagnosis interstitial cell or Leydig cell tumor has to be considered because it shares histologic features with the granular cell tumor, e. g. eosinophilic granules within the cytoplasm.[6] In hematoxylin-eosin stained slides they are almost indistinguishable. However, testicular interstitial cell tumors lack PAS-positive and diastase-resistant cytoplasmic granules as well as Luxol Fast Blue stainable granules that are indicative for a granular cell tumor.[7,11] Furthermore, granular cell tumors express neuron-specific enolase, S-100 protein, and vimentin to a variable extent[6,7,19] as was shown in the present case. Additionally, transmission electron microscopy is a suitable tool to demonstrate the typical membrane-bound granules reported for granular cell tumors, leading to their name.[6,12]

JPC Diagnosis: Testis: Granular cell tumor, dwarf rabbit, (*Oryctolagus cuniculus*).

Conference Comment: The contributor provides an excellent summary of the major features of granular cell tumors (GCT). GCTs are histologically characterized by proliferating uniform polygonal neoplastic cells that contain abundant eosinophilic granules in their cytoplasm and a round eccentrically placed nucleus.[2] Conference participants discussed differential diagnoses for neoplasms with abundant granular eosinophilic cytoplasm to include rhabdomyomas, oncocytomas, balloon cell melanoma, and interstitial cell tumors. In addition to the histochemical and immunohistochemical stains mentioned by the contributor, transmission electron microscopy remains the best way to differentiate these histologically similar neoplasms. Ultrastructurally, oncocytomas and rhabdomyomas both contain large numbers of mitochondria which explain the abundant acidophilic granular appearance histologically.[2,3,6] In balloon cell melanoma, electron microscopically reveals numerous heterogeneous melanosomes within the cytoplasm. Interstitial cell tumors (also known as Leydig cell tumors) have an abundance of smooth endoplasmic reticulum and well-developed mitochondria with tubular and vesicular cristae. In GCT, the

Testis, rabbit. Neoplastic cells show diffuse intracytoplasmic positivity fopr Melan A. (anti-Melan A, 400X)

granules are thought to be composed of numerous membrane-bound secondary lysosomes.[2,3,6]

In a 2015 *Veterinary Pathology* article, Suzuki et al. demonstrates that in canine lingual GCT, the cytoplasmic granules are positive for LC3, p62, NBR1, and ubiquitin. LC3 is localized on the membranes of autophagosomes and is a potent marker of autophagy. In addition, LC3, p62, and NBR1 are all required for production of the autophagosome.[14] This suggests that the cytoplasmic granules found in canine lingual GCT cells are autophagolysosomes which are a subset of secondary lysosomes that result from the fusion of a primary lysosome with a phagosome containing cytoplasmic
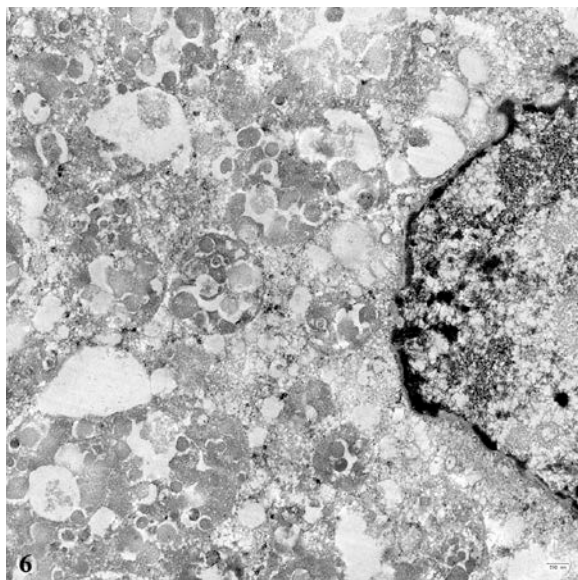
Testis, rabbit. The cytoplasm of neoplastic cells (nucleus at right is) filled with membrane-bound secondary lysosomes, which are characteristic of some types of granular cell tumors.

cellular constituents that are to be digested.[14] Recently, it has been suggested that autophagy might play a suppressive role in the initiation stages of a neoplasm by maintaining genomic stability and inducing cell senescence and autophagic death; but conversely plays a maintaining role in tumor growth in the later stages of tumorigenesis

by supplying metabolic substrate, limiting oxidative stress, and maintaining cancer stem cell population.[8]

Due to near diffuse effacement and compression of the normal testicular architecture and distortion from diffuse ductular ectasia in the adjacent epididymis, conference participants had some trouble identifying the tissue of origin in this section. The key to identification of the tissue is dependent on a close investigation of the epididymis. The epididymis is a tightly coiled mass of thin tubules that carries sperm from the testes to the ductus deferens in the male reproductive system. In this case, the epididymal tubules are markedly dilated, lined by a single layer of attenuated cuboidal epithelium and contain an abundant amount of amphophilic inspissated proteinaceous material. Some participants noted that scattered throughout the lumen are very small numbers of degenerate spermatozoa thus identifying the tissue as epididymis with adjacent testis and tunica vaginalis.

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CASE III: <u>E3133/15 (I have this as 1408 1229 and NOT E3133/15) (JPC 4084739).</u>

Signalment: Twelve-week-old, female, rabbit, (*Oryctolagus cuniculus*).

History: Per contributor: "Rabbits have been dying around 12 weeks of age. No previous clinical signs. Another rabbit had liver abscesses as well. Rule out Pasteurella, Staph, tularemia? Yellow and white abscesses."

Gross Pathology: Tissue (liver) is submitted fixed in neutral buffered formalin. The liver parenchyma contains numerous, variably-sized, 0.1-0.2cm in diameter, firm, tan, elliptical nodules.

Laboratory results: N/A

Histopathologic Description: Significant microscopic lesions are focused on portal regions. Bile ducts are markedly dilated, tortuous and encased in abundant fibrous connective tissue. The biliary epithelium is markedly hyperplastic, usually lining papillary-type projections that fill the lumen of the dilated ducts. The hyperplastic biliary epithelium contains conspicuous sexual stages of protozoal micro- and macrogametes with less conspicuous asexual developmental stages. The bile duct lumens contain myriad oocysts. The fibrous connective tissue supporting the papillary projections and surrounding the dilated bile ducts contains infiltrates by primarily lymphocytes and plasma cells. Elsewhere in the sections, the hepatocytes exhibit mild, zonal (centrilobular) atrophy.

Contributor's Morphologic Diagnosis: Liver: Marked to severe, chronic, proliferative cholangitis, lymphoplasmacytic with portal and bridging portal fibrosis and myriad, intralesional protozoa consistent with *Eimeria stiedae*.

Contributor's Comment: Hepatic coccidiosis (*Eimeria steidae*) can occur in either wild or domestic rabbits and represents an important cause of mortality in commercial rabbitries.[4] Although infections can be subclinical or even incidental findings at necropsy, significant infections result in clinical signs of anorexia, lethargy, diarrhea, distended abdomen and poor weight gain. In this case, the young rabbits were dying around 12-weeks of age without premonitory clinical signs. Because of the acute mortality and gross lesions, the attending veterinarian was concerned about
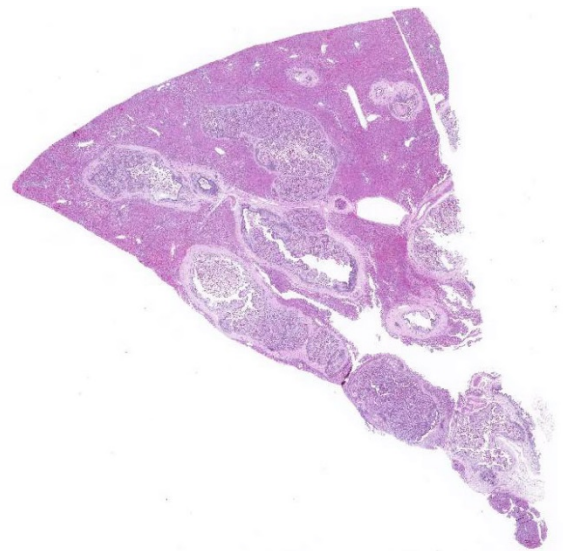
Liver, rabbit. Bile ducts are tortuous, markedly ectatic up to 4mm in diameter, and lined by markedly hyperplastic biliary epithelium. (HE, 4X)

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| Nonpathogenic	Slightly Pathogenic	Pathogenic	Highly Pathogenic
E. coecicola (appendix, sacculus rotundus, Peyers patches)	*E. perforans* (entire small intestine)	*E. media* (duodenum to jejunum)	*E. intestinalis* (lower jejunum and ileum)
	E. exingua (emall intestine	*E. magna* (jejunum and ileum)	*E. flavescens* (small intestine and cecum)
	E. vejdovskyi (ileum)	*E. piriformis* (colon)	
		E. irresidua (jejunum and ileum	

a differential diagnosis of tularemia in this case. There is some overlap of hepatic gross lesions and tularemia is not uncommon in this geographic region (Oklahoma). Concurrent infection by tularemia and hepatic coccidiosis has been previously described[2]; therefore, these gross liver lesions should always be microscopically investigated for possible tularemia, especially in wild rabbits.

After ingestion of sporulated oocysts, sporozoites penetrate the duodenal mucosa and eventually spread to the liver by lymphatic and hematogenous routes.[4] Upon reaching the liver, the sporozoites invade the biliary epithelium and enter the typical coccidian life cycle of schizogony, then gametogony with production of oocysts that are released into the bile ducts and passed into the intestine.[4] The prepatent period is approximately 15-18 days.[4]

The severity of disease appears to be related to the initial dose level of the oocyst inoculum.[3] Antemortem diagnosis is typically made by clinical signs and demonstration of oocysts in the feces. Post-mortem findings of the proliferative biliary lesions and histological identification of the organisms are pathognomonic for the disease.[4] Control of hepatic coccidiosis can be achieved with improvement of hygienic conditions, particularly removal of fecal material containing oocysts before they finish sporulation.[3] As it is probably impossible to remove all oocysts, control is augmented by prophylaxis with different anticoccidian drugs.[1,3] It should be noted that some are better than others and drugs that are effective in controlling chicken coccidiosis are not always efficacious in rabbits.

JPC Diagnosis: Liver: Cholangitis, proliferative, lymphoplasmacytic, chronic, diffuse, marked, with portal and bridging fibrosis and numerous intraepithelial coccidial schizonts, gamonts, and oocysts rabbit, *Oryctolagus cuniculus*.
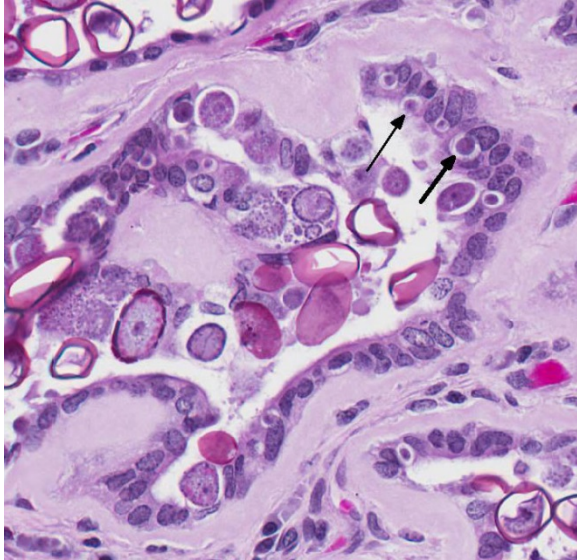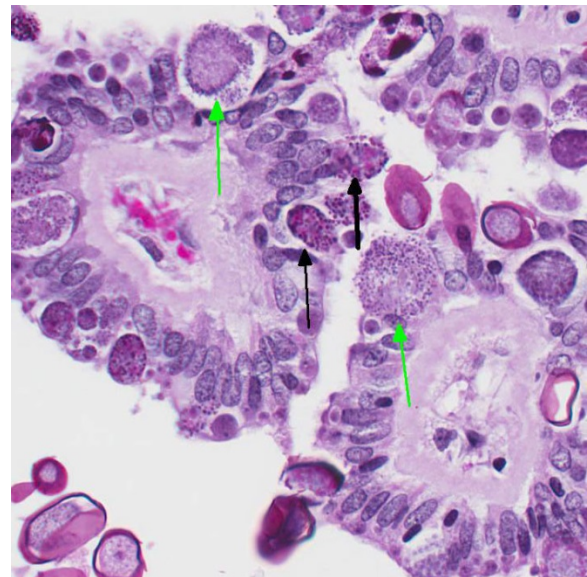
Liver, rabbit. Biliary epithelium contains developing schizonts (black arrows), gametocytes, and the lujmen is filled with shelled oocysts. (HE, 400X)

Conference Comment: The contributor provides a concise review of epidemiology, life cycle, and lesions associated with hepatic coccidiosis in rabbits. Conference participants readily identified the numerous coccidian schizonts containing merozoites, micro- and macrogametocytes, and micro- and macrogametes in the biliary epithelium and oocytes in the lumen of the markedly proliferative bile ducts. *Eimeria* sp. and *Isospora* sp. are relatively host specific coccidian parasites of the phylum Apicomplexa that typically affect the mucosal and ductular epithelial cells of the gastrointestinal tract mucosa in many different animal species.[3,4] Readers are encouraged to review WSC 2008 Conference 4 Case 3 for a list of important coccidian parasites of veterinary importance.

Eimeria sp. typically cause subclinical disease but can cause serious illness in young and immunosuppressed lagomorphs. Over 11 species of coccidian parasites have been described in rabbits and *E. stiedae* is the only coccidian parasite found in the liver and biliary epithelium.[3,4] As mentioned by the contributor, the rabbit is infected by

ingestion of sporulated oocysts in the feces with sporozoites invading the duodenal mucosa. Sporozoites have been documented in the regional lymph nodes within 12 hours, in bone marrow within 24 hours, and in the liver within 48 hours via hematogenous spread by infecting mononuclear cells.[4] Rabbits with hepatic coccidiosis are often co-infected with intestinal *Eimeria* sp. Intestinal *Eimeria* sp. target specific segments of the intestinal tract in rabbits and are divided into four groups based on pathogenicity in the following table:

Table adapted from Pakandl M[3] and Percy DH and Barthold SW[4]

Liver, rabbit. Epithelial cells contain numerous microgametocytes (green arrows) and macrogameocytes (black arrows). (HE, 400X)

The marked proliferation of the biliary epithelium, seen in this case, is secondary to destruction and regeneration of the bile duct epithelium with extensive hyperplasia of the ductular epithelium. In addition, biliary outflow may be obstructed by numerous oocysts within the lumen of the bile duct

resulting in cholestasis and further distention of the bile duct.[4]

Conference participants discussed whether this case represents cholangiohepatitis with inflammation extending into the hepatic parenchyma or simply cholangitis with secondary mild centrilobular atrophy of hepatocytes and replacement by bridging fibrous connective tissue. Conference participants overwhelmingly agreed with the contributor that while the markedly dilated bile ducts compress the adjacent hepatic parenchyma, there is no disruption of the hepatic limiting plate; thus favoring the morphologic diagnosis of lympho-plasmacytic cholangitis.
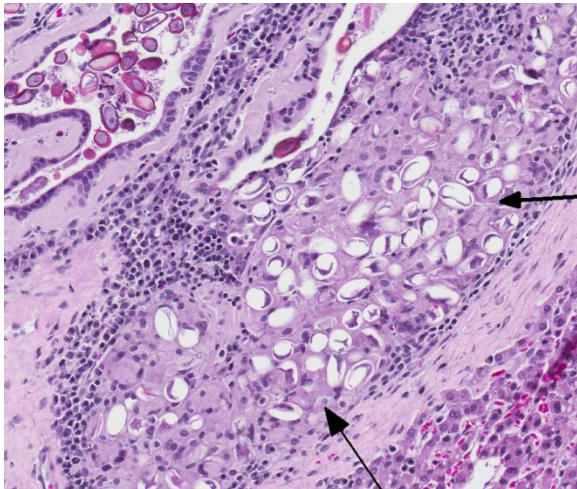
Liver, rabbit. Multifocally, aggregates of oocysts are phagocytized within epithelioid macrophages in subepithelial locations within expanded bile ducts. (HE, 400X)

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CASE IV: T4422/12 or 13 (JPC 4080931).

Signalment: Adult mole (*Talpa europaea*).

History: In the summer of 2012, a veterinarian found a dead mole in his garden in the west of the state of North-Rhine Westfalia, Germany. The animal was seen alive the day before. The vet opened and inspected the fresh carcass. He noticed lesions within lung and liver and numerous nematode larvae within the intestine. Sections of formalin-fixed liver, spleen, lung, kidney and heart were submitted for histopathological investigation.

Gross Pathology: The liver showed multiple miliary white and red foci disseminated within the parenchyma. The spleen was mildly enlarged. The lung was firm and had failed to collapse. Heart and kidneys were without any visible lesions.

Laboratory results: Histopathology of the lung revealed acute congestion, alveolar edema, and moderate suppurative broncho-

pneumonia. Nematodes are not detectable. The spleen has marked extramedullary hematopoiesis and multiple protozoal cysts within macrophages. Additionally, a chronic pericarditis and acute congestion of the kidneys is present.

Immunohistochemically, antigen of *Toxoplasma gondii* is detectable within liver and spleen using a polyclonal antiserum and the PAP method. Immunohistochemistry to detect antigen of *Neospora caninum* is negative in all tissues.

An attempt to amplify specific genomic sequences of *Toxoplasma gondii* as well as *Neospora caninum* using formalin fixed, paraffin embedded material of liver is unsuccessful (may be due to the long fixation time of the tissue samples in unbuffered formalin).

Histopathologic Description: Liver: Affecting 60% of the section, there are randomly distributed multifocal to coalescing areas of coagulative necrosis and hemorrhage, characterized by free erythrocytes in areas with hypereosinophilic hepatocytes with loss of cellular detail, pyknotic of absent nuclei but maintenance of the cell borders. Leukocytes with fragmented nuclei surround and infiltrate the necrotic regions.

Within necrotic foci and within the cytoplasm of adjacent hepatocytes are numerous protozoal cysts measuring 15 x 20 µm with a thin capsule and containing numerous 1-2 µm elongated zoites. Free within the necrotic areas are 1-2 µm elongated tachyzoites.

Portal areas are infiltrated by moderate numbers of lymphocytes, plasma cells, and eosinophils, often aggregated around bile ducts. There is a moderate increase of the number of bile ducts (hyperplasia). Within the bile duct epithelial cells, moderate
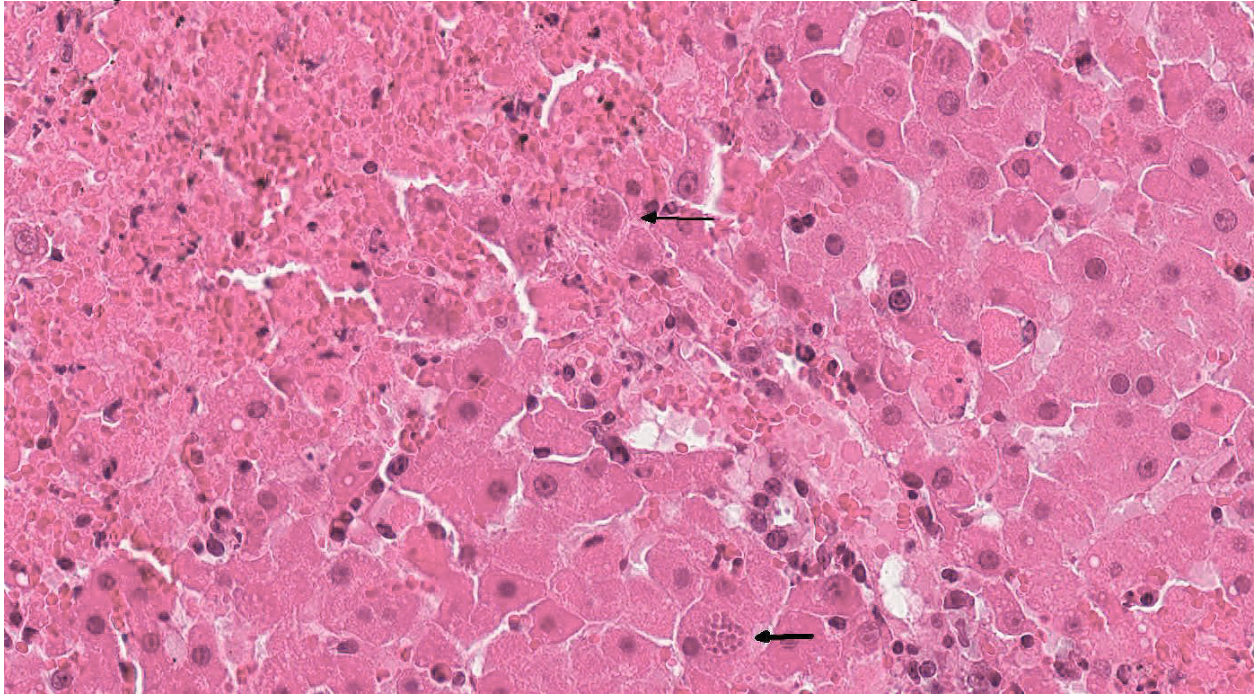
Liver, mole. Adjacent to areas of necrosis, hepatocytes contain intrcytoplasmic schizonts (black arrows) consistent with Toxoplasma gondii. (HE, 400X)

numbers of large, round to oval coccidial oocysts, about 20-40 µm in diameter, with a 1-2 µm thick eosinophilic wall, lightly basophilic granular cytoplasm, and one nucleus are visible.

Contributor's Morphologic Diagnosis: 1. Liver: Hepatitis, necrotizing, multifocal to coalescing, acute, severe, with protozoal cysts; etiology consistent with *Toxoplasma gondii*
2. Liver: Cholangitis, lymphoplasmacytic and eosinophilic, multifocal, moderate, subacute, with bile duct hyperplasia and intraepithelial coccidial oocysts: etiology consistent with *Cyclospora talpae*

Contributor's Comment: In Europe, only one member of the mole family *Talpidae* exists: a stable population of *Talpa europaea* is distributed from Spain to Russia and from Scandinavia to Greece. Moles can be found regularly in the wild (http://www.iucnredlist.org). Such small mammals are not routinely monitored regarding their health status and only rarely investigated in diagnostic laboratories; however, they are often reservoirs for disease. For example, bicolored white-toothed shrews are a proved reservoir for Borna disease virus[3] and moles may be a reservoir of hantavirus. In 60 % of captured moles in France, nova hantavirus was detected using RT-PCR.[10]

Only a few parasites have been described in moles and other insectivores.[5,11] However, it is known that *Cyclospora talpae* can be found in the epithelium of bile ducts in the liver and share similarities with *Eimeria stiedae* in rabbits. Toxoplasmosis has also been described in moles. In 1995, a similar case of combined toxoplasmosis and cyclosporiasis was documented in Bavaria.[9]

Due to their behavior, moles are a fossorial species that have extensive contact with soil and they can serve as intermediate hosts for *Toxoplasma gondii*. Moles also eat earthworms, which are identified as paratenic or transport hosts for these protozoans.[2,13] Seroprevalence for *Toxoplasma gondii* is about 40 % in wild moles in France.[1]

Toxoplasma gondii is a zoonotic protozoan which infects most mammalian and avian species. It is one of the most ubiquitous parasites and almost all homoeothermic species can be infected experimentally.[6] In domestic animals, overt disease is rare with the exception of abortion in sheep and goats. While felids are the definitive hosts (cats) and shed infective oocysts, the intermediate hosts (including cats) harbor parasitic stages in different tissue.[8,14] Predisposing factors for systemic toxoplasmosis are insufficiencies of the immune system (e.g. low levels of gamma-interferon) or concomitant infections. Recent observations regarding the pathogenesis of *Toxoplasma gondii* show that different TLRs are involved in the recognition of the parasite and there are also obvious differences between man and animal models in the effector mechanisms of toxoplasmosis.[15]
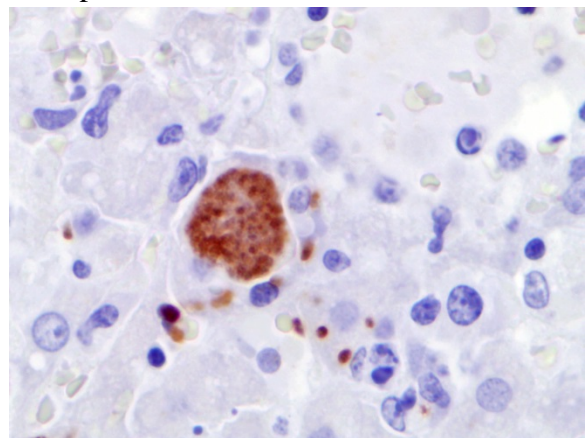
Liver, mole. Intrahepatocytic schizonts stain positively for T. gondii. (anti-T. gondii, 400X)

Macroscopic lesions in intermediate hosts infected by *Toxoplasma gondii* are variable and include splenomegaly and disseminated white foci of necrosis in liver and lung. Additional lesions may be found in myocardium and lymph nodes. In the liver toxoplasmosis is characterized histologically by foci of coagulative necrosis with less inflammatory infiltration.[8,14] Tachyzoites of *Toxoplasma gondii* may be present in hepatocytes and Kupffer cells. Special stains (Giemsa, Ziehl-Neelsen, PAS) can be applied but immunohistochemistry has been proven to detect the parasites easily within the tissue.[14] Differential diagnoses for toxoplasmosis are neosporosis and sarcosporidiosis, but the lesions, the tissue distribution as well as the affected species, are quite different.[14]

In humans, *Toxoplasma gondii* can cause abortion and stillbirth or severe neurologic and/or ocular disease in the fetus during pregnancy. The main routes of infection in man are ingestion of oocyst-contaminated soil and water or eating undercooked meat containing cysts. Other modes of transmission are less common. Most people infected after birth are asymptomatic, some may develop fever, malaise and lymphadenopathy. In immunocompromised individuals, overt disease may develop due to T cell deficiencies.[6,15] Interestingly, in human medicine, a coincidence between cerebral toxoplasmosis and mood disorders, schizophrenia, psychoses, depression or suicide and many other diseases and syndromes have been discussed.[7]

JPC Diagnosis: 1. Liver: Hepatitis, necrotizing, random, acute, marked, with protozoal cysts, mole, *Talpa europaea*.
2. Liver: Cholangitis, lymphoplasmacytic, multifocal, moderate, subacute, with biliary

hyperplasia and intraepithelial coccidian oocysts.

Conference Comment: The contributor provides an excellent review of the epidemiology, gross, and histologic lesions associated with *Toxoplasma gondii*. This obligate intracellular apicomplexan protozoan parasite is typically associated with abortion and sporadic neurologic disease in domestic animals and humans. This case is indicative of the ubiquitous nature of this parasite and its incredibly wide range of susceptible hosts. Almost all "warm-blooded" homeothermic animals are susceptible to infection with a wide variety of organ systems affected.[12] The clinical signs of toxoplasmosis are variable and depend on the organ(s) involved. Tissue cysts containing bradyzoites will not usually be associated with inflammation until the cyst ruptures inciting severe local inflammation.[12]

In addition to the differential diagnoses of neosporosis and sarcosporidiosis mentioned by the contributor, another apicomplexan protozoan parasite of European moles that should be considered is *Elleipsisoma thomsoni*. This intraerythrocytic protozoan commonly encysts in the lungs and heart, but occasionally affects the liver, spleen, and kidneys.[11] However, demonstration of protozoal tachyzoites and cysts associated with coagulative necrosis in the liver is highly suggestive of *T. gondii* infection in any homeothermic species.[12] In this case, the contributor also demonstrated *T. gondii* antigen within hepatocytes via immuno-histochemistry.

T. gondii is capable of infecting numerous cell types and its intracellular growth and replication causes eventual cell death. Attachment of the parasite to the cell occurs via the parasite major surface proteins

(SAG-1, P30) which are expressed in abundance on tachyzoites. The protozoan also binds extracellular laminin to its surface and then attaches to laminin receptors on host cells. The specialized apicomplexan club-shaped secretory rhoptry organelle then secretes lytic enzyme to facilitate cell penetration.[4,16]

The key feature of the pathogenesis of *T. gondii* is its ability to cross multiple types of barrier systems such as the intestinal mucosa, blood-brain barrier, blood-retinal barrier, and placenta by infecting endothelial cells causing vasculitis and ischemic necrosis. In addition, *T. gondii* avoids detection by the immune system by forming a parasitophorous vacuole within the host cell. The parasitophorous vacuole allows the parasite to develop while protected from the phagolysosomes of the host cell. Immuno-suppression of latently infected hosts allows cysts to rupture with reactivation of acute disease.[14,16]

Conference participants also identified coccidian oocysts within the biliary epithelium as a separate etiology from *T. gondii*. *Cyclospora talpae* is a very common extra-intestinal coccidian apicomplexan found within the biliary epithelium in wild European moles.[5] Participants noted moderate numbers of male microgamonts and female macrogamonts in the bile duct epithelium. As mentioned by the contributor, this coccidian parasite shares many similarities to *Eimeria stiedae* in rabbits.[8] Readers are encouraged to review Case 3 from this conference for a review of the epidemiology and pathogenesis of *E. stiedae* in a young immunosuppressed rabbit.

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