CASE I:  S354/10 or S370/10 (JPC 4002984).

Signalment: S354/10: Adult female barred parakeet (Bolborhynchus lineola).
S370/10: Adult male budgerigar (Melopsittacus undulatus).

History: In August 2010, sudden deaths of parakeets were noticed in an aviary close to Berlin, Germany. Three barred parakeets (Bolborhynchus lineola) and two budgerigars (Melopsittacus undulatus) died within 2-5 days after a clinical history of reduced activity and food intake. Additionally, one barred parakeet and two juvenile budgerigars were clinically affected for about two weeks but finally recovered. During the past two years, new parakeets had not been introduced into the aviary.

Gross Pathology: At necropsy, multiple petechiae were present in the myocardium, the pectoral muscles and the gizzard walls of both animals. Liver and spleen were moderately enlarged.

Laboratory results: Nested PCR and subsequent DNA sequencing of the mitochondrial cytochrome b gene derived from protozoan megalomeronts was performed. Phylogenetic comparison of 479 bp of the cytochrome b gene with published sequences of avian hematozoa found 100% identity with avian malaria parasites (Haemoproteus spp.) of European songbirds. The sequence derived from the barred parakeet was identical to the lineage TURDUS2 of Haemoproteus minutus, a
parasite previously found in the blood of the common blackbird (*Turdus merula*) in Europe. The sequence from the budgerigar was identical with the lineage TUPHI1 of a so far unnamed *Haemoproteus* sp. previously found in the blood of a song thrush (*Turdus philomelos*) in Bulgaria. In blood smears of the three only clinically affected parakeets, no parasitic structures were detectable.

**Histopathologic Description:** Skeletal muscle (S 354/10), gizzard, smooth muscle (S 370/10, both tissues with some slide variation): Numerous large, often fusiform intact and partially ruptured cyst-like protozoan structures (size up to 800 μm in diameter) are present in the skeletal muscles and the smooth muscles of the gizzards of both parakeets. The cyst-like structures (megalomeronts) have a thick pale eosinophilic outer wall, are partly compartmented by internal septae, and filled with myriads of basophilic 1-2 μm, oval merozoites. Occasionally, adjoining muscle fibers are degenerate or necrotic and scattered are mild hemorrhages. Additionally, there are multifocal infiltrations by few lymphocytes, heterophils and macrophages, individually also forming multinucleate giant cells.

**Contributor’s Morphologic Diagnosis:** Skeletal muscle (S354/10) / gizzard, smooth muscle (S370/10): Protozoan megalomeronts, numerous (variable: with myositis, minimal, lymphocytic and heterophilic and occasionally hemorrhage)

**Contributor’s Comment:** Numerous outbreaks of fatal infections with strikingly similar parasitic structures have been reported in the past decades in European aviarries.²,₄,₁₀,₁³ Psittacines from Australia, New Zealand and South America were predominantly affected. No cases of African parrots have been reported so far. Diagnosis in all reported cases was based on histopathologic detection of megalomeronts in the heart, skeletal and gizzard muscles and, to a lesser extent, in other organs such as the lung. Most often megalomeronts do only provoke minimal host response, as in the cases presented here. Previously, the parasites were described as *Leucocytozoon* spp. because of their morphologic features. Recent studies also suggest that these cases could have been infections of *Besnoitia* spp. (Sarcocystidae).²

Genetic analysis suggests *Haemoproteus* spp. from European songbirds as causative agents in both cases presented here.⁹ In Europe, asymptomatic blood infections by *Haemoproteus* spp. have been regularly observed and are especially prevalent in songbirds.¹² In the German outbreak, psittacine species endemic to South Africa and Australia were infected with two different lineages of *Haemoproteus* spp. that are known to infect blackbirds and songthrushes (Turdidae), respectively. These results suggest that infection was the result of previously unknown cross-species transmission of *Haemoproteus* spp. between birds of only distantly related phylogenetic orders.⁷,¹¹ *Haemoproteus* spp. are closely
related to *Plasmodium* spp. causing avian malaria.\(^1\) Whether the term avian malaria should be used for haemosporidian parasites other than *Plasmodium* spp. is still subject of discussion.\(^8\,^{14}\) We propose to term the disease in parrots “Haemproteosis” (G. Valkiunas, personal communication).\(^14\)

The *Haemoproteus* spp. identified are highly prevalent in the native European songbird population but normally do not cause harm to their hosts. In contrast, the cases reported here suggest that the same parasites may cause overt disease in invading species such as exotic parrots. These parasites that have adapted to European songbirds may cause fatal outbreaks in native psittacines of Australia, New Zealand, and South America that are raised in captivity. Blood-sucking insects such as biting midges (*Culicoides*), the vectors for *Haemoproteus* spp. of passerine birds in Europe may transmit the parasite from songbirds to parrots.\(^8\) Since no gametocytes in parrot blood have been found and no blood stages have ever been described in previous reports, a completely abortive development has to be assumed.

The pathogenesis in parrots is unknown. Presumably, tissue damage caused by megalomeronts in the conduction system of the heart may cause death in the affected birds.

Morphologically, the parasitic structures reported here were strikingly similar to the reported cases of numerous previous outbreaks in Europe. However, further retrospective, epidemiologic and experimental studies are needed to assess the full range of *Haemoproteus* spp. or other hematozoan parasites involved in this disease of parrots. Since blackbirds and songthrushes have been introduced to Australia and New Zealand in the 19th century, there is a concern that these parasites already have established in these areas and are potentially affecting the natural population of parrots.

**JPC Diagnosis:**
2. Proventriculus: Proventriculitis, ulcerative, focal, moderate with hemorrhage.

**Conference Comment:** There is significant slide variation in this case as a result of tissue submissions from two different psittacine birds from either the skeletal muscle or the proventriculus. Regardless of the tissue section received, conference participants readily identified numerous apicomplexan protozoal megaloschizonts undergoing various stages of degeneration within either the skeletal or smooth muscle. As mentioned by the contributor, *Haemoproteus* spp. are a large group of parasites that are found primarily in birds, but can also be seen in turtles and lizards.\(^5\) All species of this parasite are transmitted via salivary secretions of hematophagic...
biting midges, hippoboscids (louse flies), or tabanid flies containing infectious sporozoites.

Within the avian host, the sporozoites enter the vascular endothelial cells, most often within the lung, liver, bone marrow, and spleen where they undergo schizogony to form clusters of schizonts filled with merozoites. Sexual stages occur within the arthropod vector, while asexual reproduction occurs within the vertebrate host. Once the schizont ruptures, merozoites are released into the circulation, where they infect host red blood cells. Within erythrocytes, merozoites transform into macro or microgamonts. *Hemoproteus* infection is often subclinical in birds and clinical disease is associated with anemia due to parasitism of the host red blood cells. Additionally, clinically affected animals are usually concurrently immunocompromised. Severely affected animals can acutely die with no overt clinical signs.\(^{3,5,10}\)

This case likely represents a manifestation of *Haemoproteus* infection associated with the pre-erythrocyte stage, characterized by large megaloschizonts within both the skeletal and smooth muscle. The conference moderator noted that in this stage, intra-erythrocytic gametocytes are not seen, consistent with the history provided by the contributor. Rather than anemia secondary to erythrocyte parasitism, lesions are associated with megaloschizonts within a variety of cell types, such as the muscular layers of the proventriculus and skeletal muscle, in this case.\(^{3,5}\) The conference moderator also noted that similar to previously reported cases, the megaloschizonts in this case are large (200-500 um), have compartmentalized internal septae, and are often associated with hemorrhage.\(^5\) Within mature megaloschizonts, there are numerous packeted merozoites within structures known as cytomeres. As they mature, megaloschizonts rupture and merozoites are released into the blood stream. These merozoites then infect erythrocytes and become gametocytes, ready to be ingested by biting flies to complete the life cycle of the parasite.\(^{3,5,10}\) In previously reported cases, rupture of megaloschizonts and the release of merozites caused intense inflammation, hemorrhage, and necrosis; however, in this case there is only very mild inflammation. It is unclear if the ulcerative proventriculitis with hemorrhage and hemosiderin laden macrophages in the koilin membrane is related to the parasite. Despite the relatively mild lesions present in these tissue sections, conference participants agreed with the contributor that there were likely megaloschizonts in the heart that interfered with signal conduction, ultimately leading to the death of these birds.

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CASE II: 3019-15 (JPC 4081674).

Signalment: 11-month-old intact male golden retriever, (*Canis familiaris*).

History: This 11-month-old Golden retriever dog was not eating for 5 days, did not defecate, vomited once over this period, and died suddenly. Necropsy was requested and performed.

Gross Pathology: The dog was in good nutritional status (body condition score 3/5), well-hydrated and had mild rigor mortis. There was generalized pallor of mucous membrane and non-haired dermis. Thoracic cavity contained approximately 1 liter of dark red, cloudy hemorrhagic fluid. Parietal pleural surfaces of the mediastinum, diaphragmatic surface as well as the dorsal aspect of the pleural cavity were diffusely thickened, friable, mottled red and white. Few fragments of friable dark red blood clot were present in the ventral thoracic cavity.

Immediately caudal to the heart base, 14 cm length of the descending aorta was adhered to 11 cm of the immediately adjacent esophagus and formed a fibrous, dark red to white, hemorrhagic mass. Affected segment of descending aorta wall was markedly thickened and had multifocal mural nodules and tunica intima ulceration. There was focal perforation of ventral aortic wall. Tissues and subpleural parenchyma around affected segment of aorta was thickened, hemorrhagic, soft, edematous and friable. The esophageal wall was multifocally thickened by coalescing round to oval nodules. Embedded within the aortic and esophageal nodules were 4-6cm long, 1mm diameter dark red nematodes (*Spirocera lupi*).

Laboratory results: N/A

Histopathologic Description: Aorta: The aorta is characterized by variable loss of architecture with replacement by multifocal to coalescing necrosis, intense neutrophilic, eosinophilic, granulomatous and lymphoplasmacytic infiltrate that expands aorta wall. Abundant fibrin and multiple foci of dystrophic mineralization are present among the inflammatory cells. Embedded within tunica intima are cross and tangential sections of nematode organisms characterized by 0.6mm to 0.1mm diameter width, smooth cuticle, coelomyarian-polymyarian musculature, large lateral hypodermal chords, abundant amphophilic to basophilic fluid in the pseudocoelom and an intestine composed of individual cuboidal cells, each with a prominent brush border. The inflammation and necrosis are supported by dense fibrocollagenous stroma that extends into fibroadipose tissue around the aorta. Scattered within the inflammation and granulation tissue are small numbers of embryonated nematode eggs. Focally, the necrosis extends through entire aortic wall and there is continuity of aortic lumen to beyond tunica adventitia (focal rupture). The tunica adventitia is thickened by proliferation of mesothelial cells, granulation tissue and hemorrhagic exudate.
Esophagus: The grossly observed esophageal masses are characterized by expansion of the esophageal wall, mural architecture and replacement by marked necrosis, fibrin exudation and, intense mixed cell infiltrate. The inflammatory cells are intermixed with a large amount of fibrocollagenous stroma, which dissects and replaces tunica muscularis multifocally. Within the necrosis and inflammation, and embedded within esophageal wall are cross sections of 0.2 to 0.3mm diameter nematodes of similar morphology as that described above for the aorta. In a few nodules, among the inflammation and fibrocollagenous stroma are non-encapsulated lobules of atypical and dysplastic mesenchymal cells. Mesenchymal cells are spindle-shaped, arranged in streams, whorls, and bundles supported by loose fibrocollagenous to myxomatous stroma. Mitoses are present at 3 per 10 high power (400x) fields, and anisokaryosis and anisocytosis are both mild. Few scattered nematode eggs are present within the esophageal inflammation, necrosis and atypical mesenchymal cell proliferations.

Contributor’s Morphologic Diagnoses:

Aorta:
1. Severe focally extensive, chronic active, necrotizing, pyogranulomatous, eosinophilic, lymphoplasmacytic arteritis with granulating fibrosis and spirurid nematodes (*Spirocerca lupi*) and ova
2. Focal perforation.

Esophagus:
1. Severe, focally extensive, fibrinonecrotizing, eosinophilic, pyogranulomatous, lymphoplasmacytic esophagitis with marked granulating fibrosis, and intralobules *Spirocerca lupi* and ova

Aorta and esophagus, dog. There are multifocal thickenings of the ventral aortic wall and dorsal esophagus. A pair of forceps demonstrates the aortic perforation responsible for the hemothorax in this individual. Lung, penguin: Numerous small foci of necrosis, which are highlighted as they interrupt the diffuse congestion of the air capillary walls, are present throughout the section. (HE, 400X).
2. Multifocal, atypical, dysplastic mesenchymal cell proliferation (fibrosarcoma)

**Contributor’s Comment**: Major findings in this dog include the hemothorax secondary to the ruptured aorta, as well as nematode-induced inflammation and fibrosis in the aorta and esophagus. There is also evidence of neoplastic change within some of the esophageal nodule. The cause of death in this dog is most associated with the hemothorax which would also explain the generalized pallor in this dog at presentation for necropsy. The morphological features of the nematodes indicate a spirurid, most consistent with *Spirocerca lupi* based on the location and lesion associated with this dog’s pathology.

*Spirocerca lupi* is a spirurid nematode that parasitizes esophageal wall of dogs and some other carnivores and is mostly seen in warm climates where dung beetles act as intermediate hosts. The life cycle of the parasite involves passage of parasite eggs from the definitive host (canine) via feces followed by ingestion by coprophagous (dung-eating) beetle. The larva develops into infective third stage and encysts in the beetle’s tissues. The beetle is then eaten by a definitive host or by a paratenic host (e.g. lizard, chicken or rodents) which in turn is eaten by the definitive host (dog). The ingested larvae penetrate gastric mucosa and migrate to the aorta via arteries, and embed in the intima aorta and form parasitic granulomas in aortic adventitia at the caudal thoracic area. After a few months, the nematodes migrate to the immediately underlying esophagus, develop to adulthood and form cystic granulomas in distal esophagus submucosa or gastric cardia. Esophageal lesions associated with *Spirocerca* are characterized by granulomatous inflammation with highly reactive fibroblasts. These foci can develop into fibrosarcoma and osteosarcoma, with local tissue invasion and pulmonary metastasis. The parasites can extend from nodules through fistulas that lead into esophageal lumen, where female worms can oviposit into gastrointestinal tract. Aortic lesions are characterized by necrotizing, hemorrhagic, eosinophilic vasculitis, aneurysm and rare aortic rupture such as in this case. Advanced cases of *Spirocerca lupi* infection, such as in this case, are associated with guarded to poor prognosis.

Aortic rupture due to vasculitis and vascular wall compromise secondary to *Spirocerca lupi* has been reported to cause a fatal hemothorax in older literature and likely occurred in this case. Alternatively, perforation of the esophagus due to damaged esophageal wall from parasitic nodules and/or associated neoplasms can result in leakage of ingesta contents into thoracic cavity and a pyothorax.

While the majority of the limited land in Singapore consists of built up urban environment, several species of dung beetles still do exist, typically in the remaining forested areas. These insects, along with wild reptiles and rodents, are the suspected...
intermediate hosts that infect dogs that come into contact with them in the outdoor environment.

**JPC Diagnosis:** 1. Esophagus: Esophagitis, granulomatous and eosinophilic, chronic, focal, severe, with adult spirurid nematodes and eggs, Golden retriever, *Canis familiaris*. 2. Aorta: Arteritis, necrogranulomatous and eosinophilic, chronic, diffuse, severe with fibrin thrombi and marked mural fibrosis.

**Conference Comment:** There is significant slide variability in this case and not all conference participants received slides with nice cross sections of the adult nematode. However, all examined slides did, at minimum, contain variable numbers of small 20x40 um thick shelled oval embryonated eggs, highly characteristic of spirurids. The conference moderator noted that the presence of spirurid eggs within proliferative and granulomatous lesions within the esophageal wall of a canine should point observers to the diagnosis of *Spirocerca lupi*, even in the absence of adult nematode cross sections in the tissue. In sections that contained adult *Spirocerca lupi*, conference participants described nematodes as 600-800 um in diameter with an 8 um thick smooth cuticle, coelomyarian-polymyarian musculature, prominent lateral cords, a pseudocoelom containing a moderate amount of eosinophilic material, an intestine lined by uninucleate columnar epithelium with a prominent brush border, and male or female reproductive organs. Females have distended uteri containing numerous spirurid eggs.

Other esophageal parasites discussed by conference participants include *Gon-gylonema* sp. in ruminants, pigs, horses, primates, and rodents; *Gastophilus* sp. in horses; and *Hypoderma lineatum* in ruminants. With the exception of *Spirocerca lupi*, parasitic diseases of the esophagus are usually of little significance to the host.

Conference participants were unable to reach a consensus regarding the multifocal nodular proliferation of atypical mesenchymal cells within the muscular layers of the esophagus. Some agreed with the contributor, that this likely represents an early neoplastic transformation of the tunica muscularis, while others favored a proliferation of highly activated fibroblasts secondary to chronic marked inflammation. Interestingly, *Spirocerca lupi* is known for the ability to induce malignant transformation within the wall of its associated esophageal granulomas. Mesenchymal neoplasms fibrosarcoma and osteosarcoma are the most common malignancies associated with the parasite. Neoplasms that arise from areas of marked granulomatous inflammation are often highly infiltrative with frequent metastasis to the lungs. Additionally, *Spirocerca lupi* induced thoracic neoplasia has occasionally been associated with the development of hypertrophic osteopathy, a condition that results in the periosteal new bone formation.
proliferation in the distal extremities. Other potential sequelae include dysphagia, aortic aneurysm, and aortic rupture leading to acute hemothorax, seen in this case.2,4

Attendees discussed other parasites that induce neoplastic transformation in veterinary species. Residents at the Joint Pathology Center use the acronym SOCCS-T as a mnemonic device to remember which parasites are associated with neoplastic transformation. *Spirocerca lupi* causes fibrosarcoma and osteosarcoma; *Opisthorchis felineus* causes cholangiocarcinoma in cats and humans; *Cysticercus fasicolaris* can induce hepatic sarcoma in rats; *Clonorchis sinensis* is associated with cholangiocarcinoma in cats and people; *Schistosoma haematobium* is a trematode that can induce transitional cell carcinoma of urinary bladder in people; and *Trichosomoides crassicauda* can cause papillomas of the urothelium in rats.2-4

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**References:**

**CASE III:** 14-6189 (JPC 4084011).

**Signalment:** 27-year-old male African penguin, (*Spheniscus demersus*).

**History:** This captive bird was at the end of a molting period and had been depressed and lethargic for a couple of days before being found dead.

**Gross Pathology:** The lungs were described as congested by the submitting institution,
which performed the necropsy. A complete set of formalin-fixed tissues was submitted for histopathologic examination.

**Laboratory results:**

Immunohistochemistry:

In house: *Toxoplasma gondii* (commercial rabbit polyclonal antibody) – weakly positive with some non-specific background staining

CAHFS Laboratory, Davis, CA
*Sarcocystis* sp. (rabbit polyclonal antibody) – strongly positive
*Toxoplasma gondii* (rabbit polyclonal antibody) – negative

PCR:
Wildlife Conservation Society, Bronx Zoo, NY
Two assays on formalin-fixed, paraffin-embedded lung: Apicomplexan 18S rRNA gene and Sarcocystis-specific ITS-1 18S rRNA gene – both positive – sequences of products match *Sarcocystis falcata*una

**Histopathologic Description:** Lung:

Varially obscuring the parenchyma throughout the section are interstitial and luminal accumulations of inflammatory cells, eosinophilic fluid (edema), and sometimes necrotic cellular debris. Moderate numbers of macrophages and fewer heterophils are present in the lumens of parabronchi, atria and air capillaries mixed with amorphous to globular eosinophilic material and erythrocytes, and these air spaces are often lined by hypertrophied epithelial cells. Rarely, within disrupted air capillary walls, there are irregularly round to slightly elongated clusters of approximately 2-4 X 1 µm, fusiform, basophilic, protozoal zoites. There is prominent infiltration of septal and perivascular interstitium by moderate to large numbers of lymphocytes, plasma cells, and fewer macrophages, along with numerous nodular aggregates of macrophages containing brown anisotropic pigment (anthracosis).

**Contributor’s Morphologic Diagnosis:**

Lung: severe diffuse necrotizing and lymphohistiocytic interstitial pneumonia
with epithelial hypertrophy, edema, hemorrhage and protozoal schizonts.

**Contributor’s Comment:** Death of this penguin was due to a severe interstitial pneumonia caused by a protozoal infection. Occasional clusters of very small, elongated protozoal organisms present in air capillary walls throughout the lung were suggestive of apicomplexan schizonts, so differential diagnoses initially included *Sarcocystis* sp., *Toxoplasma gondii*, and *Plasmodium* sp. Both malaria and toxoplasmosis have previously been reported as causes of interstitial pneumonia in penguins,⁶,¹³ whereas sarcocystosis is a common cause of pneumonia in various avian species but has not been described in penguins. The only relevant immunohistochemical stain available in house was a rabbit polyclonal antibody against *T. gondii* (BioGenex, San Ramon, CA). Organisms exhibited weak but positive reactivity with this immunostain. Because cross-reactivity between different cyst-forming apicomplexans has been reported with polyclonal antibodies, such as between *T. gondii* and *Neospora caninum*¹,¹¹ and between *N. caninum* and *Sarcocystis* sp.,¹² more specific diagnostic methods were pursued. No fresh/frozen lung tissue was available, so formalin-fixed paraffin-embedded lung tissue was submitted to the Wildlife Conservation Society for apicomplexan and *Sarcocystis*-specific PCR assays. The DNA sequences obtained from both of these positive assays were 100% matches to *Sarcocystis falcatula*. The next closest match was *S. neurona* with a 98-99% identity. The pneumonia in this penguin is therefore attributed to *S. falcatula*. Subsequent additional immunohistochemical staining performed at the CAHFS laboratory in Davis, CA, confirmed strongly positive labelling of the protozoa with a *Sarcocystis* antibody. The *Toxoplasma gondii*
immunohistochemical stain performed by this laboratory was negative.

In North America, the definitive host for *S. falcatula* and the closely related *S. neurona* is the Virginia opossum, *Didelphis virginiana*, which sheds infective sporocysts in feces.9 Opossums had been seen on the premises of the aquarium institution where this penguin was housed. Infection of various bird species, most naturally grackles and cowbirds, as intermediate hosts is typically through ingestion of food contaminated with opossum feces, but insects such as cockroaches can serve as mechanical vectors.2 In the intermediate host, asexual reproduction (merogony/schizogony) occurs in endothelial cells in various tissues but especially in the lung.9,10 The merozoites produced eventually infect skeletal and cardiac muscle cells and form sarcocysts filled with bradyzoites, which are infective to the definitive host upon ingestion of the intermediate host tissue.9 Replication in pulmonary endothelial cells can result in severe interstitial pneumonia, the most common form of fatal infection, although two other clinical forms of disease have been characterized: a neurologic form and a muscular form.14 The neurologic form has been reported in psittacines and raptors.14,15

Fatal pulmonary infection with *S. falcatula* has been reported most often in psittacines,2-5,14 and this is thought to be the first report in a penguin. The microscopic features of the pneumonia in this penguin were similar to what has been described in other avian species infected with *S. falcatula*, namely edema, fibrin deposition, congestion, hemorrhage, mononuclear inflammatory infiltrates, endothelial cell lysis, and pneumocyte hyperplasia.7,10,14 The schizonts were rare in this case, occasionally exhibiting a “sunburst” or somewhat elongated arrangement, but characteristic serpentine forms that conform to pulmonary capillary lumens2-5,7-10,14 were not seen in this penguin. Identification of the infected cells as endothelial cells was not possible in this case from microscopic examination alone. A complete set of tissues was examined histologically from this penguin, including brain, skeletal muscle and heart, but no extrapulmonary schizonts or sarcocysts were seen. There was, however, a mild lymphohistiocytic portal hepatitis in this bird, which can be seen with *S. falcatula* infection,7,8 and for which no other etiology was identified.

**JPC Diagnosis:** Lung: Pneumonia, interstitial, necrotizing and lymphohistiocytic, multifocal, moderate with intracellular apicomplexan zoites, African penguin, *Spheniscus demersus*.

**Conference Comment:** The contributor provides an outstanding summary of avian
sarcocystosis. The genus *Sarcocystis* is a large group of cyst-forming apicomplexan protozoal coccidian parasites that affect mammals, birds, and reptiles. They have also been rarely reported in amphibians and fish.3,4,11,15 *Sarcocystis falcatula*, similar to all other members of this genus, utilizes a two-host life cycle based on the predator and prey dynamic. Carnivores and omnivores are the definitive hosts and become infected by preying upon intermediate hosts (usually herbivores) which contain mature sarcocysts in muscular or neural tissue. After digestion of the sarcocyst wall, bradyzoites are released and invade the intestinal epithelium of the carnivorous definitive host. These bradyzoites undergo sexual gametogony and develop into micro-(male), macro-(female) gamonts. Fertilization leads to the formation of infective oocysts, which sporulate in the intestinal lumen and are shed in the feces. Similar to other common apicomplexan coccidian parasites (*Besnoitia, Frenkelia, Isospora, Toxoplasma*), sporulated oocysts contain two sporocysts with four sporozoites each.3,9

Sporulated oocysts are then ingested by susceptible intermediate hosts, such as herbivorous mammals or birds. After ingestion by the intermediate host, sporozoites excyst in the intestine and undergo two generations of asexual merogony (schizogony). Sporozoites are released and first migrate to endothelial cells where they undergo the first two generations of asexual reproduction, resulting in the development of meronts.3,9,11,15 In birds, merogony is most pronounced in vascular endothelial cells within the lungs. Pulmonary capillary endothelial cells can become markedly swollen and inflamed, leading to lymphohistiocytic vasculitis, airway edema, interstitial pneumonia, and even vascular obstruction in birds with a heavy protozoan burden.11,15 Merogony has also been reported in vessels of the liver, pancreas, spleen, adrenal glands and heart.11 In animals that survive the maturation of the second generation of meronts, merozoites are liberated from meronts within capillaries and enter circulating mononuclear cells. Merozoites then undergo endodyogeny and enter muscle fibers to eventually form sarcocysts containing bradyzoites. They will remain encysted and viable in the muscular or nervous tissue until they are ingested by the definitive host to complete the lifecycle. There are over 90 *Sarcocystis* sp. known to infect mammals, birds, and reptiles. The vast majority of species are considered non-pathogenic; however, some species, such as *S. falcatula* in birds, are associated with severe clinical disease in the intermediate host.3,4,11,15

*Lung, penguin: Schizonts stain strongly positive with a non-specific anti-Sarcocystis antibody. (Photo courtesy of: Department of Pathobiology and Veterinary Science, Connecticut Veterinary Medical Diagnostic Laboratory, College of Agriculture, Health and Natural Resources, University of Connecticut, 61 North Eagleville Rd, Unit 3089 Storrs, CT 06269-3089 (anti-Sarcocyst, 200X)*
As mentioned by the contributor, the only known definitive host for this protozoan in North America is the opossum (Didelphis virginiana). Most Sarcocystis sp. infect a specific intermediate host; however, Sarcocystis falcatula is unique in that it can infect a heterogeneous population of avian intermediate hosts from numerous avian species (Passeriformes, Psittaciformes, and Columbiformes).

Birds become infected by eating feed contaminated with opossum feces containing infective oocysts or sporocysts or ingestion of cockroaches, which is the main mechanical vector for the parasite.2

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**References:**
CASE IV: E2889/15 (JPC 4084734).

**Signalment:** Six-year-old male castrated mixed-breed dog, (*Canis familiaris*).

**History:** The dog was presented to a local practitioner with suppurative conjunctivitis and permanent lacrimation. During examination a worm-like structure was found within the conjunctival sac. The worm measured about 2 cm long and 0.2 cm in diameter. The complete globe with adjacent thickened periorbital tissue was surgically removed, fixed in formalin and submitted for histological examination. Weeks later, clinical signs recurred at the other eye, again with evidence of worm-like structures within the conjunctival sac. No further clinical follow-up is available.

**Gross Pathology:** Macroscopically, the retrobulbar tissue showed marked irregular thickening, firm consistency and a greyish-yellow appearance. In the periorbital tissue, multifocal nodular accumulations of curled, thin structures were evident.

**Laboratory results:** None.

**Histopathologic Description:** The cross-section of the globe reveals severe and irregular expansion of the periorcular tissue by infiltration of numerous macrophages, fewer plasma cells, and rare lymphocytes. These infiltrates extend circumferentially into choroid, sclera, adipose tissue and periorbital musculature. Granulation tissue
composed of fibroblasts, collagen fibers, and newly formed blood vessels shows multifocal severe infiltration of neutrophilic granulocytes. Within the inflammatory infiltration, multiple cross and longitudinal sections of metazoan parasites, measuring approximately 200 – 300 µm in diameter are present. Parasites (nematodes) show a 4 – 5 µm thick, smooth, eosinophilic cuticle with cuticular ridges seen on longitudinal sections. Remnants of coelomyarian musculature are visible because the musculature is mainly atrophied and replaced by hypodermal tissue. Within the spacious body cavity, there is a faint intestine and large genital organs with remnants of eggs present. Occasionally, nematode structures display a homogenous eosinophilic appearance with structural loss (necrosis of nematodes). Furthermore, the amount of periorbital fibrous tissue composed of collagen fibers and fibroblasts is moderately increased (fibrosis). The retinal pigment epithelium displays focally a mild hypertrophy with tombstone-like appearance. The retina is detached from the retinal pigment epithelium.

**Contributor’s Morphologic Diagnosis:** Globe and peri orbital tissue: Scleritis, severe, multifocal to coalescing, chronic, granulomatous and pyogranulomatous with mild, multifocal, granulomatous choroiditis, myositis, steatitis and numerous nematodes.

**Contributor’s Comment:** During the past decades, an increasing number of cases of ocular onchocerciasis have been reported in dogs from Europe and the United States. In dogs, it is known to occur as an acute or chronic ocular disease with nodules located on the eyelids, conjunctiva, and sclera. Infections may remain undetected if verminous nodules are located within the retrobulbar areas, as in the present case. In this case, *Onchocerca* sp. are suspected as the causative pathogen, because histologic criteria including atrophied coelomyarian musculature, a very small intestine, and cuticular annulations on female nematodes similar to those described in the literature.

For a long time, ocular onchocercosis in dogs was thought to be an aberrant infection with *Onchocerca (O.) lienalis*, commonly found in cattle. The assumption that canine ocular onchocerciasis is associated with *O. lienalis* was only based on the microscopic morphology. In contrast, a previously unrecognized species of *Onchocerca* was suspected to be spilling over from wild ungulates to canines, but it turned out that the nucleotide sequences of *Onchocerca* species found in canines are unique within the genus. An analysis of the molecular genetic data suggested that the causative agent is rather *O. lupi* than *O. lienalis*.6

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*Eye, dog. Retrobulbar granuloma, eye, dog. Higher magnification of the granuloma with several coiled nematodes (circle). (Photo courtesy of: Department of Pathology, University of Veterinary Medicine, Hannover, Buenteweg 17, 30559 Hannover, Germany. [http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/].)*
A couple of nematodes are known to affect the eyes or the periorbital tissue. Thelazia, Onchocerca, Ancylostoma, Dirofilaria, Angiostrongylus vasorum, Toxocara canis and Trichinella sp. have been identified within ocular helminthic infections of dogs. O. lupi is a vector borne nematode that was first described in 1967 in a Caucasian wolf (Canis lupus) from Russia. The life cycle as well as the precise host range of canine Onchocerca is still not fully known, but it might be similar to those of other Onchocerca species. Within other Ochocerca species, blackflies and/or biting mites serve as intermediate hosts, for example Simulium tribulatum, was found to be a putative vector for O. lupi in Southern California. All other Onchocerca sp. have a long prepatent period and patency of several months or even years within their hosts.

In canine cases, gravid females, mature males, and microfilaria can be found within affected animals. Acute or chronic ocular disease with conjunctivitis, exophthalmos, periorbital swelling, photophobia, discomfort, lacrimation and discharge during the acute phase were reported as clinical signs. Chronic cases are characterized by granuloma formation in different parts of the eye and the periorbital tissue. The cuticle of females is a striking feature for the light microscopic identification of Onchocerca sp. It consists of two layers with an outer layer bearing ring-like ridges. In the anterior part of the nematode, these ridges are small and close together, becoming taller and more divergent in posterior direction. At the posterior part, they again get smaller with no visible ridges at the very end of the body. The two distinct cuticular layers are hardly identifiable within the presented case most likely due to insufficient preservation. Histopathological examination of affected tissue may reveal coiled female nematodes within a mixture of fibrotic tissue admixed with mononuclear cells. Male nematodes as well as microfilariae may occasionally be present in the periocular tissue.

Affected dogs are usually older than 1 year, which is most likely owed to the indirect life cycle of Onchocerca spp. as well as the slow development of these nematodes in their final hosts. Microfilariae can be isolated from skin samples, even if the animal has no clinical signs of onchocercosis. Microfilariae do not occur within the bloodstream, but larval concentration is high within the skin (50 - 3600 per g tissue), which might serve as a suitable screening method. Until today there is no serological test commercially available for the detection of O. lupi. O. lupi seems to be endemic in the United States. There have been also two proven cases of feline onchocercosis associated with O. lupi. Moreover, several cases of ocular onchocercosis have been reported in
humans worldwide. Onchocerca sp. infect humans in tropical regions causing severe ocular inflammation known as “river blindness”. Nevertheless, O. lupi has been identified to act as the causative agent in two human ocular cases with similar clinical features compared to those in dogs. Onchocerca sp. represent a zoonotic pathogen and should be considered as differential diagnosis in canine ocular diseases.

**JPC Diagnosis:** Globe: Scleritis, granulomatous and eosinophilic, multifocal to coalescing, severe with lymphplasmocytic conjunctivitis, and adult filarid nematodes, mixed-breed dog, Canis familiaris.

**Conference Comment:** We thank the contributor for their excellent submission and thorough review of the epidemiology, pathogenesis, histologic lesions, diagnostic modalities, and zoonotic potential associated with ocular onchocerciasis. Onchocerca lupi is an emerging zoonotic disease affecting dogs, cats, and humans in the Southwestern United States, parts of Europe, and the Middle East. Clinically, there are both acute and chronic forms of the disease. The acute form is characterized by conjunctivitis, chemosis, and periocular swelling. In chronic cases, such as in this dog, there are multiple adult filarid nematodes present within granulomatous nodules in the periocular tissue. Exophthalmos, protrusion of the nictitating membrane, anterior uveitis, corneal edema, and corneal neovascularization may be seen in chronic cases. Histologically, there are multifocal episcleral or conjunctival nodules with granulomatous and eosinophilic inflammation and fibrosis. Conference participants also astutely noted that there is perilenticular proliferation of a fibroblastic epithelial membrane causing blockage of the filtration angle (pre-iridal fibrovascular membrane). This is a common finding in the eye secondary to chronic uveitis and may result in either eversion of the pupillary margin, known as ectropion uveae, or secondary glaucoma. Grossly, proliferating periocular tissue containing adult nematodes can form pseudotumoral masses, resembling a neoplastic process.

Conference participants focused largely on the morphologic features distinguishing Onchocerca sp. in tissue section from other metazoan parasites. Filarid nematodes are small parasites that infect a number of different domestic animals. They are easily identified by their coelomyarian musculature, very small digestive tract, and larval microfilaria in the reproductive tract of gravid females. In Onchocerca sp., the coelomyarian muscles atrophy and are replaced hypodermal tissue because adults reside within a fibrous capsule and are sedentary. The key diagnostic feature that differentiates Onchocerca sp. from other filarid nematodes is the presence of evenly spaced ring-like circumferential annular cuticular ridges seen on longitudinal
This is the only nematode that has this cuticular manifestation. In contrast, *Dirofilaria immitis*, the canine heartworm, is a relatively common intraocular filarial nematode of dogs and has evenly spaced ridges that run longitudinally and can only be seen on cross section. These characteristics also help differentiate *Onchocerca* sp. from *Thelazia* sp., a spirurid nematode parasite and another common ocular parasite in a wide range of mammalian hosts.

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**References:**