

**The Armed Forces Institute of Pathology
Department of Veterinary Pathology**

Conference Coordinator
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WEDNESDAY SLIDE CONFERENCE 2010-2011

C o n f e r e n c e 5

8 September 2010

Conference Moderator:
Dale G. Dunn, DVM, Diplomate ACVP

CASE I: 396/08 (AFIP 3162240).

Signalment: 42-day-old broiler breeder chicks (*Gallus gallus domesticus*).

History: Eyes were submitted from 42-day-old broiler breeder chicks with a history of unilateral ocular disease and possible flock history of *Chlamydophila* conjunctivitis. Birds were otherwise healthy and the submitted eyes returned negative PCR for *Chlamydophila*. *Scediosporum apiospermum* was cultured from corneal tissue.

Gross Pathology: Eyes from six birds, five with macroscopic corneal changes and one grossly normal, were submitted. The damaged eyes had opaque, white cream corneas that bulged abruptly to a height of 1 to 2 mm above the limbus. All eyes were opened with temporal calottes. Corneas were thickened, and in some birds the anterior chamber was obliterated by grey-white fleshy tissue. These birds also had disruption of integrity of the iris leaflets and iridocorneal angles were obliterated. Lens changes varied from mild focal anterior opacification to severe extensive opacity with roughening of the anterior lens capsule.

Histopathologic Description: Changes in the affected eyes varied in degree but not type. There was complete loss of the epithelium over much of the cornea. The stroma was expanded by a severe superficial and midstromal infiltrate of granulocytes, lymphocytes, plasma cells and histiocytes, the latter occasionally forming prominent multinucleated giant cells. Granulocytes predominated in the superficial layers and plasma cells in deeper layers. The inflammation

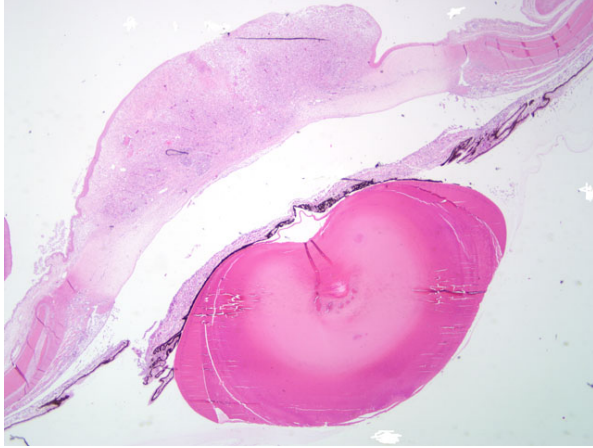
extended to full thickness in the central cornea in most birds but in more severely affected birds the inflammation was full thickness to penetrating across most of the cornea.

The stroma showed patchy to extensive necrosis, and activation and proliferation of keratocytes. Within the inflammation, particularly near areas of necrosis in the superficial and mid stroma there were few to moderate numbers of branched, septate fungal hyphae. Staining with both PAS and GMS highlights the fungi.

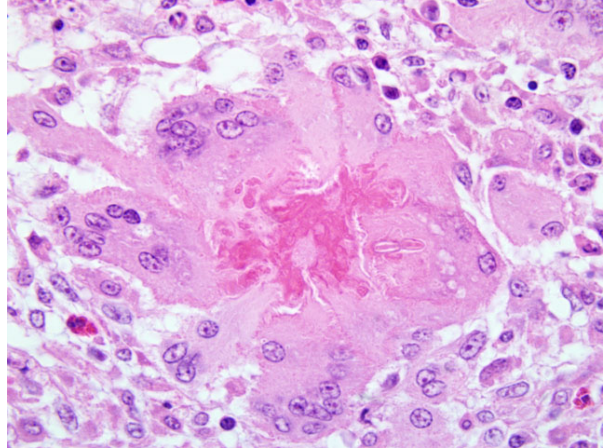
Endothelium and Descemet's membrane were missing from the central corneal region at the area of full thickness inflammation. There was a wedge of fibrous tissue bridging the gap. In the worst affected birds, Descemet's membrane, which is very thin in this age of bird, could not be detected. Where present, endothelial cell density was considered normal.

In birds with obliteration of the anterior chamber there is extensive adhesion of the iris to the posterior cornea, particularly at the areas of full thickness inflammation. In these birds the iris was only identifiable by the lines of pigment. In other birds there is moderate to severe infiltration of the iris and ciliary body with inflammatory cells of similar mixture to those in the cornea. Granulocytes predominate with few giant cells.

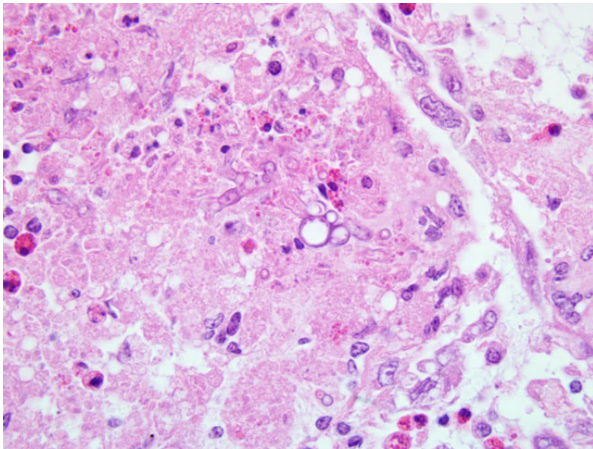
Lens epithelium was irregular and heaped up centrally and at one pole in more moderately affected birds. The worst affected birds had wrinkles and sometimes fragmented lens capsule outlining a pocket of proliferative epithelial cells.



1-1. Eye, chick. The corneal stroma is focally expanded by a cellular infiltrate, and there is loss of the overlying corneal epithelium. The iris is adhered to the lens (posterior synechia). (HE 20X)



1-2. Eye, chick. The corneal stroma is expanded and replaced by many lymphocytes, plasma cells, epithelioid macrophages, few heterophils, and occasional multinucleated giant cells. (HE 1000X)



1-3. Eye, chick. Within areas of necrosis and inflammation in the corneal stroma there are few, branched septate fungal hyphae. (HE 1000X)

These birds had marked lymphocytic inflammation of the pecten but the posterior segment in all birds was otherwise unremarkable except for some retinal folding, which may be normal for this age.

There was moderate to severe lymphocytic conjunctivitis in some birds more evident ventrally, in the birds that had it, than in the dorsal lids. There was no evidence in any submitted eye of chlamydophilosis.

Contributor's Morphologic Diagnosis: Severe chronic diffuse, mixed cellular, mycotic keratitis. Severe chronic active iritis with extensive anterior synechia. Cataract and pectenitis secondary to intraocular inflammation.

Contributor's Comment: Reports of fungal keratitis in poultry are few. Reis (1940)^{10,11} described lesions involving much of the anterior segment in birds 2 to 5 weeks of age which were reproducible by dropping a suspension of *Aspergillus fumigatus* onto a deeply scarified cornea. Subsequent reports of outbreaks of *Aspergillus* describe the development of a caseous pellet under the nictitating membrane in up to 10% of chicks in a flock, often at around a week of age. Itakura et al. (1972), reporting on natural cases, showed that infection was related to the environment of the birds; morbidity coincided with the introduction of chip litter.¹⁰ Likewise, Beckman showed corneal lesions caused by excessive ammonia fumes may permit fungal colonization in chickens.¹ In humans, the species in which mycotic keratitis is best described, damage to the corneal surface is most commonly caused by surgery and extended contact lens wear.¹⁶

Fungal lesions arising in the cornea may extend to involve the anterior segment, but rarely progress into the posterior part of the eye; in contrast, endophthalmitis, associated with fungal respiratory disease, typically affects the retina and vitreous, but the cornea remains unaffected. Interestingly, corneal infections are almost always unilateral.

In all previous reported cases of fungal keratitis in chickens, the isolated agent has been *Aspergillus fumigatus* which makes this outbreak unique. *Scedosporium apiospermum* (and its teleomorph, or sexual stage, *Pseudallescheria boydii*) is a filamentous fungus of widespread distribution, recently described as "one of the clinically significant emerging mycoses."¹⁷ It has, however, a long history, being first recognized in human otitis in the late 19th century, and in the mycetoma known as "madura foot" in the early 20th century. Taxonomy has been confused, but the present designation is *S. apiospermum* for the asexual state and *P. boydii* for the sexual.

S. apiospermum is best known for its association with near drowning events and subsequent pneumonia and disseminated disease.^{5,17} Its tolerance to cold, low oxygen tension and high salinity make it a survivor in polluted environments and recovery of the species from unpolluted environments is rare.⁵ Cytological and histological distinction from *Aspergillus* spp. is difficult. The two genera are closely related and share antigenic epitopes in formalin fixed tissues.¹⁷

Corneal infections have been reported in humans and are associated with a guarded prognosis for sight because of the resistance of this fungus to commonly used therapeutic agents.¹⁶ To date no other species has been reported with this infection in the cornea or ocular tissues, but the increasing rate of environmental detection of *S. apiospermum* suggests that infection may become more common in domestic species and poultry as it has in humans.

AFIP Diagnosis: 1. Eye: Keratitis, ulcerative, granulomatous and heterophilic, diffuse, severe with ulcerative conjunctivitis and fungal hyphae.
2. Eye, iris: Anterior uveitis, heterophilic and lymphoplasmacytic, diffuse, marked with synechia.

Conference Comment: Conference attendees reviewed the histologic anatomy of the avian eye, to include the 10 layers of the retina. Unlike the mammalian eye, in many species of birds the cartilaginous sclera ossifies with age and there are bony ossicles at the corneal-scleral junction. Consequently, care should be taken to decalcify avian eye specimens to prevent damage to the microtome when sectioning occurs. Another difference between the avian and mammalian eye is that the avian eye has a specialized pigmented and vascular structure that projects from the retina into the vitreous humor known as the pecten.¹⁴ The pecten's function is thought to be to provide nutrients to the avascular retina.

Most attendees identified the fungal hyphae as *Aspergillus* species. Given the morphologic similarity between *S. apiospermum* and *Aspergillus* spp., the confusion is understandable and highlights the utility of microbial culture in arriving at a definitive diagnosis when mycotic organisms are observed during histologic examination of tissues. Because mild conjunctivitis was present in some conference attendees' slides, participants briefly reviewed several common causes of conjunctivitis in avian species, among which include: *Chlamydomydia psittaci*, Newcastle disease virus, avian influenza virus, infectious bronchitis virus, infectious laryngotracheitis virus, and fowl poxvirus. Likewise, causes of cataract formation in the bird eye were discussed, two of which include vitamin E deficiency and avian encephalomyelitis virus.¹⁴ Additionally, congenital cataracts of unknown etiology sporadically occur in commercial turkeys.¹⁴

The contributor provides detailed information on *S. apiospermum* as an important emerging mycotic disease.

The moderator stressed that, in addition to the outbreak of *S. apiospermum* in this flock of chickens, *S. apiospermum* (*Pseudallescheria boydii*) has been noted to occur in elephant seals⁸ and in the nasal passages of cattle,¹³ horses,⁶ and dogs.^{2,3,4} In addition to the nasal passage, bones and joints appear to be other common locations for infection in dogs.^{7,9} Other species of *Scedosporium* have been reported to cause osteomyelitis in animals, including *S. prolificans* in a horse¹⁵ and *S. inflatum* in a dog.¹² In contrast to humans, *S. apiospermum* infection in animals has not yet been linked to immunosuppression.¹³

Multiple eyes from several different animals are submitted, and hence anterior or posterior synechia may be present, depending on the section evaluated. Additionally, the lens in some sections contains cataractous change.

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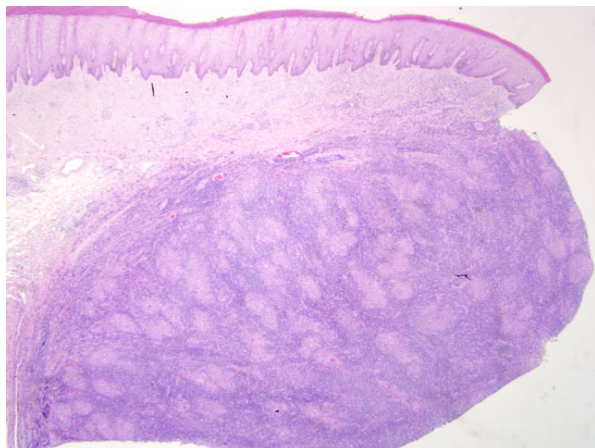
CASE II: C10-629 (AFIP 3165176).

Signalment: 13-year-old male, castrated, warmblooded horse (*Equus caballus*).

History: The owner noted two masses on the inside of the prepuce that have been present for two months and are rapidly growing.

Gross Pathology: There are two pieces of non-haired skin that are disrupted by a central tan, firm, nodule, measuring 2 x 1.5 x 1 cm in one piece, and 11 x 11 x 8 mm in the other.

2-1. *Glabrous skin, prepuce, horse. The dermis is markedly expanded by a nodular focus of granulomatous inflammation. Diffusely, the overlying epidermis is hyperplastic and hyperkeratotic. (HE 20X)*



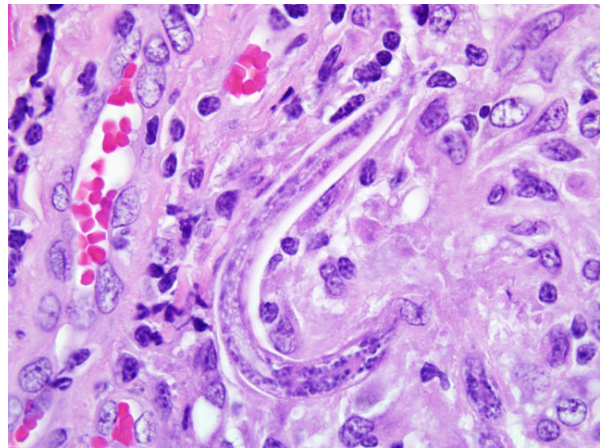
Histopathologic Description: Prepuce: The dermis is markedly expanded by large, dense, nodular aggregates of lymphocytes and plasma cells containing multiple smaller dense aggregates of epithelioid macrophages and multinucleated giant cells, with fewer scattered eosinophils. Multifocally within the inflammatory aggregates and frequently surrounded by macrophages and giant cells are cross-sections of nematodes, ranging up to 15 µm in diameter. These have a very thin cuticle, with low, indistinct platymyarian-meromyerian musculature, a pointed tail, numerous deeply basophilic 2-3 µm internal structures, and an esophagus with a prominent corpus, isthmus, and bulb (rhabditiform esophagus). There are moderate numbers of lymphocytes and plasma cells and fewer eosinophils scattered throughout the dermis surrounding the nodule. There is marked epidermal hyperplasia with compact hyperkeratosis overlying the nodule.

Contributor's Morphologic Diagnosis: Posthitis, lymphoplasmacytic, eosinophilic, and granulomatous, severe, chronic, multifocal, with intralesional rhabditiform nematodes

Contributor's Comment: *Halicephalobus gingivalis* (formerly known as *Micronema deletrix* or *Halicephalobus delatrix*) is a free-living rhabditiform nematode. Infections in horses are infrequently reported; when found, organisms typically infiltrate the central nervous system,^{2,3} but have also been reported in the prepuce,^{4,10} kidney,^{1,15} eye,¹¹ bone,⁵ and one case of systemic infection.¹³ In addition to *Halicephalobus*, other free-living rhabditoid nematodes, such as the genus *Cephalobus*⁶ and *Pelodera*,¹² have been reported to cause skin infections in horses, and cannot be ruled out in this case. In addition to horses, other equids, such as zebras, have also been reported to be infected.⁸

To date, only female *Halicephalobus* have been found in tissue samples.¹⁰ While the route of infection and

2-2. *Glabrous skin, prepuce, horse. Lymphocytes, plasma cells, and epithelioid macrophages surround an adult nematode that has a thin cuticle, indistinct platymyarian-meromyerian musculature, pointed tail, and numerous deeply basophilic 2-3 µm internal structures. (HE 1000X)*



pathogenesis of disease are poorly understood, it is suspected that the organism gains entry via existing wounds. In some cases, viable *Halicephalobus* organisms have been detected in sperm and urine;⁹ while no transmission via this route has been proven, it is another possible source of infection. Organisms access the central nervous system via blood vessels, and cause necrosis and inflammation due to migration through the tissue.

Under light microscopy, adult females are typically approximately 20 µm in diameter and 350 µm long, with a thin, smooth cuticle and tapered, pointed tail. They have platymyarian-meromyarian musculature, a rhabditiform esophagus, and an intestine lined by low cuboidal cells. Larvae are approximately 10 µm in diameter, with tapered, pointed tails. Typically, tissue sections have numerous parasites; this may be explained by the fact that females are parthenogenetic and thus can produce offspring in the absence of males.

Other rule outs for rhabditoid parasites in tissue sections include *Cephalobus* spp., *Strongyloides westeri*, and *Pelodera strongyloides*.¹² *Cephalobus* spp. can be differentiated from *Halicephalobus* spp. by examination of the tail, which is blunt in *Cephalobus*.⁶ *Strongyloides westeri* have alae, which *Halicephalobus* spp. lack.⁶ *Pelodera strongyloides* also have two lateral alae, as well as two lateral cords noted by two dense nuclei.⁷

AFIP Diagnosis: Glabrous skin, prepuce: Posthitis, granulomatous and eosinophilic, focally extensive, marked with rhabditiform nematode adults, larvae and eggs, etiology consistent with *Halicephalobus gingivalis*.

Conference Comment: The contributor provides an excellent overview of halicephalobiasis in the horse. Most conference participants correctly identified the nematode as *H. gingivalis*, though some participants identified the tissue as gingiva rather than skin from the prepuce. As *H. gingivalis* causes granulomatous inflammation at both anatomic locations, tissue identification is important. Upon closer examination of the tissue sample, all participants subsequently identified scattered sebaceous and apocrine glands, and some sections contain rare hair follicles, consistent with glabrous skin. Conference participants also reviewed the differential diagnosis for this lesion, which the contributor discusses in detail. In addition to the morphologic features provided by the contributor, the moderator commented that a unique anatomic feature of *H. gingivalis* is the presence of a dorsally reflexed ovary, which is often difficult to appreciate on routine histologic examination, but when present is diagnostic.

In addition to the histomorphologic differences among the various nematodes, clinicopathologic presentation may assist with differentiating them. Although *H. gingivalis* dermatitis may occur anywhere on the skin of horses, it generally localizes near or on the prepuce; lesions tend to be papules or nodules that are well circumscribed, measure 0.5 cm to 8 cm

in diameter, and are often ulcerated.¹⁴ In contrast, *Pelodera* dermatitis is characterized by papules, pustules, ulcers, crusts, alopecia, and scales on areas of the skin that typically have contact with damp soil and decaying organic matter, such as the limbs, ventral thorax, and abdomen;¹⁴ pruritus is usually moderate to intense.

We thank Dr. Christopher Gardiner, Consulting Parasitologist for the AFIP's Department of Veterinary Pathology, for his study and diagnostic commentary for this case.

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CASE III: Colorado State University CSU-CVMBS 090-TK01 (AFIP 3166610)

Signalment: Young male intact commercial grower turkey.

History: A male commercial production turkey was submitted for necropsy (donation) by a large commercial production company. The company has multiple complexes in multiple states in the United States. This bird came from a premise in Arkansas with a flock size of 40,000 birds. All birds in this flock are males and are sent to slaughter around 20 weeks of age. This bird was found dead. The company did not report a higher than normal rate of turkey mortality.

Gross Pathology: Liver: There are multifocal to coalescing circular regions of necrosis with depressed pale tan/yellow



3-1. Liver, turkey: There are multifocal to coalescing circular regions of necrosis with depressed pale tan/yellow centers surrounded by a slightly raised, often hemorrhagic rim. Lesions range in size from 0.5 to 3.0 cm in diameter and are disseminated randomly throughout the parenchyma. Photographs courtesy of Colorado State Diagnostic Medicine Center, 300 W. Drake Rd., Fort Collins, CO 80523, ej.ehrhart@colostate.edu

centers surrounded by a slightly raised, often hemorrhagic rim. Lesions range in size from 0.5 to 3.0 cm in diameter and are disseminated randomly throughout the parenchyma.

Ceca: Both ceca are moderately distended and fluid filled with thickened hyperemic walls mottled tan to dark red. The mucosal surface is multifocally ulcerated with occasional

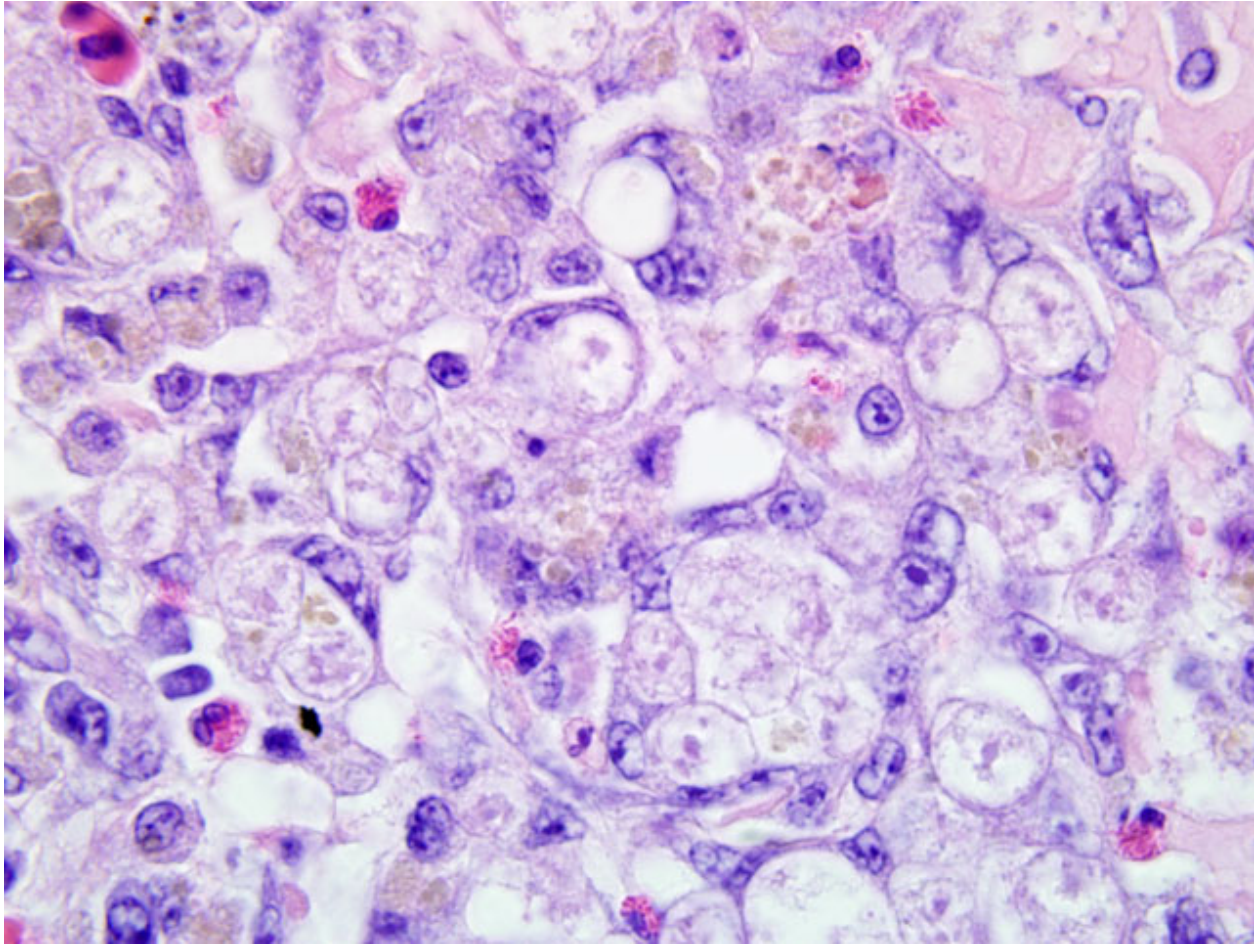


3-2. Cecum, turkey: Both ceca are moderately distended and fluid filled with thickened hyperemic walls mottled tan to dark red. The mucosal surface is multifocally ulcerated with occasional partially adhered caseous debris amidst abundant luminal hemorrhagic exudate. Photographs courtesy of Colorado State Diagnostic Medicine Center, 300 W. Drake Rd., Fort Collins, CO 80523, ej.ehrhart@colostate.edu

partially adhered caseous debris amidst abundant luminal hemorrhagic exudate.

Histopathologic Description: Liver: Approximately 50 to 80% of the parenchyma is largely obliterated by multifocal, random, discrete and coalescing to regionally extensive areas of coagulative hepatic necrosis. Affected areas are characterized by shrunken hepatocytes with intensely eosinophilic cytoplasm and pyknotic to lytic nuclei or loss of hepatocytes, disruption of hepatic cords, and replacement with eosinophilic cellular and karyorrhectic debris. Mainly within necrotic foci and variably expanding portal triads are pleocellular infiltrates composed of moderate to large numbers of macrophages (many hemosiderin-laden), lymphocytes, heterophils, few plasma cells and variable numbers of foreign body-type multinucleate giant cells. Phagocytized by multinucleated giant cells as well as free and within the lumen of ectatic, hyperplastic bile ducts are individual to clustered 12-20 μ m in diameter eosinophilic to amphophilic protozoal trophozoites. Trophozoites may contain a 2-4 μ m diameter oval shaped basophilic central to slightly eccentric nucleus. Pericholangial inflammation and fibrosis is prominent around large sized bile ducts with remaining hepatic parenchyma interrupted by numerous variably sized newly formed bile ductules. Numbers of multinucleate cells and organisms vary depending on the section received.

Cecum: Diffusely expanding the lamina propria and multifocally extending transmurally with effacement of up to 50% of the muscular coat is an inflammatory infiltrate composed predominantly of lymphocytes and macrophages with fewer plasma cells and heterophils admixed with numerous 12-20 μ m diameter protozoal trophozoites similar



3-3. Liver; turkey. Areas of hepatic necrosis contain many hemosiderin-laden macrophages, lymphocytes, heterophils, fewer plasma cells, and numerous intrahistiocytic and extracellular; 12–20µm trophozoites. Trophozoites may contain a 2-4 µm oval shaped basophilic central to slightly eccentric nucleus. (HE 1000X)

to those described within the liver. There is extensive loss of crypts and remnant crypts are hyperplastic, tortuous and ectatic with piling of 2-3 epithelial cells layers, and luminal aggregates of detached epithelial cells and degenerate leukocytes, mainly heterophils (crypt microabscesses). There is multifocal extensive mucosal ulceration, and in some sections the cecal lumen contains a dense core of eosinophilic cellular and karyorrhectic debris, erythrocytes, fibrin, degenerate protozoal trophozoites, macrophages and heterophils, and aggregates of rod shaped bacteria (cecal core).

Contributor's Morphologic Diagnosis: Liver: Multifocal and coalescing, random, necrotizing, sub-acute-to-chronic, lymphohistiocytic and heterophilic hepatitis with biliary hyperplasia and intralesional protozoal trophozoites, etiology consistent with *Histomonas meleagridis*.

Cecum: Transmural, marked, sub-acute-to-chronic lymphohistiocytic and heterophilic typhlitis with fibrinonecrotic core (variable) and intralesional protozoal trophozoites consistent with *H. meleagridis*.

Contributor's Comment: Histomoniasis, also known as blackhead and infectious enterohepatitis, is a disease of gallinaceous birds caused by *Histomonas meleagridis*, a flagellated amoeboid protozoan which remains an important disease economically for the commercial poultry industry. Two aspects of this disease worthy of brief review are 1) the unique life cycle of the organism and 2) differences in transmission and extent of disease in turkeys and chickens.

The role of the cecal worm *Heterakis gallinarum* as an intermediate host has been well-described.^{2,3} The exact mechanism of infection of *Heterakis* eggs with histomonads remains unknown, but it has been suggested that the protozoan may be transferred to the female worm during mating and are then incorporated into embryonated eggs.² Infected eggs pass in feces where they may be ingested directly by birds or by earthworms who may serve as a transport host. Ingestion of either egg-laden feces or earthworms by the bird results in transport to the cecum where flagellated trophozoites are released from the

nematode egg, multiply in the cecal lumen and penetrate the cecal wall. The tissue stage loses the flagella becoming amoeboid. Eventually the histomonads gain entry into the bloodstream and are carried to the liver via the hepatic-portal system. It is important to note that the fragile trophozoite of *Histomonas*, which cannot survive long outside of any of its hosts, would be unable to survive passage through the stomach if not within a nematode egg or an earthworm. Therefore, fecal oral transmission is not thought to be an important route of transmission. Interestingly, some recent publications have shown the existence of a cyst stage in some species of *Histomonas in vitro* sparking interest in the possibility of this happening under certain conditions in the natural disease allowing for persistence in the environment and the possibility of oral transmission.^{4,7}

Extension to the liver as described above occurs at a higher rate in turkeys than chickens, with a much higher associated mortality rate. Concurrent infection with *Eimeria tenella* results in increased liver lesions in chickens. Histomonad virulence also requires the presence of cecal bacteria such as *Escherichia coli*, *Clostridium perfringens*, and/or *Bacillus subtilis*, especially in turkeys.^{2,3} As mentioned previously, turkeys suffer from a much higher mortality rate than chickens, with the latter being better able to control the disease but still suffering decreased productivity. A recent publication showed that chickens mount a more effective innate immune response to *H. meleagridis* in the ceca than do turkeys resulting in better control of parasite numbers in chickens.⁵ Furthermore, unregulated cell-mediated immunity in the liver is more pronounced in the turkey than the chicken often leading to lymphoid depletion of the spleen.⁵ Another difference of histomoniasis in turkeys is that, in addition to transmission via ingestion of *Heterakis* eggs, turkeys appear to be able to transmit histomonads directly via “cloacal drinking” where cloacal droppings from an infected bird can be pulled retrograde into the ceca of a susceptible bird if the droppings contact the vent of the uninfected bird.^{2,3}

AFIP Diagnosis: 1. Liver: Hepatitis, random, necrotizing, lymphohistiocytic and heterophilic, multifocal coalescing, severe, with biliary hyperplasia and protozoal trophozoites, etiology consistent with *Histomonas meleagridis*.
2. Cecum: Typhlitis, transmural, lymphohistiocytic and heterophilic, diffuse, severe, with fibrinonecrotic core and protozoal trophozoites, etiology consistent with *Histomonas meleagridis*.

Conference Comment: The contributor provides an excellent review of the pathology and life cycle of *Histomonas meleagridis*. While attendees found this to be a relatively uncomplicated histologic diagnosis given the species and abundance of trophozoites, all were interested and engaged in the discussion that the case precipitated.

Conference participants noted that “blackhead” is an imprecise colloquialism in that cyanosis of the head is neither

a constant feature nor a unique clinical sign of histomoniasis.⁶ Other diseases, such as turkey enteric coronavirus (bluecomb disease), avian influenza, and various respiratory pathogens may cause a cyanotic head in turkeys and chickens. Gross digital images were shown during the conference to reinforce the point that the macroscopic lesions of histomoniasis are very distinctive, and when classical lesions are present in both the liver and cecum simultaneously, the findings are considered pathognomonic for the condition. When only cecal cores are present, other etiologic considerations would include salmonellosis for both chickens and turkeys, and *Eimeria tenella* in chickens.

In addition to histomoniasis, cecal heterakiasis was also discussed by participants. Although *H. gallinarum* can cause thickened cecal walls, nodule formation, and inflammation in its own right in turkeys and chickens, its practical and economic importance is the nematode’s ability to serve as a carrier for *H. meleagridis*.¹ In pheasants, however, *Heterakis isolonche* causes severe cecal disease with mortality rates that may exceed 50%.² The disease in pheasants is characterized by a marked inflammatory response, fibrosis, and coalescing cecal wall nodules.

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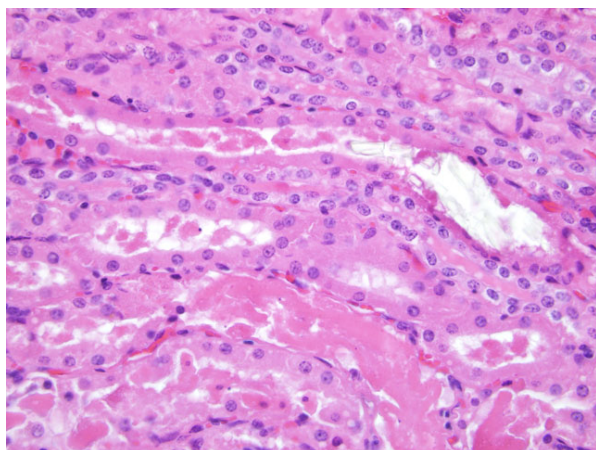
CASE IV: V10-02733 (AFIP 3167339).

Signalment: 3-year-old, female, Rambouillet, *Ovis aries*.

History: This animal was one from a herd of approximately 1200 sheep on open range in the “Four Corners” region of northwestern New Mexico. During a winter snowstorm in the area, the shepherd moved the animals from the mesa tops down into surrounding lower country (arroyos, valleys). During the day and night following the move, it had snowed approximately 8 inches. The following morning the shepherd found 50 animals dead and 5 animals that were down, debilitated, and showing “neurological” signs. The water source was snow; sheep were ranged on the country described above. Trace mineral salt was supplemented. The shepherd was with sheep at all times.

Gross Pathology: This ewe was presented alive, but down, and with neuromuscular fasciculations of the head and neck. The animal was unable to get up, and was euthanized. On gross exam, the rumen was engorged with a somewhat woody or brushy type plant material; this had a somewhat pungent odor. Both the rumen and reticulum were somewhat edematous. The kidneys were pale, swollen and bulged on cut surface.

Histopathologic Description: The kidneys had flattening and destruction of proximal convoluted tubular epithelial cells, with prodigious aggregates of birefringent crystals in



4-1. Kidney, ewe. Multifocally in the cortex, the epithelium of the proximal and distal convoluted tubules is degenerate and necrotic, and there are many intraluminal birefringent oxalate crystals (HE 400X)

these tubules. These crystals also were seen in distal convoluted tubules, but in much lower numbers. Crystals had layers or sheaves aggregated together; these were consistent with oxalates histologically.

Contributor’s Morphologic Diagnosis: Nephrosis, toxic tubular, kidney, ovine due to potassium oxalate intoxication from excessive ingestion of “greasewood” (*Sarcobatus vermiculatus*).

Contributor’s Comment: Brushy plants found in the rumen were identified as greasewood (*Sarcobatus*

vermiculatus), a plant commonly found in the western and Southwestern range country. This is a plant browsed extensively by sheep, usually without any problems when eaten in combination with other forages. However, when eaten as an exclusive diet (and thus in excess), it is highly toxic and can cause massive die-off, such as with this case. In this particular situation, greasewood was the only browse poking thru the recent snowfall, and sheep are not prone to paw or dig for forage like cattle. Hence, in this circumstance of weather, terrain, and ready availability of a poisonous plant, coupled with grazing habits of sheep, a “perfect storm” of all the above ingredients resulted in the excessive ingestion of *Sarcobatus* spp. and subsequent intoxication and die-off of animals. The shepherd had moved the sheep into a fenced pasture and put out high grade alfalfa hay prior to bringing this animal in for necropsy, as he was suspicious of greasewood being the cause of the problem. A few more animals died, but the change in diet resolved any further problem.

Histologic lesions are quite similar to those of antifreeze (ethylene glycol) intoxication in a number of species; however, these are potassium oxalates with the *Sarcobatus* spp. intoxication, versus calcium oxalates crystals seen with antifreeze poisoning. Histologically, the lesions and crystals are very similar.

Sarcobatus spp. contains a mixture of neutral sodium and potassium oxalates - about 10-15% d.w. in leaves and in smaller amounts in stems and fruits. Oxalate concentrations reach a peak in early fall. Aqueous extracts of the plant containing 40% oxalates, when given to sheep, produced the same signs and lesions as when the plant was eaten.¹

Other oxalate producing plants that can cause poisoning in sheep and cattle include halogeton (*Halogeton glomeratus*), common rhubarb (*Rheum rhaponticum*), soursob (*Oxalis cernua*), and sorrel dock (*Rumex* spp).²

AFIP Diagnosis: Kidney: Tubular necrosis, acute, diffuse, with intratubular oxalate crystals.

Conference Comment: Case discussion focused on the pathogenesis of oxalate nephrosis in animals. Sheep are able to consume oxalate-containing plants without toxicity due to the rumen’s ability to metabolize oxalates to bicarbonate and carbonate; however, changes in the microbial balance of the rumen may reduce this ability. In this case, unmetabolized oxalates are absorbed into the circulation where they chelate calcium ions, and thus forming insoluble calcium oxalate complexes. In the kidney, these complexes may crystallize in vessel walls, or within the lumens of blood vessels or renal tubules. Deposition within vessels results in renal hemorrhage and necrosis, while deposition in renal tubules results in obstruction and acute renal failure. Furthermore, intracellular chelation of calcium and magnesium ions may interfere with oxidative phosphorylation, resulting in nephrotoxicity in addition to physical tubular obstruction.²

Electrolyte disturbances in affected animals include increased plasma sodium, potassium and calcium; of these changes, the cardiotoxic potential of hyperkalemia is most life-threatening. In addition, ingestion of oxalate-containing plants by sheep results in hypocalcemia due to chelation and may result in tetany. Acidosis and azotemia are other common clinicopathologic findings in acute renal failure, with acidosis in affected animals often being the most significant contributory factor in the cause of death.²

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