WEDNESDAY SLIDE CONFERENCE 2017-2018

Conference 2

30 August 2017

CASE I: 16040206 (JPC 4084138).

Signalment: 1 month-old, intact female, Thoroughbred horse (*Equus caballus).*

History: The owner found her dead in the stall. She was behaving normally the night before. (per rDVM)

Gross Pathology: The conjunctiva, sclera, and adipose tissue are yellow. The liver is enlarged and skeletal muscle is pale. (per rDVM)

Laboratory results: None provided.

Microscopic Description: Liver (H&E): The liver contains large, individual to coalescing, randomly-placed foci of degenerate neutrophils with fewer macrophages surrounding foci of necrotic hepatocytes admixed with cellular debris. Adjacent peripheral hepatocytes are shrunken, angular, and hypereosinophilic with small, pyknotic nuclei and contain intracyto-plasmic, long, slender, parallel to cross-hatched bundles of lightly basophilic to negatively staining, 3.5 to 14 microns in length bacilli. Bile canaliculi in areas less affected by inflammation are distended multifocally with golden brown intra-

canalicular bile. Randomly, many sinusoids are modestly to moderately congested.

Liver (Steiner's silver stain): Myriads of multifocal to coalescing intracytoplasmic, long, slender, parallel to crosshatched bundles of argyrophilic bacilli measuring 3.5 to 14 microns in length are within hepatocytes peripheral to foci of necrosis and inflammation throughout the section.


Liver, foal. There are numerous coalescing areas of pallor and hypercellularity (necrosis) forming a retiform pattern through the tissue. (HE, 6X)

Contributor's Morphologic Diagnosis: Liver: Severe, acute, multifocal to coalescing, random, necrosuppurative hepatitis with intracellular bacilli and moderate bile stasis.

Contributor's Comment: Tyzzer's disease is a bacterial infection that has been reported to affect many different species[4]. The etiologic agent, originally deemed *Bacillus pilifomis,* was reclassified in 1993 to the class *Clostridia* based on genetic sequencing of the 16S rRNA[4]. *Clostridium piliforme* is a filamentous, spore forming, gram negative, argyrophilic, obligate intracellular bacterium.

Adult horses are relatively resistant to clinical infections and harbor *C. piliforme* as part of their normal gastrointestinal microbiota. The upregulation of IL-12 has been explored in laboratory mice experimentally infected with *C. piliforme* which aids in resistance to this infection and others[7]. Foals under 6 weeks of age however are typically fatally affected. Transmission in foals is thought to be via a fecal-oral route due to coprophagia of the dam's feces during the early weeks of neonatal life[6].

Bacteria gain access to intestinal mucosal cells and by an unknown mechanism travel to and infect hepatocytes where it replicates and causes hepatocellular death. The incubation period can be as long as seven days until clinical signs first appear. At this time, nonspecific signs including anorexia, depression, fever, jaundice, and diarrhea may be observed[6]. These signs can be subtle, which is why many foals present as a "found dead" case. Due to the rapid course of disease, treatment typically is unrewarding.
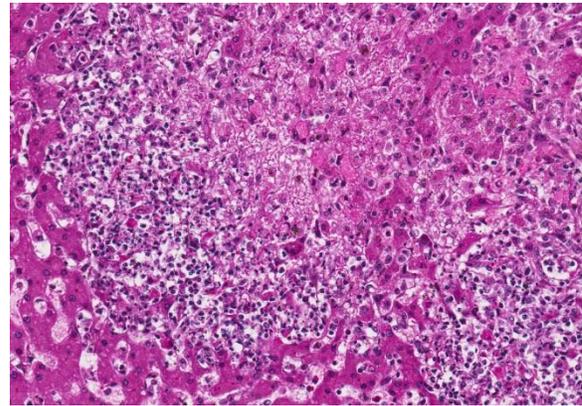

Liver, foal. Lytic necrosis predominates within the liver, with small foci of coagulative necrosis scattered throughout the section. (HE, 296X)

This case was a classic presentation of clinical disease. The age of the foal (4 weeks old) places it in the susceptible age range for acquiring the bacteria from coprophagic practices[6] and allows for a week long incubation period. Foals become coprophagic during the second week of life and continue the practice through the fifth week[6]. Typically foals born late in foaling season, from mid-March through May, have a higher prevalence of infection.[5, 6] Seventy-seven percent of cases occur in these foals where as 23% of cases occur foals born between January to early March[6]. This is thought to be due to environmental changes and diet which impact the mare's microbiota[6]. Heavy rainfalls, wildlife reservoirs, and spores contaminating the soil perpetuate the bacteria within the environment for extended periods of time[7]. Nutrient-dense diets are thought to increase incidence of infection as well and correlate with the overrepresentation of Thorough-breds and other performance breeds associated with this disease.

The provided history of behaving normally and then finding the foal dead is also typical of this infection, especially in younger foals. Foals closer to the six weeks of age typically

display the nonspecific signs described above for 24-48 hours before death; whereas younger foals usually are found dead with no outward signs of illness[6]. Icterus is a common sequela of hepatic damage; however, hepatomegaly is not a common gross finding. We interpret this to be due to the prominent inflammatory cell infiltrate in the necrotic lesions (fig. 1). Our initial suspected diagnosis of *Clostridium piliforme* (fig. 2) was confirmed by the positive Steiner's silver stain demonstrating black, argyrophilic bacilli bordering foci of necrosis (figs. 3, 4). An antemortem diagnosis of *Clostridium piliforme* has been historically challenging as the bacteria does not grow on conventional media; however, a nested PCR has been developed for in vivo clinical diagnosis using feces as an inexpensive way of confirming the *Clostridium piliforme*[5].
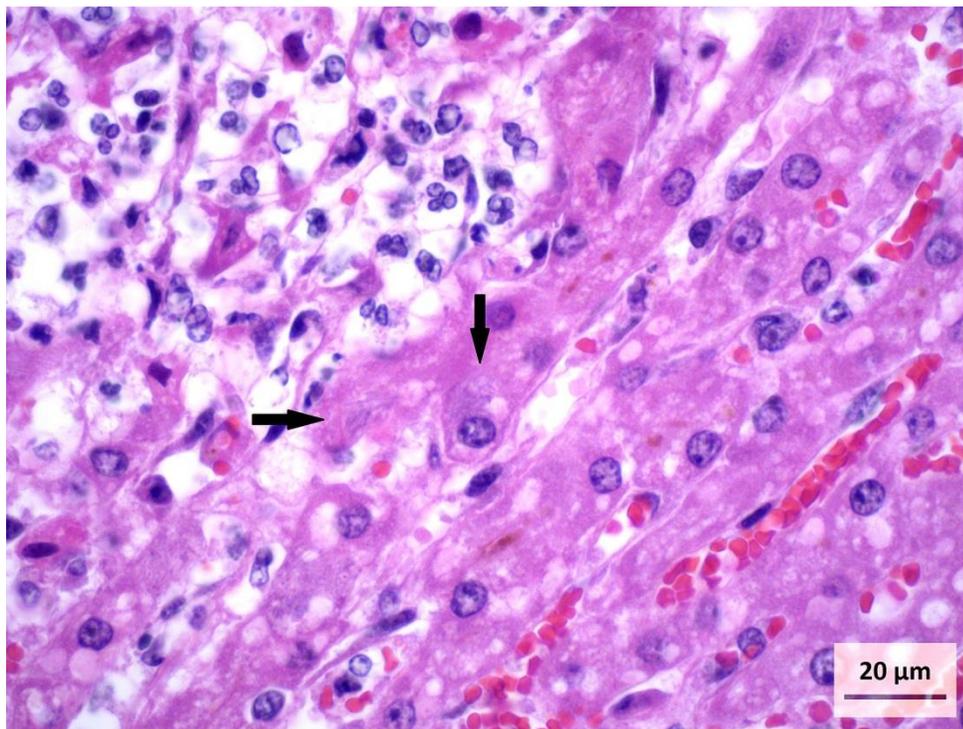
Liver, foal. At the edges of necrotic lesions, hepatocytes contain aggregates of faintly-staining intracytoplasmic filamentous bacilli within their cytoplasm. (HE, 600X) (Photo courtesy of: Oklahoma State University Center for Veterinary Health Sciences, Department of Pathobiology, http://cvhs.okstate.edu/Veterinary_Pathobiology).

JPC Diagnosis: Liver: Hepatitis, necrotizing, multifocal to coalescing, random, marked, with numerous intracytoplasmic bacilli, Thoroughbred, equine.
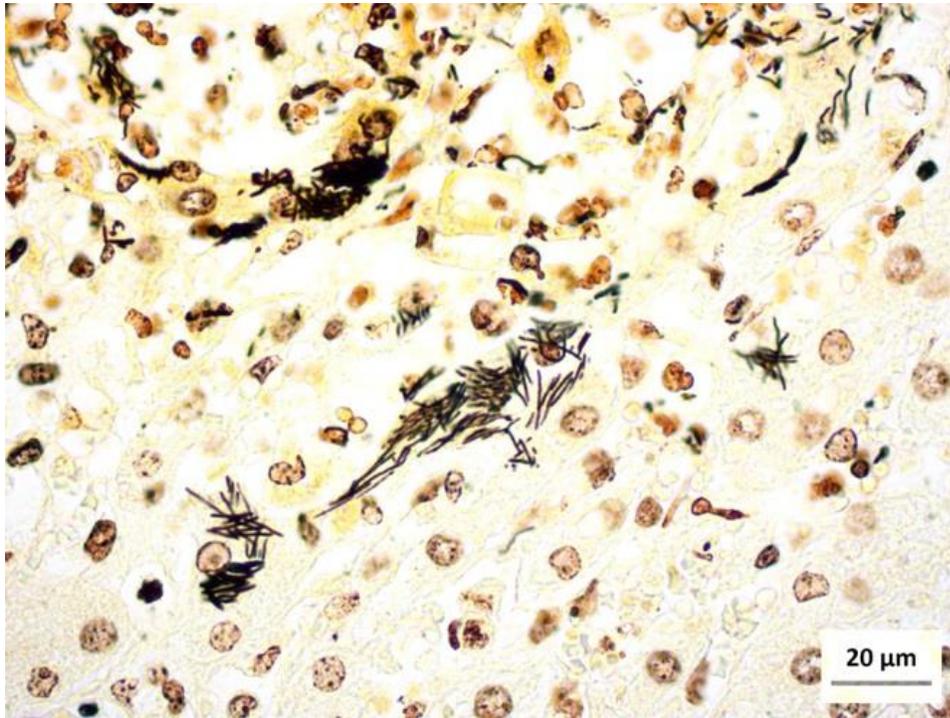
Conference Comment: This case demonstrates a classic example of Tyzzer's disease in a foal resulting in acute necrotizing hepatitis. Participants' description and discussion mirrored much of what was offered by the contributor. A Warthin-Starry stain was viewed during the conference which beautifully demonstrates the argyrophilic intracellular bacteria lying in sheaves or bundles within hepatocytes located at the periphery of necrotic foci.[3] As the contributor aptly notes, *C. piliforme*-induced lesions in the liver are generally acute and began as foci of coagulative necrosis. As neutrophils are recruited to phagocytize necrotic hepatocellular debris, additional tissue damage results in foci of lytic necrosis, the predominant type of necrosis in this case.

The contributor provides an excellent review of the clinical findings, pathogenesis, gross and microscopic lesions in foals. Additionally, the conference moderator reviewed several susceptible laboratory animal species which present with characteristic lesions not

necessarily associated with the classic target tissues (liver, intestine, and heart). Mongolian gerbils are particularly susceptible to *C. piliforme* infection which can additionally cause diffuse suppurative encephalitis. In rats, infection usually occurs in young animals during the postweaning period and is typically an enterohepatic

Liver, foal. Intracytoplasmic C. piliforme within hepatocytes are well-demonstrated with silver stains. (Steiner, 600X) (Photo courtesy of: Oklahoma State University Center for Veterinary Health Sciences, Department of Pathobiology, http://cvhs.okstate.edu/Veterinary_Pathobiology).

disease. The typical manifestation in rats is necrotizing and hemorrhagic ileitis with adynamic ileus (also known as megaloileitis). Rabbits usually present with severe, watery colitis that result in high mortality. Microscopically, the characteristic foci of hepatic necrosis are present, as well as, severe focal to segmental necrosis of the cecal mucosa that usually extends transmurally.[1]

Finally, participants discussed differential diagnoses for random necrotizing hepatitis in foals. Equine herpesvirus-1 causes systemic disease in neonates including multifocal necrosis in liver, spleen, adrenal glands and other tissues with concurrent bronchointerstitial pneumonia. However, there are often prominent intranuclear inclusion bodies in hepatocytes and occasional syncytial cells in the lungs.[2] Septicemia due to *Salmonella* sp. or *E. coli* results in watery foul smelling diarrhea as well as joint lesions, pneumonia, and meningitis (with *Salmonella* sp.).[10] Lastly, sleepy foal disease, caused by *Actinobacillus equuli,* causes multifocal hepatitis, severe enteritis, and embolic nephritis.[3]

Contributing Institution:
Oklahoma State University Center for Veterinary Health Sciences Department of Pathobiology

http://cvhs.okstate.edu/Veterinary_Pathobiology

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CASE II: 1235813-002 (JPC 4101299).

Signalment: Juvenile female cynomolgus macaque, non-human primate (*Macaca fascicularis).*

History: This animal had decreased body weight, and a history of soft watery feces. It was found hypothermic and lying on floor of the cage and was humanely euthanized.

Gross Pathology: No gross pathology lesions were observed.

Laboratory results: None provided.

Microscopic Description: Colon: There are multifocal areas of segmental to full-thickness loss of mucosal architecture, with colonic crypts replaced by pale staining eosinophilia with pyknotic and karyorrhectic nuclear debris (necrosis) and some viable neutrophils. Crypts adjacent to the necrotic areas are variably lined by detached epithelial cells with pyknotic nuclei and
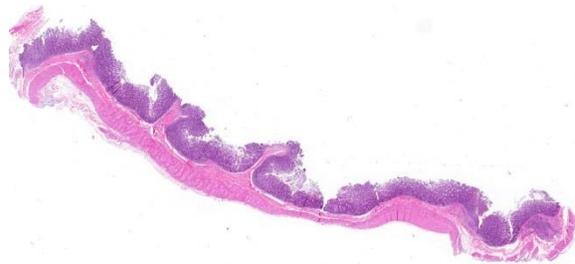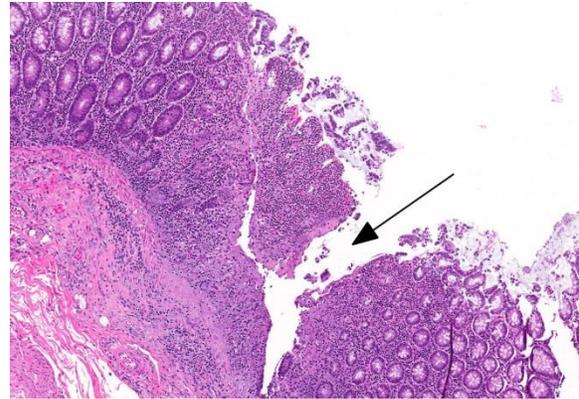

Colon, cynomolgus macaque. A section of colon is presented for evaluation (HE, 5X).

scant eosinophilic cytoplasm. Frequently scattered within the areas of necrosis, as well as within the submucosa, there are clusters of round protozoal trophozoites, which partially efface occasional crypts. Clusters of bacterial colonies are noted within the superficial to mid-mucosa in some areas of necrosis. The colonic lamina propria is infiltrated by a mixed population of inflammatory cells, including moderate numbers of plasma cells, fewer lympho-cytes, and rare eosinophils. In some slides, the colonic serosa is infiltrated by a similar inflammatory cell population.

Some sections of colon contain gut-associated lymphoid tissue with loss of lymphocytes and variable necrosis. The protozoa stained positive with PAS and the bacteria were identified as a mixture of gram negative and gram positive organisms with a Gram Twort stain.

Contributor's Morphologic Diagnosis: Colon: Acute, multifocal, necrotizing colitis, with intralesional protozoa consistent with *Entamoeba histolytica*.

Contributor's Comment: Non-human primates held in laboratory and research settings harbor a variety of intestinal parasites that rarely cause clinical infection.[5] *Entamoeba histolytica* is a protozoan parasite that is a part of the normal fauna, but has also been shown to be pathogenic in captive non-human primates. Amebiasis affects the colon, causing diarrhea and can also cause liver abscesses, and ultimately lead to death.[1] It is of particular concern because of its zoonotic potential.[3] Clinical disease secondary to this pathogen is uncommon in the biomedical research laboratory setting and is usually secondary to stress or immunosuppression.

Colon, cynomolgus macaque. There are multifocal areas of full thickness mucosal necrosis (arrow) (HE, 168X).

Definitive speciation of *Entamoeba* spp. can be made using PCR-reverse line hybridization blot (RLHB), as differentiation of this protozoan from *Entamoeba dispar*, *Entamoeba nutalli*, and other species of this genera using light microscopy can be challenging.[1,3] Because of this difficulty, it has been suggested to make a combined diagnosis of *Entamoeba histolytica* and *Entamoeba dispar* unless molecular differentiation can be made. *Entamoeba nutalli* was found to be pathogenic in in Japanese macaques in a study where *Entamoeba dispar* was not identified, despite being reported to be prevalent in captive macaques. This suggests that colonies of macaques in captivity may have differing predominant causes of amebiasis.[2]

A definitive diagnosis of intestinal amebiasis may be complicated in cases of macaques infected with *Entamoeba chattoni*, as it has been shown to breach the mucosa of the cecum shortly after death.[4] In the case presented here, protozoal trophozoites are present in the submucosa with and without any associated pathology in varying areas of the slide. However, the organisms present within the mucosa and the GALT (present in some slides) are associated with pathology.
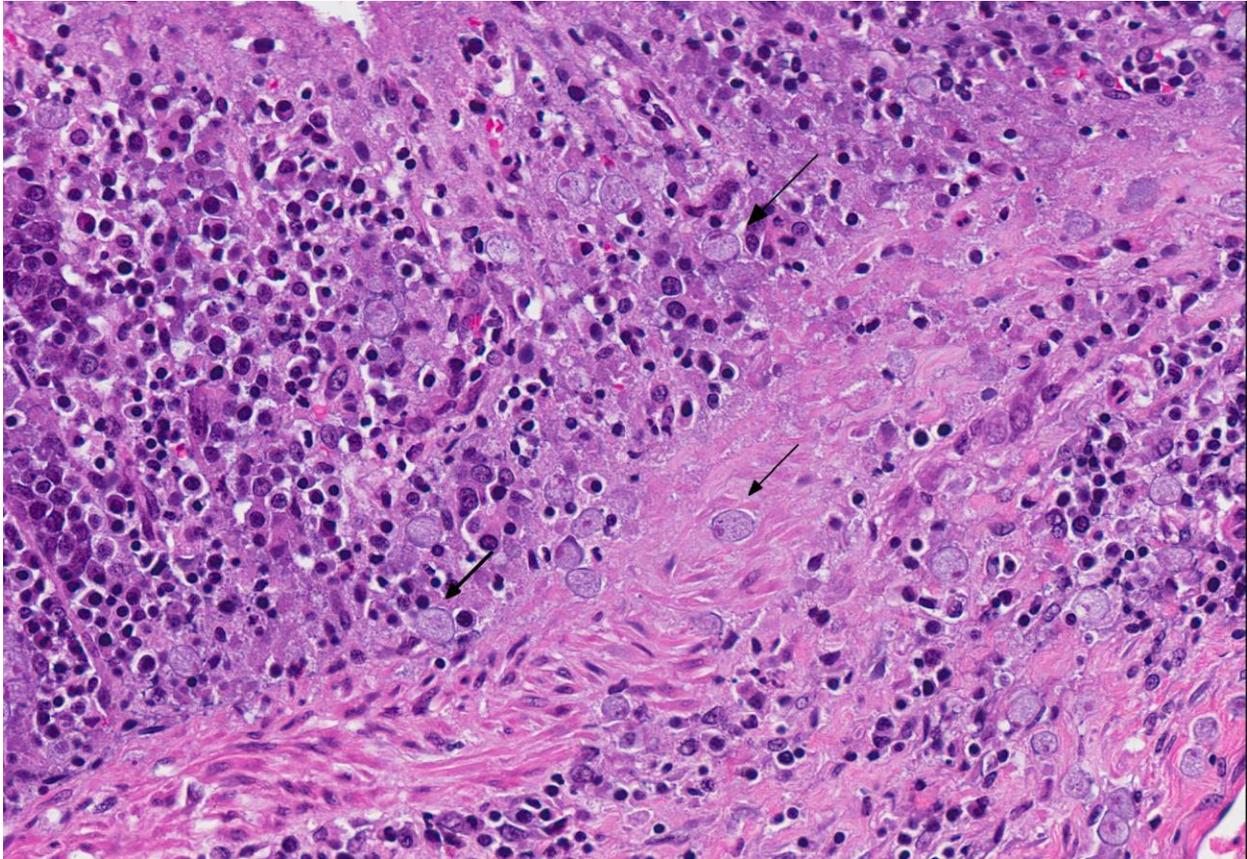
Colon, cynomolgus macaque. Within the necrotic mucosa and extending into the unaffected submucosa, there are numerous amebic trophozoites. (arrows) (HE, 400X).

JPC Diagnosis: Colon: Colitis, necrotizing, multifocal, moderate with numerous amoebic trophozoites, cynomolgus macaque, non-human primate (*Macaca fascicularis).*

Conference Comment: Conference participants discussed two species of Entamoeba: *E. histolytica* and *E. dispar* (the primary form of amoeba found in macaques).[4] Amoebic dysentery caused by *Entamoeba histolytica* is relatively common in humans and nonhuman primates, but rarely infects other species. Cats are susceptible to experimental infection, and infection in dogs is sporadic and most likely caused by ingestion of infected human feces. Dogs act as dead end hosts since they don't pass encysted amebae, and thus present little public health hazard.

Amebae are usually nonpathogenic organisms that inhabit the lumen of the large intestine, but may cause colitis due to changes in host diet or immune status, or the virulence attributes of the organism. Additionally, disease appears to be more common in animals with concurrent *Trichuris* or *Ancylostoma* infections.

The essential steps leading to tissue damage by amebae are: adhesion to mucus by lectins, enzymatic breakdown of protective mucus, and lectin-mediated adherence to host epithelium. *E. histolytica* releases cysteine proteases that cause damage to mucosal epithelium and attract inflammatory

cells, both of which lead to characteristic ulcerative colitis with "flask shaped" ulcers. Microscopically, amebae are surrounded by a clear halo with extensive pseudopodia and possess a nucleus with a dark karyosome. The cytoplasm appears foamy and they frequently phagocytize erythrocytes, which makes them difficult to distinguish from activated macrophages.[7] A periodic acid-Schiff stain was viewed during the conference which nicely highlighted intra-cytoplasmic glycogen granules within amebae; it also lightly stained goblet cells within the mucosa. Trichrome and Giemsa stains can also be used to highlight amoebic trophozoites. Due to the presence of intracytoplasmic glycogen (starch), Lugol's iodine can also be used to diagnose the presence of trophozoites via direct smear.

In humans and non-human primates, primary sites of dissemination are the liver (through the portal circulation) and less commonly, lung and brain. Fatal amebiasis with abscess formation has been reported in various primate species.[5] Recent reports in transgenic mice that overexpress Bcl-2 (anti-apoptotic gene) reveal that epithelial cell apoptosis facilitates *E. histolytica* infection in the intestinal tract.[1]
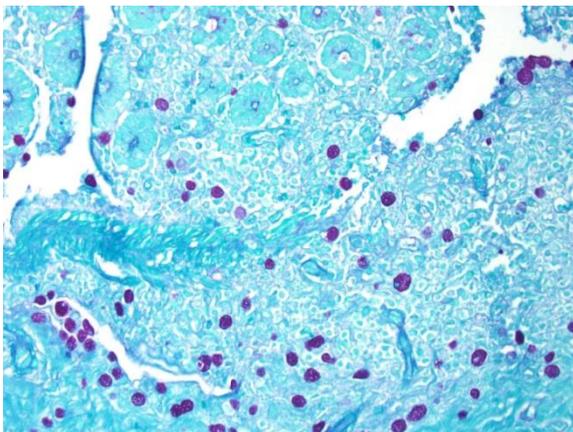
Colon, cynomolgus macaque. A PAS easily demonstrates the presence of the trophozoites. (Periodic acid-Schiff, 400X).

Conference participants discussed several ruleouts including: *Shigella flexneri* and *S. sonnei* (both are gram-negative bacilli that cause necrohemorrhagic colitis and can lead to submucosal ulceration and perforation), *Salmonella enteritidis* and *S. typhimurium* (which, although less common, cause necrotizing suppurative enterocolitis with paratyphoid nodule formation and can lead to septicemia with pyogranulomas in other organs), *Campylobacter jejuni* and *C. coli* (these are spiral bacteria evident with silver stains, and the most frequently isolated enteric pathogens causing mild colonic mucosal hyperplasia), *Yersinia enterocolitica* and *Y. pseudotuberculosis* (large colonies of gram-negative bacteria within necroulcerative enterocolitis), and *Balantidium coli* (ciliated trophozoites with a kidney-shaped macronucleus, can cause ulcerative enterocolitis which is fatal in great apes).[5] Leaf-eating primates (colobus monkey, silver-leafed monkey) are particularly susceptible to erosive and ulcerative gastritis due to a higher gastric pH (similar to the colon in other species) which is conductive to the survival of the amebae.[5] Comparatively, *Entamoeba invadens* was discussed as causing significant disease in snakes. *E. invadens* is typically spread via fecal-oral transmission and results in hemorrhagic enteritis and colitis, with subsequent spread to the liver via portal circulation to cause hepatic abscesses.[2]

Lastly, free-living (leptomyxid) amoebae were reviewed, which rarely cause disease, but may in immunosuppressed animals. *Acanthamoeba* sp., *Balamuthia mandrillaris* and *Naegleria fowleri* may result encephalitis. *Acanthamoeba* sp. and *B. mandrillaris* both cause granulomatous amoebic encephalitis. In contrast, *N. fowleri* infection is such an acute process that there are very few inflammatory cells associated with infection.[5]

Contributing Institution:
http://www.mpiresearch.com

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CASE III: 14-1424 (JPC 4066348).

Signalment: 16-year-old, female, Hafflinger horse (*Equus caballus*).

History: The animal was presented in emergency care center for acute neurological disorders (unsteadiness, fallings and decubitus, amaurosis). Despite critical care, development of a semi-comatose state and convulsions led to euthanasia.

Gross Pathology: At necropsy, the liver had an increased consistency with a variegated aspect and somewhat bulging tissue at section. The brain did not show visible changes.
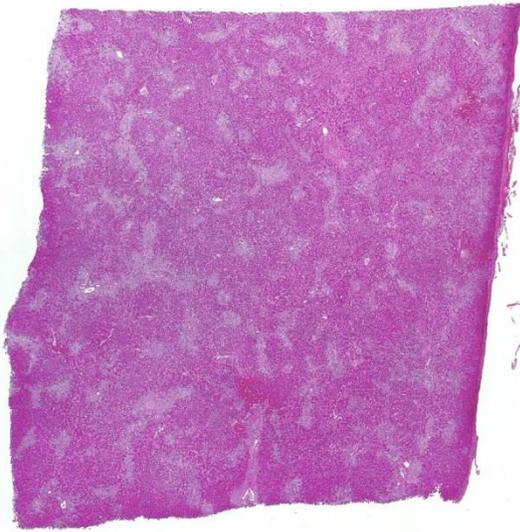
Liver, horse. At subgross magnification, bridging fibrosis between portal areas forms a retiform pattern within the section. (HE, 168X)

Laboratory results: Blood analyses showed severe neutrophilic leukocytosis (Leukocytes 32,99.109/L, neutrophils 28,5.109/L, lymphocytes 2,67.109/L).
Biochemistry exam showed hyperproteinemia (80 g/L), hypoalbuminemia (25 g/L) and hyperglobulinemia (54 g/L).
A severe augmentation of gamma-GT was present (510 U/L), and increased values for GLDH (11,7 UI/L), total bilirubin (89 µmol/L), CK (> 2036 U/L), and lactates (> 12 mmol/L).
CSF fluid analysis did not show significant changes.
PCR (blood for Babesia sp., Theileria sp., and blood and CSF for Borrelia sp.) were negative.

Microscopic Description: Liver: Diffusely, there is marked fibrosis, mostly restricted to portal tract, and multifocal bridging between these portal tracts, distorting normal hepatic architecture. Isolation of individual hepatocytes by fibrosis is also present at the edges of the lobules. Diffusely, there are hepatocytes which are moderately to severely enlarged, with swollen nuclei. Cytoplasm is also enlarged and is vacuolated. In several portal tracts, bile duct proliferation is present. Multifocally, little hemorrhages and discrete infiltration of neutrophils, lymphocytes and macrophages are present.

Contributor's Morphologic Diagnosis: Liver: Hepatocellular degeneration, diffuse, marked, with megalocytosis. Generalized portal and bridging fibrosis and moderate bile duct proliferation, Haflinger, equine.

Contributor's Comment: Such changes observed in the liver are suggestive of pyrrolizidine alkaloids intoxication. These alkaloids are toxic to the liver, leading to irreversible lesions when intoxication comes to chronicity, especially in pigs, horses and cattle. These toxic alkaloids are not directly toxic, and necessitate bioactivation in hepatocytes (especially those in centrilobular region), leading to binding of these agents to proteins and nucleic acids. It results then in inhibition of mitosis, without inhibiting DNA synthesis, leading to megalocytosis. Associated to megalocytosis, there is fibroplasia and bile duct proliferation (fibroplasia is marked in cattle,
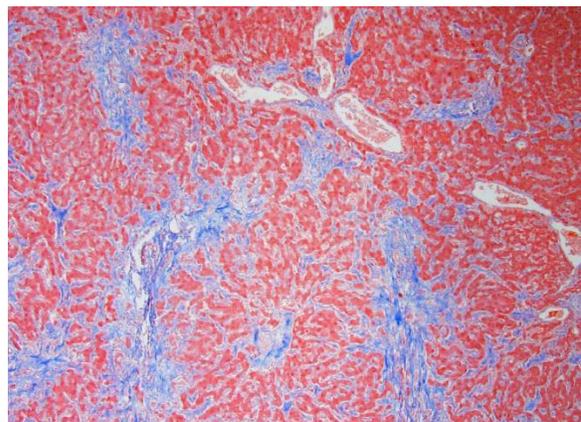
Liver, horse. A Masson's Trichrome stain highlights the extent of portal fibrosis in this section. (Masson's Trichrome, 40X)

moderate in horses and often minimal in sheep). Pyrrolizidine alkaloids are not the only toxic substances to cause such megalocytosis. Indeed, aflatoxins and nitrosamines can lead to this change in liver.[1,2]

Clinically, chronic intoxication by pyrrolizidine alkaloids is characterized by liver failure and its possible consequences (icterus and photosensitization). Secondary neurological signs can develop, known as "hepatic encephalopathy". Histopathological analysis of the brain of this horse showed presence of Alzheimer type II cells, consistent with this syndrome. In species other than the horse, spongiosis is also present in addition to Alzheimer type II cells.[3]

Many plants containing pyrrolizidine alkaloids can be ingested (*Senecio* spp*., Crotalaria* spp*., Heliotropium* spp.). In this particular case, the plant responsible for these lesions was not identified; given the wide distribution of *Senecio vulgaris* in the region where the horse lived, its consumption was very likely the origin of this chronic intoxication.[4]

JPC Diagnosis: Liver: Fibrosis, portal and bridging, diffuse, moderate with hepatocellular loss, karyomegaly and megalocytosis, and biliary hyperplasia, Haflinger, equine.

Conference Comment: This case provides a classic example of pyrrolizidine alkaloid toxicity in the liver. Participants described the dense bridging fibrosis from periportal
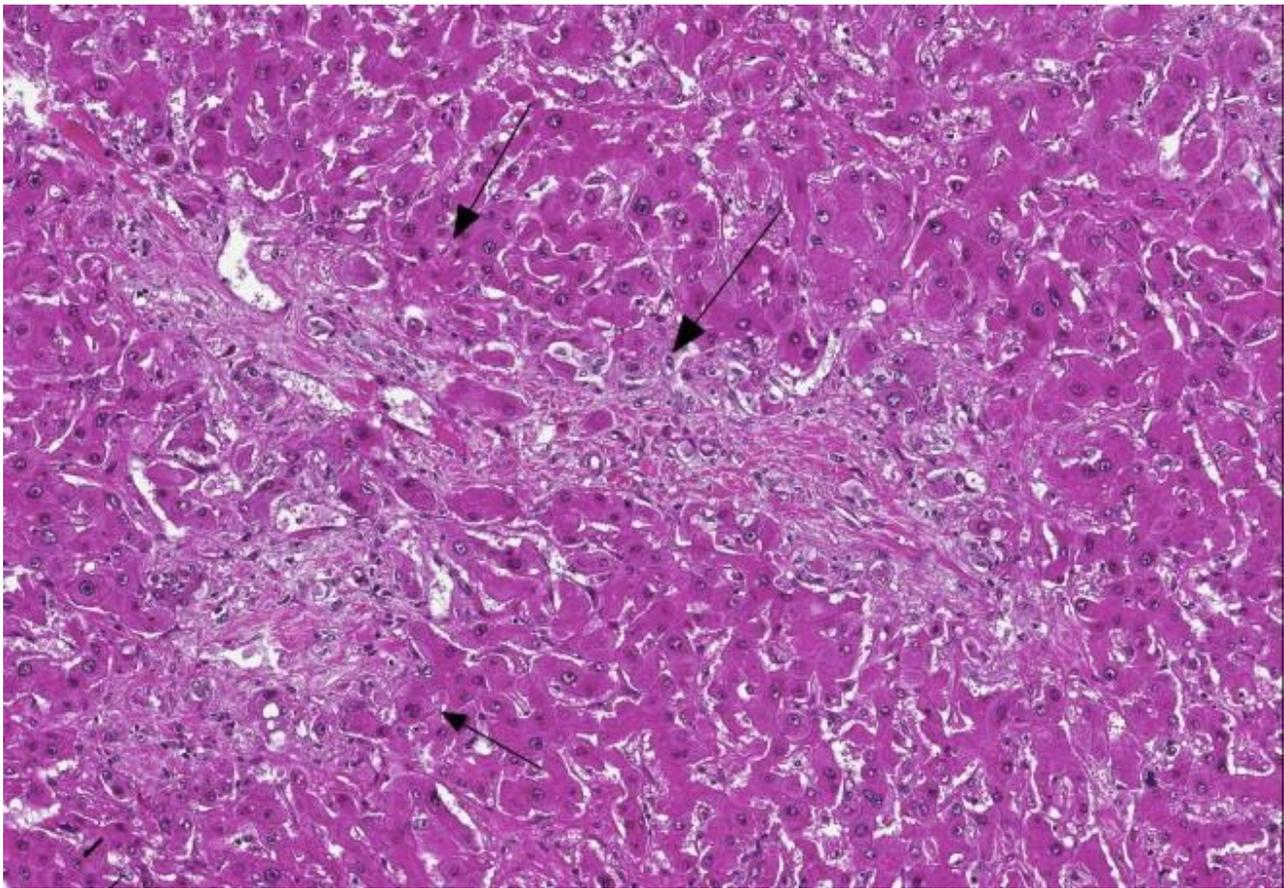
Liver, horse. Higher magnification of the portal areas demonstrates the extent of fibrosis, which encircles and replaces the hepatocytes of the limiting place, and the mild associated biliary hyperplasia. (HE, 168X)

regions that surrounds and separates hepatocytes and effaces the limiting plate as well as the ductular reaction with cholestasis. Occasionally obliterated centrilobular veins were suggestive of veno-occlusive disease. Particularly prominent are the perinuclear accumulations of bile pigment within hepatocytes and multifocal karymegalic and/or multinucleated hepatocytes which are a characteristic finding in several varieties of toxic hepatic diseases.

The contributor offers a concise review of pyrrolizzidine alkaloid toxicity including pathogenesis and clinical signs. The submitted blood work was reviewed during the conference, illustrating the degree of cholestasis and hepatocellular damage (elevated GGT and bilirubin) as well as the musculoskeletal damage and reversion to anaerobic metabolism due to persistent convulsions (elevated CK and lactate).

The moderator led a brief discussion of hepatic encephalopathy caused by hyper-ammonemia. Within the large intestine, breakdown of protein and urea by microflora occurs routinely to produce ammonia. Ammonia is also produced within the liver (hepatic deamination of amino acids) and in peripheral tissues (from metabolism of glutamate). Normally, ammonia is removed the first time through the liver via portal circulation, whereupon it enters the urea cycle. However, acute hepatic disease can result in buildup of ammonia within the circulation which passes through the blood-brain barrier, and causes a decrease in energy metabolism, astrocyte injury and edema formation, and neuronal injury. Overworked, damaged astrocytes cluster together in pairs with enlarged swollen nuclei, margination of chromatin, and prominent nucleoli to form Alzheimer type II astrocytes.[3]

Various plant species that produce different types of alkaloids were reviewed: *Compositae* (*Senecio* spp.), *Leguminosae* (*Crotalaria* spp., *Tephrosia* spp.), and *Boranginaceae* (*Heliotropium*, *Cynoglossum*, *Amsinckia*, *Echium*, *Trichodesma* and *Symphytum* spp.). *Croatalaria* sp. affects the widest range of tissues. Pyrrolizidine alkaloid toxicity depends on four factors: which alkaloids are produced, which organ is affected and the metabolic activity of target cells, the rate the alkaloid is converted to toxin versus the efficiency of glutathione conjugation, and the species, sex, and age of the animal. Monogastric species are the more susceptible to toxicity, as they lack a rumenal degradative pathways for toxin; sheep and goats have the greatest resistance. Horses are more likely to develop hepatic encephalopathy which causes head-pressing and compulsive walking and leads to idiomatic names like "walkabout" and "walking disease".[3]

Several differential diagnoses for hepatotoxins were discussed during the conference, including aflatoxins, nitrosamines, triterpenes, methylazoxy-methanol, and indospicine.

Toxin	Produced by	Microscopic changes
Aflatoxin (B1 most potent)	*Aspergillus flavus, A. parasiticus, Penicillium puberulum*	Biliary hyperplasia and hemorrhagic necrosis; megalocytosis is less prominent
Nitrosamines (dimethylnitrosamine)	Reaction product of trimethylamine with sodium nitrite (preservative) in herring meal	Not specific – slowly developing hepatotoxicity; megalocytosis, fatty change, bile accumulation
Triterpenes (predominately Lantadene A and C)	*Lantana camara*, an ornamental shrub native to the Americas and Africa	Focal hepatic necrosis, canalicular cholestasis; icterus and photosensitization
Methylazoxymethanol	*Cycas* or *Zamiaceae* spp. plants produce toxin	Centrilobular necrosis Cycads also produce neurotoxic animo acid β-N-methylamino-L-alanine (BMAA) – cause CNS lesions due to excitotoxicity
Indospicine (6-amidino-2-hexanoic acid)	Legumes of the genus *Indigofera*	Cattle/dogs: centrilobular necrosis, cholestasis, hepatic encephalopathy Horses: neurologic disorder "Birdsville horse disease"

Chart adapted from Cullen JM, Stalker MJ. Liver and biliary system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol 2. 6th ed. Philadelphia, PA: Elsevier; 2016:330-343.

Species differences with regard to target tissues and histologic lesions were also discussed. Cattle tend to develop more severe fibrosis that can lead to veno-occlusion. Sheep affected by *Heliotropium europaeum* and *Echium plantagineum* may develop severe intravascular hemolysis if liver copper content is high and hepatic mass is decreased. In pigs, pyrrolizidine alkaloids cause pulmonary emphysema with diffuse fibrosis and potential renal insufficiency. Experimental exposure in rats leads to progressive pulmonary disease, pulmonary hypertension, and cor pulmonale with necrotizing vasculitis of the pulmonary arterioles. Lastly, goats are relatively resistant, but with exceptionally high doses of *Croatalaria retusa* they develop acute lesions consisting of centrilobular hemorrhagic necrosis, midzonal hepatocyte swelling and vacuolation.

Finally, a review of photosensitization was provided by the moderator. Photo-sensitization is the inflammation of unpigmented skin (usually) due to the reaction of ultraviolet light of wavelengths 290-400 nm on photodynamic compounds that have become bound to dermal cells. Type I or primary photosensitization those

photodynamic compounds were deposited unchanged in the skin before the liver (healthy) could excrete it. Examples were given of photosensitizing plants that contain pigments from the helianthrone (St. John's Wort, buckwheat) or furocoumarin (spring parsley, bishop's weed, Dutchman's breeches, giant hogweed) family. The pigments produced by helianthrone are hypericin and fagopyrin, and furocoumarin are psoralens.

Type II photosensitization is due to defective pigment synthesis associated with several congenital conditions is specific breeds. Bovine congenital hepatopoietic porphyria ("pink tooth") is most common in Shorthorn, Ayrshire, Holstein and Jamaican cattle and causes a deficiency in uroporphyrinogen III cosynthetase which results in red-brown coloration of porphyrin in dentin and bone and skin lesions due to accumulated uroporphyrins which absorb UVA radiation. Siamese cats are also prone to congenital photosensitization and the deficiency is also thought to be uroporphyrinogen III cosynthetase. Another inherited deficiency is seen in Limousin cattle that develop bovine erythropoietic protoporphyria due to ferrochelatase deficiency with leads to protoporphyrin IX accumulation in blood and tissue. In this disease, photodermatitis is the only lesion. Type III (hepatogenous) photosensitization is the most common form and usually accompanies cholestasis of more than a few days' duration in herbivores eating green feed that are kept in direct sunlight. Phytoporphyrin (phylloerythrin), a porphyrin produced in herbivores by rumenal microflora as a breakdown product
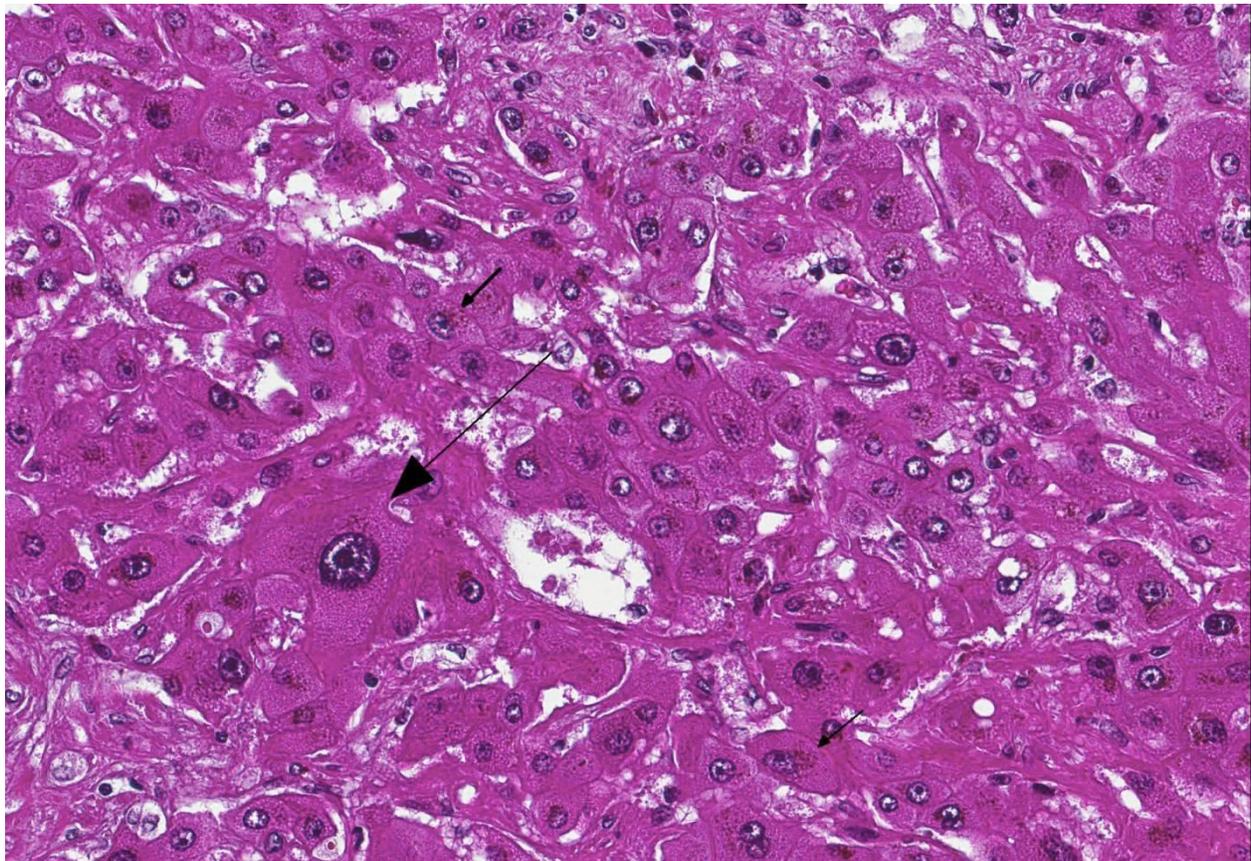
Liver, horse. Hepatocytes are diffusely swollen, effacing normal plate architecture by the accumulation of numerous intracytoplasmic vacuoles, and cell borders are distinct. Occasionally hepatocytes are increased up to 5x normal, with a single large nucleus (megalocyte) (large arrow). Hepatocytes often contain an aggregate of lipofuscin granules in proximity to the nucleus (small arrows). (HE, 400X)

of chlorophyll, is released into portal circulation, removed by hepatocytes, and excreted in bile. Cholestasis increases retention of phytoporphyrin in the blood and result in dermatitis of unpigmented areas of skin exposed to sunlight.[3]

Contributing Institution:
Anatomie Pathologique
Vetagro sup
Campus vétérinaire

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CASE IV: T17-15923 (JPC 4101085).

Signalment: 8-month-old, female, German Shepherd Dog (*Canis familiaris*).

History: A 5 x 5 x 3 cm gingival mass was observed on right mandible.

Gross Pathology: A multilobulated exophytic growth.

Laboratory results: None provided.

Microscopic Description: Gingiva: The mass was a non-demarcated and non-capsulated mass that contained epithelial and mesenchymal elements composed of islands of odontogenic tissue lined with a palisading columnar epithelium along a basement membrane consistent with ameloblasts supported on ample spindled to stellate mesenchymal cells (dental pulp). Multiple section consistent with a
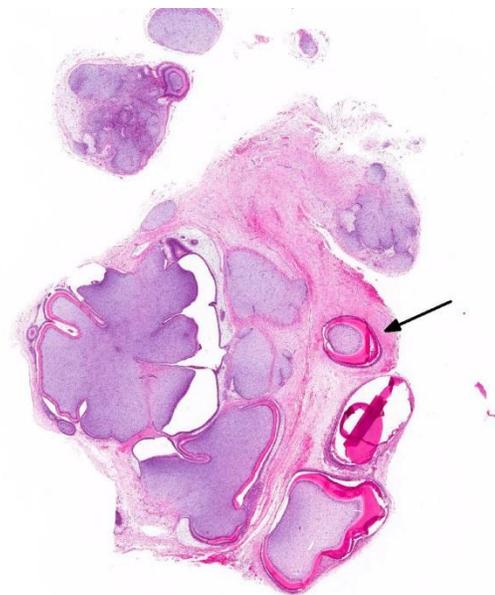
Mandible, dog: The submitted submucosal tissue demonstrates numerous well-formed tooth like structures (denticles). An arrow demonstrates one structure bearing an extremely strong resemblance to a developing tooth. (HE, 11X)

developing tooth like-structures (denticles) composed of odontoblasts, dentin and enamel were observed. Mitotic cells and malignant features were not present.

Contributor's Morphologic Diagnosis: Gingiva: Odontoma, German Shepherd dog, canine (*Canis familiaris*).

Contributor's Comment: Differential diagnoses for maxillary and mandibular swelling in immature dogs include trauma, infection, developmental disorders, and neoplastic lesions. Neoplastic oral maxillary or mandibular masses in immature, young dogs are typically of odontogenic origin.[2] Odontomas are rare[1] slow-growing masses that occur during odontogenesis, mainly in young dogs, resulting in dentition disruption and in prevention of eruption or displacement of normal teeth.[3] The masses cause swelling and deformity of mandible and/or maxilla. The cause and pathogenesis of odontomas, either in humans or nonhuman species, is unknown.[2,3] However, hereditary factors, genetic alterations during dental development, infections or trauma have been suggested to be involved in odontoma formation.[3]

Odontomas are odontogenic tumors with features of resembling the embryonic pattern of tooth development. Production of enamel, dentin, cementin, and sometimes small teeth

Mandible, dog: Higher magnification of tooth-like structures (denticles). PU= dental pulp; E=Enamel; D=Dentin (black arrows). (HE, 400X) (Photo courtesy of: The University of Georgia, College of Veterinary Medicine, Department of Pathology, Tifton Veterinary Diagnostic & Investigational Laboratory, Tifton, GA 31793; http://www.vet.uga.edu/dlab/tifton/index.php)

are characteristic. Tumors with patterns resembling normal teeth are compound odontomas, whereas a more disorganized arrangement is a characteristic of complex odontomas. Complex odontomas are composed of well-differentiated but disorganized dental hard tissues bearing no resemblance to a tooth and surrounded by a thin fibrous capsule. Compound odontomas are composed of many separate, small tooth-like structures known as "denticles", each one containing enamel, dentin, cementum, and pulp which are anatomically similar to normal teeth.[3,4] Compound odontomas are localized in the mandible or maxilla. However, in most reported cases in dogs, compound odontomas are localized in the mandible[3].

In dogs and other species, clinical signs associated with odontomas include facial or mandibular swelling, ocular symptoms, missing teeth, or tooth-like structures erupted into the oral cavity. Odontomas can become extremely large, even in very young dogs.[2]

Surgical excision is the treatment of choice for odontomas. Complete surgical excision of the mass in combination with aggressive curettage would result in a favorable outcome.[2,3] For prognostic and therapeutic considerations, it is important to differentiate odontomas from ameloblastic odontoma and ameloblastoma, which develop in old dogs.[4]

JPC Diagnosis: Gingiva: Compound odontoma, German Shepherd Dog, canine.

Conference Comment: This case provides an excellent example of a compound odontoma in a dog. The contributor's review of odontomas is excellent and mirrors much of the initial conference discussion. Conference participants described hamar-

tomatous proliferation of odontogenic epithelium and primitive mesenchyme forming variably sized tooth-like structures in variable stages of development (denticles).

The moderator began with a review of tooth development which begins with two embryonic tissues: buccal cavity squamous epithelium (BCSE) and embryonic mesenchyme (EM). The EM forms dentin, cementum, and pulp, and the BCSE invaginates into the EM to form the dental lamina. The dental lamina grows into the adjacent tissue to form the dental bud which progresses through cap and bell stages eventually leading to crown eruption. The epithelium (BCSE) differentiates to form the ameloblastic cell layer at the outer surface of the tooth that produce enamel and the mesenchymal layer (EM) differentiates into odontoblasts, cementoblasts, and pulp (also periodontal ligament and alveolar bone) which move toward the center of the tooth and makes dentin and cementum respectively. Enamel covers the outside of the tooth and is composed of hydroxyapatite (mineral) crystal and heavily mineralized calcium salts. Dentin comprises the bulk of the tooth mass under the enamel and is slightly softer with tubules that contain odontoblast processes. Cementum covers the root of the tooth (doesn't project above gumline) and is a bone-like substance (contains basophilic reversal lines) with embedded cementocytes.[5]

The microscopic characteristics of epithelium of odontogenic origin (also known as ameloblastic epithelium) are: peripheral palisading, anti-basilar nuclei, basilar epithelial cytoplasmic clearing, and non-basilar epithelial cells (stellate reticulum) have intercellular bridges which separate the inner and outer layer of enamel epithelium during development.[3] There are

Tumor	Odontogenic epithelium	Stroma	Mesenchyme	Matrix	Species affected	Misc.
Ameloblastoma	Yes	Not essential for diagnosis	None	None	Dog, cat, horse	Keratinization may occur
Amyloid producing odontogenic tumor	Yes	Not essential for diagnosis	None	Amyloid	Dog, cat, horse	Matrix composed of enamel proteins which are still Congophilic and exhibit apple-green birefringence; IHC + for laminin
Canine acanthomatous ameloblastoma	Yes	Stellate fibroblasts in dense collagen; regularly spaced dilated, empty blood vessels	Periodontal ligament	None	Dog	Interconnected sheets of odontogenic epithelium
Ameloblastic fibroma	Yes	Loose, collagen poor, resembles dental pulp	Dental pulp	None	Young animals, cattle	Most common oral neoplasm in cattle
Ameloblastic fibro-odontoma	Yes	Loose, collagen poor, resembles dental pulp	Dental pulp	Dentin or enamel	Young animals, cattle	
Complex odontoma	Yes	Well-differentiated dentinal tissue	Dental pulp	Dentin, enamel (may be mineralized)	Dog, rodent, primates, horse	Horse, rodents produce cementum; "balls of disorganized dental hard substance"
Compound odontoma	Yes	Well-differentiated dentinal tissue; dense collagen and vascular connective tissue	Dental pulp	Dentin, mineralized enamel	Young dogs	Multiple tooth-like structures (denticles)

Chart adapted from Munday JS, Lohr CV, Kiupel M. Tumors of the alimentary tract. In: Meuten DJ, ed. *Tumors in Domestic Animals*. 5th ed. Ames, IA: John Wiley & Sons, Inc.; 2017:530-542.

two types of teeth in mammals, brachydont (carnivores) and hypsodont (ruminants and horses). Brachydont teeth are characterized by a short crown above the gingiva, constricted neck at the gumline, and a root embedded in the jawbone. Hypsodont teeth are high-crowned teeth extending past the gum line that continue to erupt throughout life.[5]

The moderator compiled a very complete description of various dental tumors classified as: epithelial with mature fibrous stroma (no odontogenic mesenchyme, not inductive) and mixed epithelial and mesenchymal (odontogenic epithelium and ectomesenchyme, inductive).

Tumors of mesenchyme or odontogenic ectomesenchyme with or without sparse odontogenic epithelium were briefly presented: Peripheral odontogenic fibromas (POFs) are composed of connective tissue consisting of delicate fibrillar collagen of varying density and evenly spaced stellate cells. Relatively small amounts of odontogenic epithelium are present in the connective tissue. At times, this tumor can be difficult to differentiate from fibrous gingival hyperplasia. However, POFs present as a single mass whereas gingival hyperplasia is multifocal and extend linearly along the gingiva.

Within the domestic animal species, cementomas have only been reported in the horse. They appear as radiopaque masses surrounding tooth roots of the incisors (most common). Microscopically they are composed of thick deposits of irregular cementum-like material with basophilic reversal lines that are fused to the base of the tooth. Osteomas can look similar but will be fused with adjacent bone, not with the tooth.

Finally, the various types of odontogenic cysts, both developmental and inflammatory, were discussed. Developmental or dentigerous cysts are the most common and occur usually in sheep and brachycephalic dogs. The epithelial lining of dentigerous cysts is thought to be derived from the reduced enamel epithelium. There are two types of inflammatory cysts: periapical (radicular) cysts which form around the apex of non-vital teeth and are due to periodontal or endodontal disease, and lateral periodontal cysts which are less common and occur lateral to the root of a vital tooth. In general, all types of odontogenic cysts have a true epithelial lining and usually an underlying loose fibrous wall.[3]

Contributing Institution:

The University of Georgia
College of Veterinary Medicine
Department of Pathology
Tifton Veterinary Diagnostic & Investigational Laboratory
Tifton, GA 31793
http://www.vet.uga.edu/dlab/tifton/index.php

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