The Armed Forces Institute of Pathology
Department of Veterinary Pathology

Conference Coordinator
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WEDNESDAY SLIDE CONFERENCE 2010-2011

Conference 3

25 August 2010

Conference Moderator:
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CASE I: 05-8848 (AFIP 2986919).

Signalment: 10-week-old, female, Yorkshire x Landrace, pig (*Sus scrofa domesticus*).

History: This feeder operation with 1200 pigs experienced repeated episodes of scouring and high rates of weight loss in younger pigs. The farm had changed production to "natural" approximately 12 months previously. Diseased pigs had not been treated.   The ration was a corn/soybean mix supplemented with vitamins and mineral concentrate. Six, 10-week old pigs were submitted alive.   The vaccination status of the submitted animals was unknown.

Gross Pathology: All six piglets were runted, dehydrated and had fecal stains around the anus.   The tissues on the conference slide were taken from a single piglet (piglet #1) and all subsequent descriptions focus on this animal.   The soles of the feet and the right hind food had up to 0.5 cm in diameter, sharply demarcated depressions that lacked epithelial covering. Both palatine tonsils each had a single, sharply demarcated, red-green, 0.5 to 1.0 cm in diameter, deep depression that lacked epithelial covering. The mucosa of the caudal ileum was diffusely thickened to 2 mm and slightly granular.   The contents of all intestinal segments were watery. The colonic and cecal contents also had thick aggregates of yellow, rubbery material that was multifocally slightly adherent to the mucosa.   A few other piglets had cranioventral consolidation of the lungs and rare, small, renal cortical cysts.

Laboratory Results: Coronavirus was detected on electron microscopic examination of ileal mucosal scrapings. *Lawsonia intracellularis* was detected by immunohistochemistry in sections of ileum.   A specific pathogen was not isolated from cecal mucosal scrapings. Endoparasites were not identified on fecal flotation.   PCR was positive for porcine circovirus type 2 (PCV). PCR was negative for swine influenza virus and porcine respiratory and reproductive syndrome virus (PRRSV).

Histopathologic Description: Sections were cut from two blocks resulting in slight variation between slides. Nonetheless, all slides should show the three main lesions to some degree.

Diffusely, small intestinal villi are moderately to severely blunted and many are fused. Ileal segments have a thickened lamina epithelialis mucosae with long, branching crypts. The lamina propria mucosae has multifocal, mild, neutrophilic infiltrates.   Peyer's patches are moderately depleted and rarely have individual multinucleate giant cells. The colon has multifocal crypt necrosis and dilation and segmental ulceration with massive bacterial colonization. On Warthin-Starry silver impregnated re-cuts, large numbers of short, comma-shaped bacteria colonize the apical aspect of affected ileal enterocytes.

Contributor's Morphologic Diagnosis: 1. Ileum: Severe diffuse proliferative ileitis (with intraepithelial, comma-shaped bacteria as per special stain).
2. Small intestine: Moderate to severe diffuse villous atrophy.
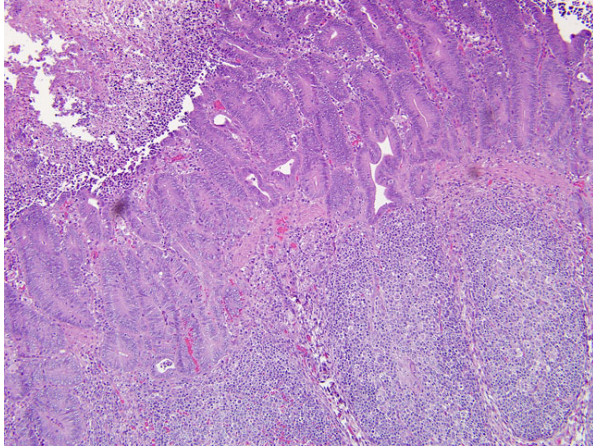
1-1. Ileum, pig. Diffusely in the mucosa there is blunting and fusion of villi, and intestinal crypts are elongated and branching (epithelial proliferation). (HE 100X)
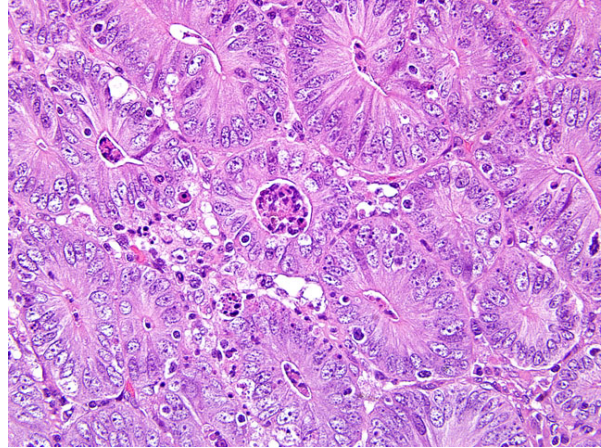
1-2. Colon, pig: In the mucosa, there is multifocal crypt herniation and crypt abscesses. Crypts are lined by closely packed, tall columnar epithelial cells with amphophilic to basophilic cytoplasm (hyperplasia). (HE 200X)

3. Colon: Moderate multifocal, acute, fibrino-necrotizing colitis with intralesional colonies of mixed bacteria.

Contributor's Comment: This case is a good example for the complexity of enteric disease in pigs. Clearly there are (at least) three disease processes.

1. The diffuse villus atrophy of the small intestine is characteristic of a viral infection. It is a bit surprising that a coronavirus was still detectable in this 10-week-old pig.

2. The proliferative ileitis and the morphology of the intraepithelial bacteria on silver impregnation are suggestive of an infection with either *Lawsonia intracellularis* or *Campylobacter* spp. The presence of *L. intracellularis* was confirmed by immunohistochemistry.

The nomenclature of this bacterial pathogen has been changed numerous times in the past decades, most recently from "ileal symbiont intracellularis" to *Lawsonia intracellularis*.[9] Proliferative enteropathy (PE) with intraenterocytic *L. intracellularis* has been described in a wide range of hosts. The distribution of lesions varies with the host ranging from ileum (white tailed deer, horse, guinea pig, and rhesus macaques), to caudal ileum and colon (pig and hamster), cecum and colon (rabbit, blue fox, and ferret), to rectum (emu). Four forms of enteric lesions have been associated with *L. intracellularis* infection in pigs.[7] Weaners or young growing pigs are most commonly affected by a persistent uncomplicated proliferation sometimes described as porcine intestinal adenomatosis (PIA). The lower ileum and – less commonly – colon have small, raised, opaque islands to an irregular nodular or folded surface. Histologically, proliferating crowded immature enterocytes form branched and elongate crypts replacing the normal villous epithelium. Villus loss and the lack of a brush border on affected cells clearly interfere with intestinal physiology. Affected enterocytes are colonized by apical, comma-shaped

bacteria that reside free in the cytoplasm. In mature animals (>4 months of age), infection results in proliferative hemorrhagic enteropathy (PHE), an acute clinical disease in which major intestinal hemorrhage arises from a proliferative ileal lesion. Proliferation may not be as severe as in PAI and bacteria may be seen also extracellular and in macrophages. Necrotic enteritis (NE) and regional ileitis (RI) represent a proliferative lesion that has been subject to further insult. Necrotic enteritis is an extensive coagulative necrosis of the epithelium that often results in rapid death. Regional ileitis is thought to be the outcome of NE with replacement of the damaged mucosa by granulation tissue associated with hypertrophy of the tunica muscularis ("hosepipe gut"). In a recent study on the infection dynamics of *L. intracellularis* under field conditions, shedding was detected by PCR in 75% of 100 pigs at 10-12 weeks of age (22-29 kg) and had ceased by 18 weeks of age. Seroconversion was detected after the expected lag time at 12-14 weeks of age and 92% of the pigs remained seropositive until slaughter.[11] A negative effect on growth rate was documented shortly before and during early infection followed by a compensatory impact. In another study, seroconversion and – prevalence of intragastrically with *L. intracellularis* challenged piglets from *L. intracellularis*-seropositive and seronegative gilts were compared.[2] Piglets from seronegative gilts had highest seroprevalence at 5 weeks (84%) that declined gradually from week 11 to week 26 (10%). Offspring of seropositive gilts had only a seroprevalence of 23% at 5 weeks that decayed much faster to 0% at week 17. Cellular immune response *to L. intracellularis* infection has been documented in piglets challenged intragastrically with wild type and attenuated live vaccine by ELISPOT assay for IFN-gamma.[4]

Very little is known about the pathogenesis of *L. intracellularis* infection. This is largely due to the obligate intracellular nature of the pathogen, which does not allow application of standard molecular techniques.[8] An outer membrane protein LsaA (Lawsonia surface antigen) has

recently been identified and partially characterized. It is a member of the TlyA family, which are proteins present in a wide range of bacteria that in a few cases cause hemolysis. LsaA does not mediate hemolysis, but is expressed during infection and monoclonal antibodies to LsaA significantly inhibit in vitro infectivity of *L. intracellularis*. This suggests a role of LsaA in attachment and/or entry of *L. intracellularis*. The site of attachment and entry in vivo are immature enterocytes at the base of crypts. How the proliferative character of the lesion comes about has yet to be determined. Some data suggests that bacterial replication and dissemination is tied to replication of the host cell. (data reviewed in[7])

L. intracellularis is difficult to culture (requires co-culture with mammalian cells). Histopathology in conjunction with demonstration of bacteria in tissues by silver impregnation can only suggest an infection with *L. intracellularis* as *Campylobacter* spp. share morphologic characteristics and can be encountered in enterocytes of proliferative enteritis cases in pigs. *L intracellularis* can be identified by PCR on feces or diseased intestine; immunohistochemistry; or immunofluorescence on sections of affected intestine.[5,6]

3. The fibrino-necrotizing colitis is interpreted as a sequel of the proliferative enteropathy. The colonization with bacteria is thought to be a tertiary problem as a specific pathogen was not isolated. The differential diagnosis for fibrinonecrotic colitis should include salmonellosis and European [classical] and African swine fever.

The significance of the detection of porcine circovirus type 2 (PCV) is uncertain, as it can be detected in clinically healthy pigs. The only lesion present that could be associated with a PCV2 infection in this piglet was the rare multinucleate giant cells in Peyer's patches, yet they lacked cytoplasmic inclusion bodies.

AFIP Diagnosis: 1. Ileum: Enteritis, proliferative, diffuse, moderate, with crypt necrosis and abscesses, and villar atrophy, blunting, and fusion.
2. Colon: Colitis, proliferative and necrotizing, diffuse, moderate, with crypt herniation.
3. Jejunum: Enteritis, lymphoplasmacytic, diffuse, moderate, with villar blunting and fusion.

Conference Comment: Conference participants appreciated this real-world case with overlapping lesions containing more than one etiology. Since the submission of this case in 2005, there have been several advances concerning the pathogenesis of *L. intracellularis* infection in pigs; for example, it has been shown that bacterial entry into host cells requires host cell activity and actin polymerization. Additionally, there is speculation that a type III bacterial secretion system may be involved, similar to that used by *Salmonella* spp.[1] Briefly, this is a process by which bacterial proteins are transferred into enterocytes which then activate host cell Rho GTPases and triggering actin rearrangement

and bacterial uptake.[12] The method by which the *L. intracellularis* bacterium stimulates host cell replication remains unknown.

Lawsonia intracellularis shares many features with other obligate intracellular pathogens in the orders *Chlamydiales* and *Rickettsiales*. Obligate intracellular bacterial pathogens often obtain a portion of their energy needs from the host cell; acquiring all of their metabolic demands from the host cell would be disadvantageous as this would reduce host cell proliferative potential. While both rickettsial and chlamyidal organisms are capable of generating their own energy, they also use the host cell as a supplementary source of energy, as well as a source of nucleotides. Based on genomic analysis, there is speculation that *L. intracellularis* can generate its own energy; however, the bacterium still requires supplementation from the host cell, and this is accomplished through the use of an ATP/ADP translocase whereby bacterial ADP is exchanged for host cell ATP. The ATP/ADP translocase enzyme belongs to a broad class of translocases termed nucleotide transport (NTT) proteins which import nucleotides or ATP from the host cell into the bacterium.[10]

In the past decade, *L. intracellularis* has been seen with increasing frequency in foals and weanlings. A retrospective study of infections in Kentucky between 2005 and 2007 found infection to be most common in two to eight-month-old thoroughbreds between the months of August and January. The most common clinical findings were ventral edema and hypoalbuminemia.[3]

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CASE II: 9759624 (AFIP 3162239).

Signalment: A 6-year-old, male, neutered, domestic short hair, cat (*Felis silvestris catus*).

History: Chronic history of respiratory disease.   Chest radiographs showed pneumonia or possible pulmonary mass.

Gross Pathology: The postmortem was performed by the referring veterinarian. There were multifocal firm regions in the right middle lung lobe and a mass compressing the right medial bronchus. Lung and mediastinal lymph nodes were submitted for histopathological examination.

Laboratory Results: Not available.

Histopathologic Description: Lung: There are multifocal to coalescing inflammatory areas effacing the normal lung parenchyma. These areas are characterized by large numbers of neutrophils and macrophages with abundant foamy cytoplasm that occasionally form multinucleated cells. Within the inflammatory foci there are many round large, 30-60 μm in diameter spherules with a thick double wall (up to 2μm) fungal yeast. Some of these organisms represent the variable morphology typical of *Coccidioides immitis*, such as mature spherules with round sporangia with thick birefringent capsule containing endospores or immature spherules containing blue flocculent material.

Contributor's Morphologic Diagnosis: Lung: Severe multifocal to coalescing necrotizing and granulomatous pneumonia with intralesional yeast organisms consistent with *Coccidioides* spp.

Contributor's Comment: Coccidioidomycosis is non-contagious fungal disease caused by a dimorphic soil-borne fungus called *Coccidioides immitis* or *C. posadassi*.   The disease is also known as San Joaquin Valley Fever (in California), Desert fever, and Valley Fever. It is an endemic disease in specific ecological regions that include the southern United States, northern parts of Mexico and some countries in South America.   Coccidioidomycosis is a dimorphic fungus; in soil it presents as a mycelium or arthrospore form that behaves as a saprophytic organism, and when the fungus gains access to tissues and body fluids it becomes the spherule form where it behaves as a parasitic organism.   The pathogenesis starts with inhalation of the infective arthrospores (common in dry environment especially after a long dry weather season followed by heavy rain) or direct inoculation to the skin.

Even though the disease affects humans it is not considered a zoonosis. This disease is not transmitted through animal-animal contact. The only mode of transmission is through contaminated soil or dust with mature arthrospores. Coccidioidomycosis could develop infective arthrospores during fungal culture, and laboratory workers should be cautioned for possible source of infection.

Cats are not particularly susceptible to coccidioidomycosis compared to dogs. The most common presentation in cats is skin and pulmonary. Clinical signs are not very specific, as this organism can affect different tissues including skin, lung, eyes, nervous tissue.

2-1. Lung, cat. Many viable and degenerate neutrophils and macrophages efface the lung and surround large (30-60 um), round fungal yeasts that have thick double contoured walls. Fungal sporangia contain endospores or immature spherules containing blue flocculent material. (HE 400X)
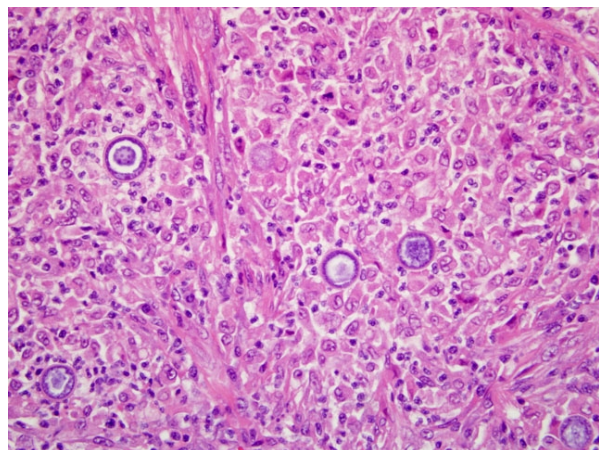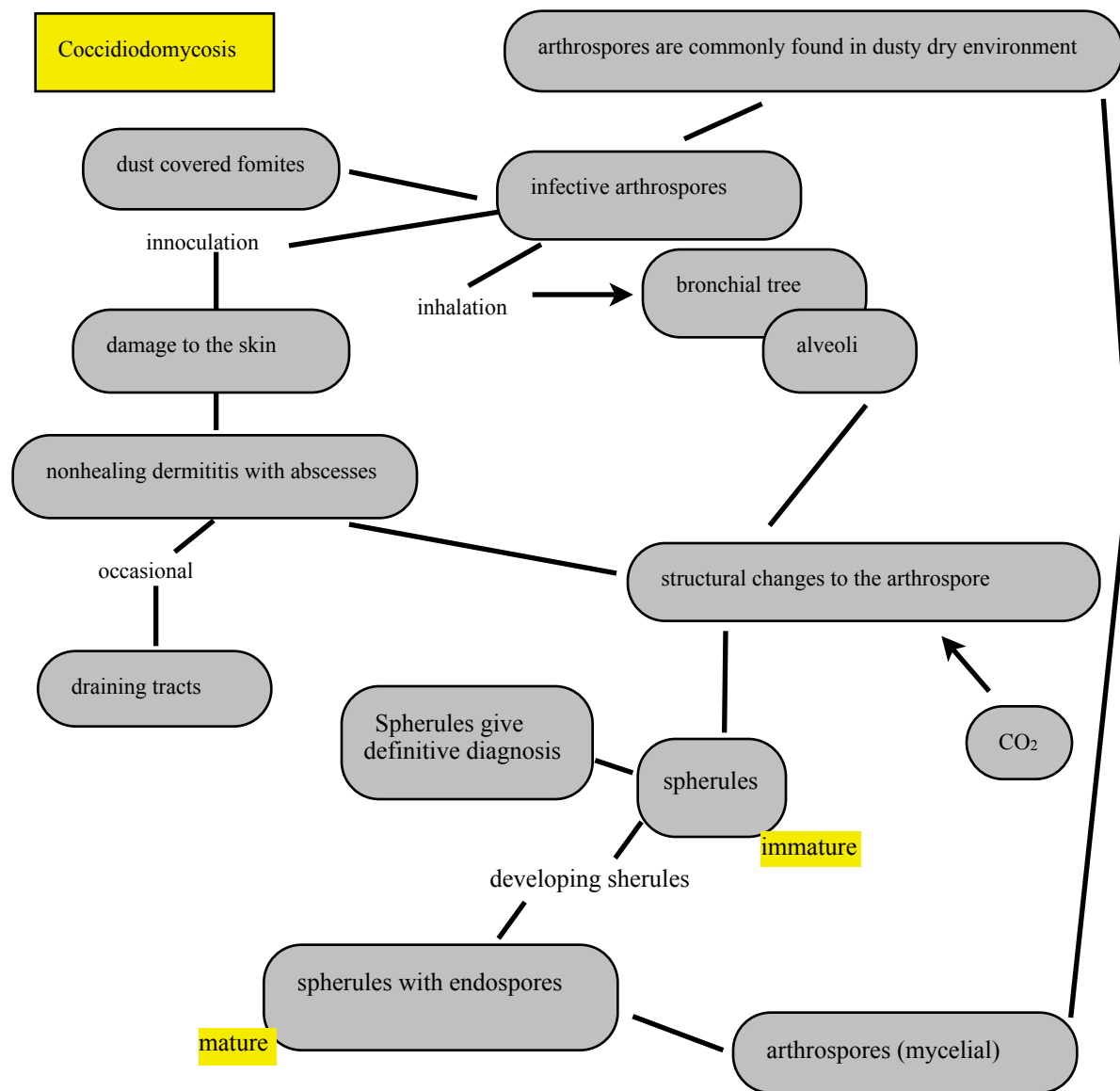
Diagnostic tests available include:

1. Cytology smears/ FNA with identification of spherules (definitive diagnosis)
2. Histopathology with identification of spherules (definitive diagnosis). The variable morphology of *C immitis* is diagnostic: Sporangia 30-200 um in diameter containing 2-4 um endospores (mature spherules); immature spherules
3. Agar gel immunodeficiency (AGID) assay for IgG and IgM- specific test but not sensitive enough
4. ELISA to detect IgG and IgM- sensitive test but false positive results are common. Serological tests appear to be poor in cats

Greene described 48 feline coccidioicomycosis cases with skin (56%), respiratory (25%) musculoskeletal (19%), and CNS and ophthalmic (19%) involvement. There is no current effective treatment available. Fungal culture is not a useful diagnostic tool since definitive identification depends on spherule formation which is the only form in tissues during the parasitic phase of the life cycle of this peculiar fungus. Species affected by this fungus include many mammalian species such as dogs, cats, horses, sheep, cattle, pigs, non-human primates and South American camelids.

The OIE reports this disease as a differential for tuberculosis since the granulomatous, multifocal to coalescing inflammatory process will efface the normal architecture in lymph nodes and lung similar to the pattern encountered in tuberculosis.

Coccidiodomycosis

arthrospores are commonly found in dusty dry environment

dust covered fomites

infective arthrospores

innoculation

inhalation

bronchial tree

alveoli

damage to the skin

nonhealing dermititis with abscesses

structural changes to the arthrospore

occasional

CO₂

draining tracts

Spherules give definitive diagnosis

spherules

immature

developing sherules

spherules with endospores

mature

arthrospores (mycelial)

AFIP Diagnosis: Lung: Pneumonia, pyogranulomatous, diffuse, severe with numerous endosporulating yeast, etiology consistent with *Coccidioides* spp.

Conference Comment: Nearly all conference participants diagnosed pyogranulomatous pneumonia, though a few participants interpreted some of the histologic lesions as granulomas. The moderator discussed the distinguishing histologic features between granulomas versus granulomatous/pyogranulomatous inflammation; the former are histologically well-organized and characterized by a central aggregate of epithelioid macrophages surrounded by a collar of mononuclear leukocytes, principally lymphocytes and occasionally plasma cells; older granulomas may be bounded by an outer rim of reactive fibroblasts and connective tissue.[8] Granulomatous and pyogranulomatous inflammation are not characterized by the same level of histologic organization.

Conference participants discussed the typical clinical presentation of *Coccidioides immitis* infection in the dog and cat. Most infections in the dog are pulmonary, with occasional systemic dissemination to multiple organs including bones and the skin. Bone infections tend to occur in long bones, and result in both lytic and productive lesions; thus, radiographically, coccidioidal osteomyelitis is included in the differential diagnosis for osteosarcoma. In contrast to dogs, the lesions in cats are primarily cutaneous and clinically characterized by multiple draining nodules without underlying bone involvement.

The moderator shared some experiential histopathologic features of pulmonary blastomycosis, coccidioidomycosis, and cryptococcosis. In general, pulmonary blastomycosis tends to be fibrosing; coccidioidomycosis is necrotizing; and cryptococcosis incites very little inflammatory response.

Conference participants also briefly reviewed some of the microorganisms which reproduce via endosporulation, including :

- *Coccidioides immitis*
- *Rhinosporidium seeberi*
- *Prototheca wickerhamii, P. zopfi*
- *Chlorella* spp.
- *Batrachochytrium dendrobatidis* (chytridiomycosis)

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CASE III: BK1 (AFIP 3166500).

Signalment: 1-year-old, male, pit bull terrier/boxer mix, dog (*Canis familiaris*).

History: This mixed breed dog was rescued and adopted at one year of age, and was initially energetic and in good health. The patient developed and was treated for diarrhea and a wheezing cough shortly after adoption. At this time, he developed a cyst-like nodule on his left front paw at the first digit nail bed. He was treated with Epsom salt bath soaks and Benadryl®. This lesion progressed multifocally to the other paws, forming papillary, exophytic growths that spread proximally up the legs to the elbows. At the time of biopsy submission, the patient was having difficulty ambulating. Concurrently, the patient developed a hive-like rash around the face with red welts. Fine needle aspirates, cultures, and biopsies of the affected areas were submitted.

Gross Pathology: Multifocal to coalescing, well demarcated, papillary, proliferative, nodular masses expand the plantar surfaces of the feet, expanding from the foot pads, nail beds, and proximal haired skin of the lower leg. Many of the masses are reddened, ulcerated, and hyperkeratotic.

Histopathologic Description: Haired skin: In this section of haired skin, there is a circumscribed, unencapsulated, shallow, bowl-shaped endophytic, neoplastic proliferation of the surface epithelium compressing and displacing adnexal

3-1, 3-2. Haired skin, Inverted viral papilloma, dog. Multifocal to coalescing, well demarcated, papillary, proliferative, nodular masses expand the plantar surfaces of the feet, extending from the foot pads, nail beds, and proximal haired skin of the lower leg. Many of the masses are reddened, ulcerated and hyperkeratotic. Photographs courtesy of Johns Hopkins School of Medicine, Department of Molecular and Comparative Pathobiology, 733 N. Broadway St., Baltimore, Maryland 21205, nnath2@jhmi.edu

structures in the underlying dermis. Neoplastic cells are arranged in broad infolds and papillary projections supported on thin fibrovascular cores. There is hyperplasia of the basal cells with differentiation to large polygonal, hyperplastic epithelial cells with distinct borders, abundant basophilic cytoplasm, and round to oval central nuclei with finely stippled chromatin. Frequent cells have amorphous eosinophilic intranuclear inclusions that measure 10-15 μm in diameter peripherally marginating chromatin (papillomavirus inclusions). Many other nuclei have a glassy appearance with intranuclear cytoplasmic invagination. Mitoses are 1-2 per hpf. Rare epithelial cells, especially in the stratum spinosum, have clear to pale cytoplasm with eccentric nuclei (koilocytes, viral cytopathic effect). Inverted epithelial papillary fronds are covered with a variably thick band of parakeratotic cells and some keratin material continous with acanthosis, parakeratotic and orthokeratotic hyperkeratosis of the adjacent epithelium. Inflammation infiltrating the stroma is comprised of macrophages, neutrophils, and fewer lymphocytes. In some sections, associated superficial epithelium has an overlying thick serocellular crust composed of degenerate keratinocytes, keratin material, red blood cells, degenerate neutrophils and eosinophilic cellular and pyknotic material. Neoplastic cells approach lateral surgical margins in many sections.

Contributor's Morphologic Diagnosis: Haired skin: inverted papilloma, viral

Contributor's Comment: The papillary nature of this tumor with prominent intranuclear papillomavirus inclusions suggests that this inverted papilloma is caused by papillomavirus (PV) infection. Immunohistochemistry using monoclonal antibody against human papillomaviruses (HPV-1, 6, 11, 16, 18, and 31) using SDS-disrupted bovine papillomavirus type 1 immunogen (Millipore Billerica, MA) was positive with multifocal intense intranuclear staining.[1]

At least four different PVs are believed to infect dogs.[2] Classification of PVs is often based on the L1 gene, which encodes the viral capsid and packages viral DNA with L2, because it is the most conserved region of the PV genome.[11] Oral papillomatosis in dogs, characterized by multifocal cauliflower growths affecting the tongue, gingival, buccal mucosa, lips, and pharynx, is believed to be caused by the lambda papillomavirus COPV.[2] Dogs that clinically manifest oral papillomas are generally less than 3 years old, but papillomas can appear in immunosuppressed and older dogs. Papillomatosis in dogs is considered to be a self-limiting disease with spontaneous regression of tumors, so treatment is generally not recommended.[2] Occasionally, these tumors persist and undergo malignant transformation. PVs are associated with cutaneous neoplastic transformation in several species, including sarcoids in cats and horses, and squamous cell carcinoma in dogs, cats, rabbits, bandicoots, and rodents.[7]
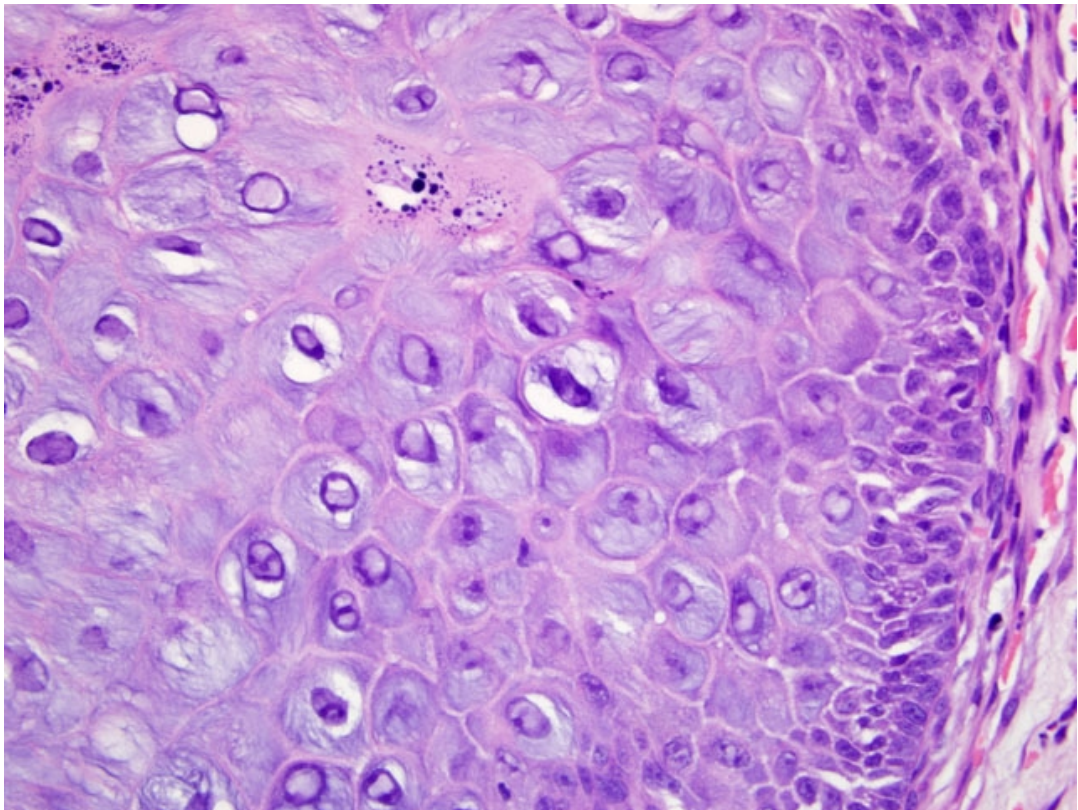
It has been proposed that cutaneous papillomatosis is caused by a different PV. A novel, epidermotropic PV has been recently described, termed CfPV-2.[10] Unlike COPV, lesions associated with CfPV-2 appear to be restricted to the footpads, with more chronic lesions lasting greater than 6 months. Experimentally, CfPV-2 is unable to induce oral papillomas in immunocompetent dogs, and vaccination against COPV is not effective against CfPV-2.[10] In addition, chronic infection with CfPV-2 is associated with highly malignant squamous cell carcinoma with distal metastases, although the exact pathogenesis of CfPV-2-associated neoplastic transformation is not known. The link between different PVs and specific tumor manifestations is also unclear. Recently, genotyping of PVs associated with canine inverted papillomas discovered the presence of either CfPV-2, COPV, or unknown canine PVs, suggesting that more than one type of PV may cause inverted papillomas.[6]
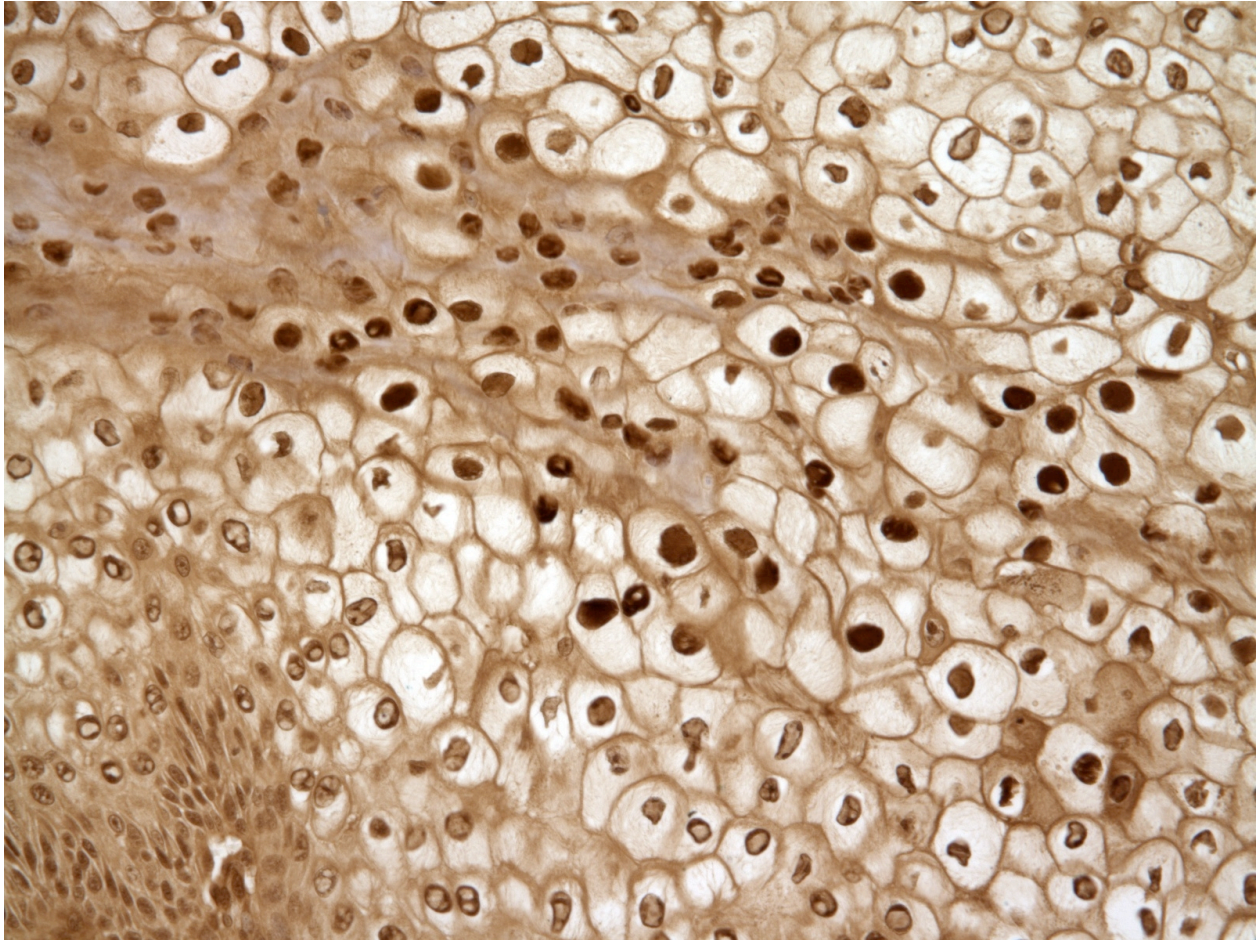
Canine papillomatosis can provide a model for studying regression of warts in human PV-associated cervical papillomatosis, because the predictable nature of lesion

3-3. Haired skin, Inverted viral papilloma, dog. Expanding the dermis is a well circumscribed, unencapsulated, endophytic (bowl-shaped), epithelial neoplasm that compresses and displaces adnexal structures. Neoplastic cells are arranged in broad infolds and papillary projections supported by a thin fibrovascular core. (HE 40X)
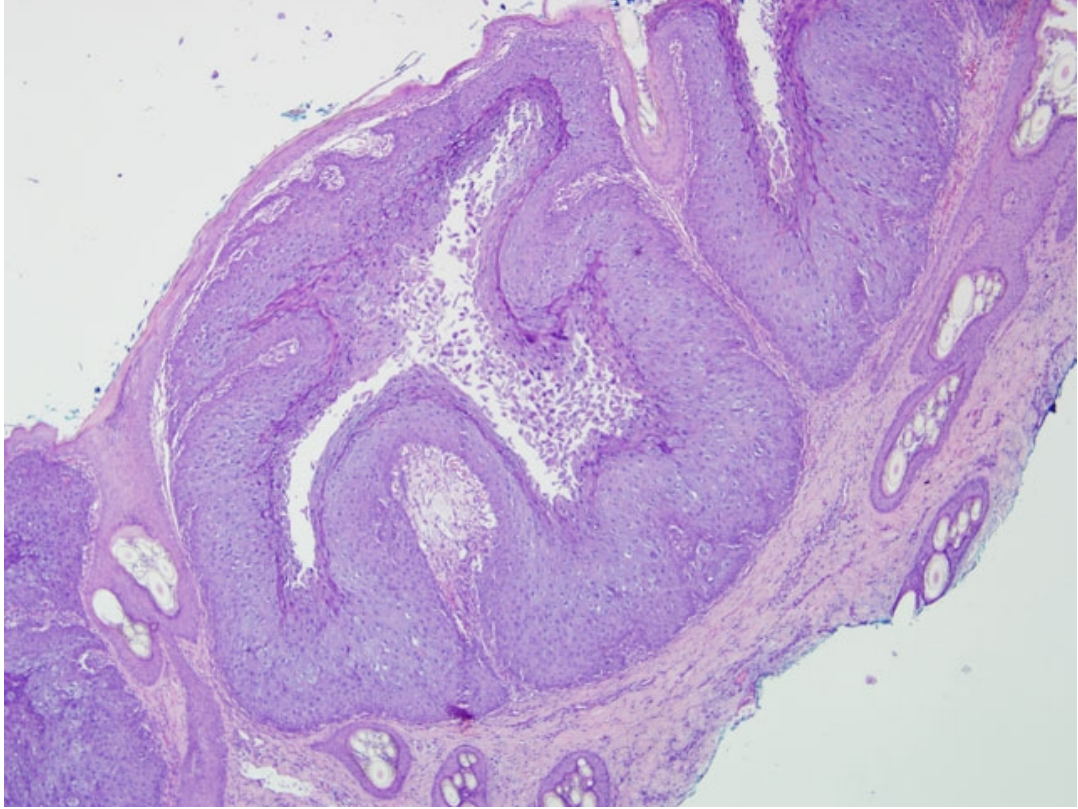
3-4. Haired skin, Inverted viral papilloma, dog. Neoplastic basal cells differentiate into larger epithelial cells that have distinct borders, abundant amphophilic cytoplasm, and round to oval nuclei with finely stippled chromatin. Occasionally, there are eosinophilic intranuclear inclusion bodies that marginate the chromatin. Few epithelial cells in the stratum spinosum have abundant clear to pale cytoplasm with an eccentric nucleus (koilocytes, viral cytopathic effect). (HE 400X)

3-5. Haired skin, Inverted papilloma, dog. Neoplastic epithelial cells are immunopositive for papillomavirus. Photographs courtesy of Johns Hopkins School of Medicine, Department of Molecular and Comparative Pathobiology, 733 N. Broadway St., Baltimore, Maryland 21205, nnath2@jhmi.edu

regression in canine papillomas closely mimics regression in cervical warts. In canines, papilloma regression is associated with leukocyte influx, with an abundance of CD4+ and CD8+ lymphocytes.[8]

Differentials for this case include nail bed inverted squamous papilloma. These masses arise from nail bed epithelium, and histologically contain laminated, compact keratin within a hollow mass.[9] The nails are also grossly broken or missing; however, viral inclusions, lack of involvement of the nails, and lack of cyst formation rule out nail bed inverted papilloma. In this case, the papilloma likely originated from the skin adjacent to the nail bed, consistent with previous reports.[2] In the absence of viral inclusions, other differentials include cutaneous squamous papilloma of non-viral origin.

AFIP Diagnosis: Haired skin: Inverted papilloma, viral.

Conference Comment: Two prominent histologic characteristics of viral papillomas are koilocytes and intranuclear inclusion bodies. Koilocytes, seen primarily in the spinous layer, are enlarged epithelial cells that have eccentric pyknotic nuclei surrounded by a clear "halo"; their ghosts may be seen in the more superficial stratum corneum.[4] In the stratum spinosum, the normal eosinophilic cytoplasm is replaced by more basophilic cytoplasm; the nuclei of the cells may contain pale basophilic (amphophilic) or smaller eosinophilic viral inclusions.[4] Some degenerating keratinocytes may also contain intracytoplasmic eosinophilic material resembling inclusions; these are not true viral inclusions, but rather merely aggregates of keratin thought to be a byproduct of the viral cytopathic effect.[4,7]

The precise role that papillomaviruses play in the development of cutaneous neoplasia in animals is not entirely understood. It has been proposed that ultraviolet light and papillomaviruses may act as co-carcinogens. Ultraviolet light causes damage to nuclear DNA, which increases the likelihood of oncogenic transformation.[3] Simultaneously, papillomavirus infection promotes epithelial proliferation.[4]

Species	Virus	Tissue(s) affected	Disease(s)
Ox	Bovine papilloma virus (BPV) 1, 2 & 5	Udder, teats, head, neck, shoulders, omasum, vagina, vulva, penis and anus; BHV-2 also affects the urinary bladder	"Teat frond" warts; cutaneous warts; "rice grain" fibropapillomas; transmissible fibropapilloma in bulls
Ox	BPV-3	Skin	Papillomas of the skin
Ox	BPV-4	Alimentary tract e.g. mouth, pharynx and upper alimentary tract	"Bovine alimentary papillomata;" associated with squamous cell carcinoma and squamous papilloma of the alimentary tract and urinary bladder
Equine	BPV-1 & -2	Skin	Sarcoid
Sheep	OvPV-1	Skin	Fibropapillomas
Feline	FdPV-1 & -2	Skin	Viral plaque progressing to Bowenoid carcinoma in-situ; FdPV-2 linked to squamous cell carcinoma
Feline	FdPV-2	Skin	Squamous cell carcinoma (SCC)
Canine	CfPV-3 & -4	Skin; mucous membranes of the eye, skin and genitals	Papillomas; viral plaque progressing to Bowenoid carcinoma in-situ and SCC
Canine	CfPV-2	Skin	Inverted papilloma; SCC
Rabbit	CRPV	Tongue; skin	Lingual papillomas; SCC
Western barred Bandicoot	BPCV1	Skin	SCC

Many important papilloma viruses of animals exist and are beyond the scope of this discussion; those interested are invited to consult the references.[3,7] The chart above summarizes several papilloma viruses in animal species.

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CASE IV: H38407 (AFIP 3166566).

Signalment: Age unknown, sex unknown, red fox (*Vulpes vulpes*).

History: A free-ranging red fox (*Vulpes vulpes*) (age and sex not recorded) was submitted to a veterinary practice in Cheshire, England in May 2000. The animal was depressed and exhibited mild jaundice at the time of admission. It died one day later.

Gross Pathology: At necropsy, the fox was mildly jaundiced and had a congested liver, with mild accentuation of the hepatic lobular pattern. The mesenteric and hepatic lymph nodes were mildly enlarged and congested.

Histopathologic Description: Liver: Histopathologic examination of the liver reveals numerous amphophilic intranuclear inclusion bodies in hepatocytes. Hepatocytes are swollen, mildly vacuolated and dissociated. There is individual degeneration and necrosis of hepatocytes. Hepatic sinusoids are congested and contain fibrin deposits. There is expansion of the space of Dissé.

Contributor's Morphologic Diagnosis: Hepatocellular degeneration, acute, diffuse, moderate, with numerous intranuclear inclusion bodies, infectious canine hepatitis (canine adenovirus type 1).

Contributor's Comment: The presence of intranuclear inclusion bodies and degeneration of hepatocytes in this case were consistent with infectious canine hepatitis (ICH), which is caused by canine adenovirus type 1 (CAV-1). Intranuclear inclusion bodies were also observed in renal glomeruli, renal tubular epithelial cells and endothelial cells lining blood vessels. CAV-1 was isolated from a liver sample of the affected red fox.[7]

Spontaneous ICH has been reported mostly in domestic dogs, farmed foxes and other captive carnivores.[7] The disease was first identified in North America in captive silver

ranging red foxes in Europe.[7]

Clinical signs and pathologic findings in red foxes with ICH are similar to those described in other species of foxes and in dogs.[2,7] Clinical signs in foxes appear after an incubation period of 2 to 6 days and may include anorexia, rhinitis, hemorrhagic diarrhea, hyperexcitability, seizures, paralysis, coma and death. Death may occur after a brief clinical course or may occur suddenly without prior clinical signs. Uveitis and keratitis ("blue eye") may develop in non-fatal cases of ICH in silver foxes. Gross lesions in foxes with ICH are considered to be less distinctive than in dogs, with generalized congestion and mild enlargement of the liver, spleen and adrenal glands. On histopathologic examination, vasculitis is considered to be a prominent feature of ICH in foxes, but was not a major finding in three red foxes with ICH examined in the United Kingdom.[7] Necrosis of hepatocytes and renal tubular epithelial cells are evident in foxes with ICH, but hepatic necrosis may be less severe than in dogs.

There is serologic evidence of exposure to CAV-1 in free-ranging red and gray foxes in North America, Germany, Australia and the United Kingdom.[1,3,4,7,8] Antibodies against CAV-1 have been detected in serum from 2/57 (3%) free-ranging North American red foxes in Wisconsin, USA, 17/485 (3.5%) free-ranging red foxes in Germany and 308/1326 (23.2%) free-ranging naturalized red foxes in Australia.[1,4,8] Antibodies against canine adenovirus type 1 were detected in postmortem fluid extracts from 11/58 (19%) frozen red fox carcasses from the United Kingdom.[7]
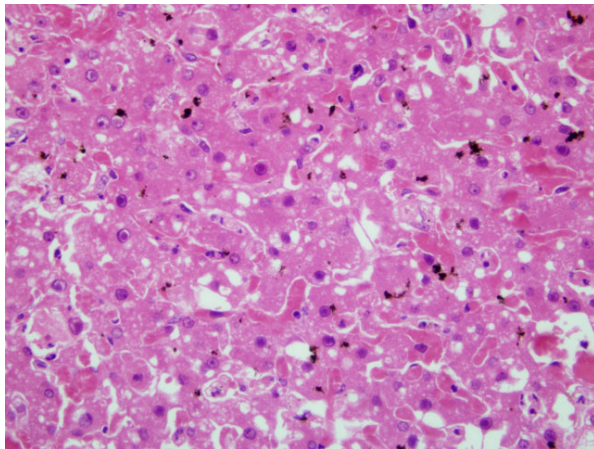
4-1. Liver, fox. Diffusely hepatocytes are swollen and vacuolated (degeneration) or disassociated and necrotic. (HE 400X)
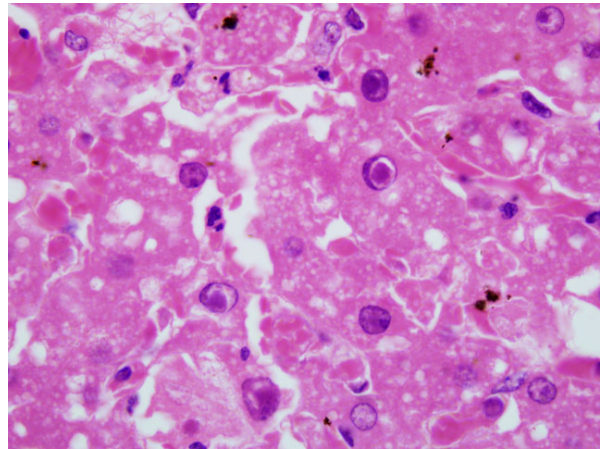
4-2. Liver, fox. Many degenerate hepatocytes contain eosinophilic intranuclear inclusion bodies that marginate the chromatin. (HE 1000X)

foxes, a colour variant of the red fox (*Vulpes vulpes*), and has also has been reported in farmed Arctic (blue) foxes (*Alopex lagopus*). Red foxes and gray foxes (*Urocyon cinereoargenteus*) are susceptible to experimental infection with CAV-1. The first case of ICH in a free-ranging gray fox was identified in 2004 in Georgia, USA.[2] The present case represents one of the first recorded cases of ICH in free-

Antibodies against CAV-1 have also been detected in 24/27 (88%) free-ranging gray foxes in the USA.[3]

The roles of red and gray foxes in the epidemiology of ICH are uncertain. It is not known if foxes are an important

reservoir of infection with CAV-1 and thus a source of infection for domestic dogs, or vice versa.

AFIP Diagnosis: Liver: Hepatocellular degeneration and necrosis, diffuse, moderate, with numerous hepatocellular viral intranuclear inclusions and sinusoidal fibrin thrombi.

Conference Comment: The structural unit of the liver is classically referred to as the hepatic lobule; however, when viewed with respect to its functionality and proximity to the blood supply, it is commonly referred to in the literature as a hepatic acinus. Both the hepatic lobule and acinus are further subdivided anatomically and physiologically into areas or zones. Hepatocytes in zone 1 of the acinus are closest to the incoming supply of oxygenated blood; in terms of lobular structure, this is the periportal area. Zone 2 corresponds to mid-zonal hepatocytes. Zone 3 (periacinar) hepatocytes are furthest from the oxygenated blood, and surround the portal vein; from an anatomic standpoint, this area is referred to as centrilobular. A single layer of hepatocytes at the periphery of the lobule forms a histologically distinct zone referred to as the limiting plate.[5]

When examining the liver, the pattern and extent of necrosis, can provide insight into potential etiologies. Necrosis is often classified based on the part(s) of the lobule affected. Centrilobular necrosis is common with viral infection, many toxins, and as cells of this region are the last in the body to receive oxygenated blood, processes resulting in hypoxemia (anemia or circulatory failure) often result in centrilobular necrosis. Pure mid-zonal lesions are extremely rare. Periportal necrosis is indicative of direct-acting toxins that do not need to be metabolized to a toxic intermediate via the cytochrome P450 system.[6]

The World Small Animal Veterinary Association Liver Standardization group published an accepted, standardized nomenclature and corresponding diagnostic criteria of hepatic disease.[5] Within sites of hepatic inflammation, there can be individual apoptotic or necrotic cells referred to as **apoptotic** or **acidophil** bodies. **Confluent** necrosis involves large areas of the liver, may have a random or zonal distribution and, when bridging vasculature structures, is more aptly termed **bridging** necrosis. When cells in all regions of the acinus/lobule are necrotic, the term **massive** necrosis is often employed, such as that observed in hepatosis dietetica, cocklebur intoxication and *Amanita* spp. intoxication. The pattern of **piecemeal necrosis** is characterized by hepatocyte death at the interface of parenchyma and connective tissue.[5,6]

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