



WEDNESDAY SLIDE CONFERENCE 2012-2013

Conference 25

15 May 2013

CASE I: A12-3507 (JPC 4017798).

Signalment: 11-year-old Saddlebred gelding, horse, *Equus caballus*.

History: This horse was referred to Purdue University Veterinary Teaching Hospital after being rescued from a barn fire. The horse had been treated

with intranasal oxygen by firefighters, with dexamethasone and furosemide by the attending veterinarian. Of four other horses in the barn, two died and two remained stable.

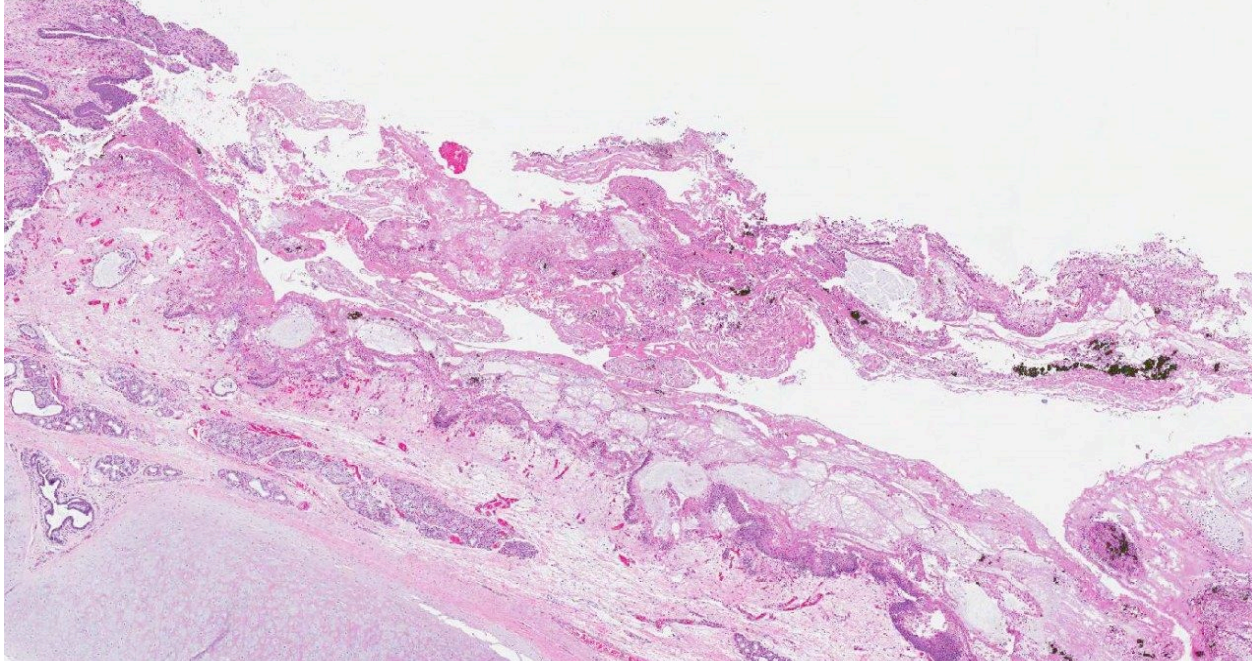
On admission, the horse had marked respiratory distress (manifested by nostril flaring, increased abdominal efforts, and cyanotic mucous



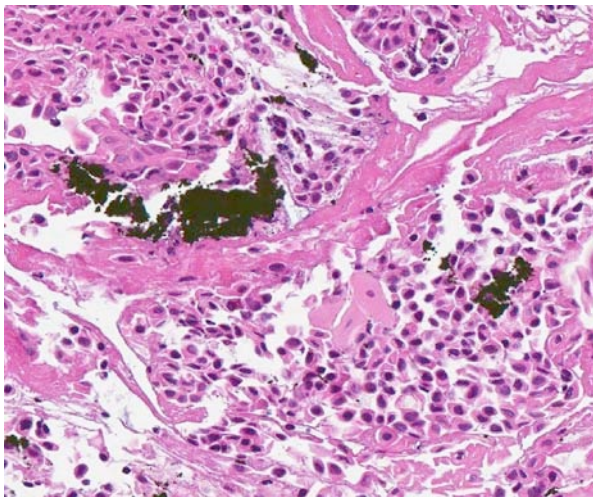
1-1. Larynx, horse: The larynx was diffusely thickened by edema with streaks and there are specks of black granular material on the mucosal surface. The lumen contains abundant froth. (Photo courtesy of: Purdue University Department of Comparative Pathobiology, 725 Harrison St., West Lafayette, IN 47907 (<http://www.vet.purdue.edu/cpb/>))



1-2. Lung, horse: The lungs were heavy, wet, and non-collapsing. On cut section, abundant clear colorless to red frothy fluid flowed from the airways and pulmonary parenchyma. Aggregates of black fibrillar to granular specks were in the pulmonary parenchyma and tracheobronchial lymph nodes. (Photo courtesy of: Purdue University Department of Comparative Pathobiology, 725 Harrison St., West Lafayette, IN 47907 (<http://www.vet.purdue.edu/cpb/>))



1-3. Larynx, horse: There is partial- to full-thickness necrosis of the laryngeal epithelium (normal at extreme left). The submucosa is markedly edematous. (HE 15X)



1-4. Larynx, horse: The sloughed and necrotic epithelium contains multifocal aggregates of dark granular carbon particles (soot). (HE 232X)

membranes) and evidence of pulmonary edema (frothy nasal discharge and bilateral crackles on thoracic auscultation). It also had tachycardia with hypovolemia and marked hypoxemia. Despite supportive treatment, the horse's condition did not improve, so it was humanely euthanized.

Gross Pathology: The nasal and pharyngeal mucosa was diffusely thickened, dark red to green, wet, and glistening with streaks or mats of black granular material. The larynx was diffusely

thickened by edema with streaks or specks of black granular material on the mucosal surface. The tracheal lumen was filled with clear frothy fluid; tracheal submucosa was diffusely expanded with transparent yellow edema fluid. The lungs were heavy, wet, and non-collapsing. On cut section, abundant clear colorless to red frothy fluid flowed from the airways and pulmonary parenchyma. Aggregates of black fibrillar to granular specks were in the pulmonary parenchyma and tracheobronchial lymph nodes. The pericardium contained approximately 50 ml of serosanguineous fluid.

Histopathologic Description: Pharyngeal and laryngeal mucosa is eroded or ulcerated with fibrinosuppurative exudate, free erythrocytes and carbon particle accumulation. The propria-submucosa is expanded by poorly stained edema fluid and variably sized aggregates of neutrophils. Blood vessels are distended with blood. Lymphatic vessels and some glands and ducts contain numerous neutrophils and macrophages. The remaining epithelial lining has a few necrotic ciliated epithelial cells with pyknosis and hyper eosinophilic cytoplasm. In the lung (not included in the submitted slide), bronchi and bronchioles were partially or totally obstructed by thick, amphophilic mucus mixed with black carbon particles, numerous leukocytes, including neutrophils, mast cells, and eosinophils, and

sloughed epithelial cells. The associated bronchial/bronchiolar epithelium was multifocally attenuated or sloughed. The interlobular septa were diffusely expanded by poorly stained edema fluid.

Contributor's Morphologic Diagnosis: Necrotizing pharyngitis and laryngitis with intralesional carbon particles.

Contributor's Comment: Inhalation of smoke and associated gases in a closed space can cause severe acute respiratory injury, which is a major cause of death in fires.^{2,4,5,6} The severe acute respiratory injury that occurs in barn fires can be explained through three pathophysiological mechanisms; direct thermal injury, carbon monoxide intoxication, and chemical irritation.^{4,6} Thermal injury causes local edema, necrosis, inflammation and upper airway obstruction by direct insult to the microvasculature and coagulation of tissue.⁶ Carbon monoxide, which is produced by incomplete combustion, leads to delayed or interrupted oxygen delivery due to low blood oxygen content, which can lead to pulmonary vasoconstriction and hypoxia.^{4,6} The severity of the chemical insult depends on the burned products, which may include hydrogen cyanide, hydrochloric acid, sulfuric acid, or aldehydes. These compounds initially induce mucosal hyperemia, but as long as the carbon particles containing the chemical remain on the mucosal surface, they can cause severe inflammation and mechanical pressure resulting in peribronchial edema or mucosal sloughing and necrosis. The damage to respiratory mucosal epithelium also results in decreased mucociliary transport and clearance of bacteria or foreign material in the airways.^{4,6}

JPC Diagnosis: Pharyngeal and laryngeal mucosa: Necrosis, multifocal to coalescing, with marked edema and numerous aggregates of carbon particles.

Conference Comment: Conference participants found tissue identification somewhat difficult, as much of the respiratory epithelium has been effaced by necrosis. Discussion centered on classification and morphology of direct thermal injury. Direct thermal burns are classified into the following categories based on the depth of their involvement: superficial burns are restricted to the epidermis; partial thickness burns involve the dermis; and in full thickness burns there is damage to subcutaneous tissue or underlying muscle tissue. Grossly, partial-thickness burns are pink, blistered, and painful;

whereas full thickness burns are white, charred, and non-painful due to the destruction of nerve endings in the tissue. As seen in this case, thermal burns are characterized histologically by coagulative necrosis that is often sharply demarcated from vital tissue.³

Conference participants also discussed the phases of injury associated with smoke inhalation, noting that during the early phase (first 24 hours) of smoke inhalation, there is acute pulmonary insufficiency due to bronchoconstriction and upper airway damage. This is followed within the next 24 to 72 hours by the middle phase, characterized by the development of pulmonary edema and laryngeal reflux. The pulmonary edema obstructs nasal passages and can lead to pneumonia. During the late phase, bronchopneumonia develops and worsens due to several factors, including the loss of protective barriers such as mucociliary clearance and surfactant as well as the blockage of effective inflammatory response owing to compromised blood flow in the area.³

Contributing Institution: Purdue University
Animal Disease Diagnostic Laboratory: <http://www.addl.purdue.edu/>
Department of Comparative Pathobiology: <http://www.vet.purdue.edu/cpb/>
Purdue University Department of Comparative Pathobiology
725 Harrison St.
West Lafayette, IN 47907

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CASE II: TP 11 089 (JPC 4020070).

Signalment: 1.5-year-old male non-castrated colony beagle, dog (*Canis familiaris*).

History: The dog was received from the provider when it was eight months old. Until the onset of clinical signs, it was never part of a toxicology study. One month before euthanasia, this dog presented with bloody stool, which was refractory to metronidazole and fenbendazole. Colonoscopy revealed multiple erosive lesions in distal colon.

Gross Pathologic Findings: Body condition was thin. There were small amounts of adipose tissue in the subcutis and abdomen. Mesenteric and submandibular lymph nodes were slightly enlarged and red. The cecum had numerous, sometimes coalescing, 0.3 to 0.6 cm diameter erosions-ulcers in the mucosa. Similar findings were observed in the distal colon, 5 to 12 cm cranial to the anus, where the mucosa was thickened, reddened and numerous 0.3 to 0.8 cm erosions-ulcers were present.

Histopathologic Description: Multifocally expanding the mucosa of the colon, separating mucosal crypts and extending into the submucosa, there is a dense inflammatory infiltrate composed of large numbers of macrophages and fewer neutrophils, and occasional lymphocytes and plasma cells. These macrophages have abundant eosinophilic cytoplasm and occasionally contain pale eosinophilic, variably sized vacuoles, bacillary

bacteria or cellular debris in the cytoplasm. Along the mucosal surface, there are multiple erosions of the epithelium, with the presence of numerous bacillary bacteria, and associated with necrotic cellular debris in the mucosal and apical lamina propria. Colonic crypts are often dilated with desquamated and necrotic cellular debris, and lined by flattened epithelium. Additionally, there are focal hemorrhages at the apical portion of the mucosa. Numerous foamy macrophages are separating the basal crypts from the muscularis mucosa and are present in the submucosa, surrounding mucosa associated lymphoid tissue. Frequently in the crypts, there are numerous spirochetes.

In the cecum (not submitted), findings are similar as described above.

Macrophages infiltrating the colonic mucosa and submucosa are PAS-positive (data not shown). Immunohistochemical stains using polyclonal anti-*Escherichia coli* antibody highlighted numerous bacillary bacteria present free in the upper lamina propria and in the cytoplasm of macrophages.

Contributor's Morphologic Diagnosis: Colon and cecum: Marked, multifocal to coalescing, chronic histiocytic typhlocolitis, with intralesional *Escherichia coli*.

Contributor's Comment: Canine histiocytic ulcerative colitis is an idiopathic, probably multifactorial, inflammatory condition of the cecum,



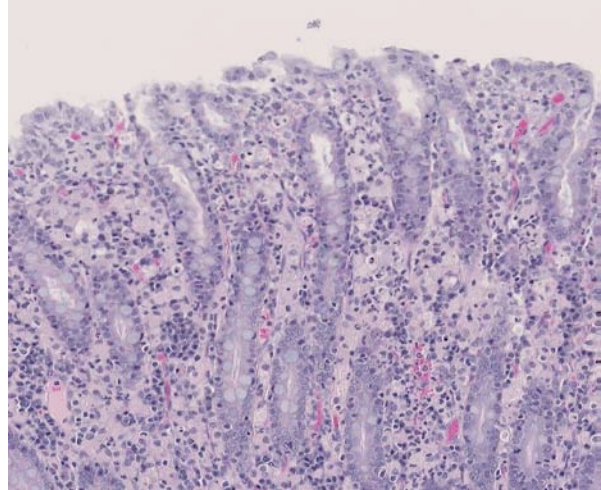
2-1. Cecum, dog: The cecum had numerous, sometimes coalescing, 0.3 to 0.6 cm diameter erosions/ulcers in the mucosa. (Photo courtesy of: Pfizer Inc., Global Research and Development, Groton/New London Laboratories, Eastern Point Road MS 8274-1330, Groton, CT (www.pfizer.com))



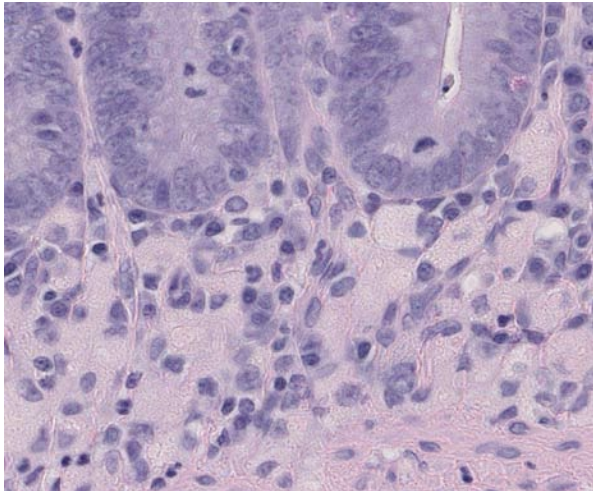
2-2. Colon, dog: Similar findings were observed in the distal colon, 5 to 12 cm cranial to the anus, where the mucosa was thickened, reddened and numerous 0.3 to 0.8 cm erosions-ulcers were present. (Photo courtesy of: Pfizer Inc., Global Research and Development, Groton/New London Laboratories, Eastern Point Road MS 8274-1330, Groton, CT (www.pfizer.com))



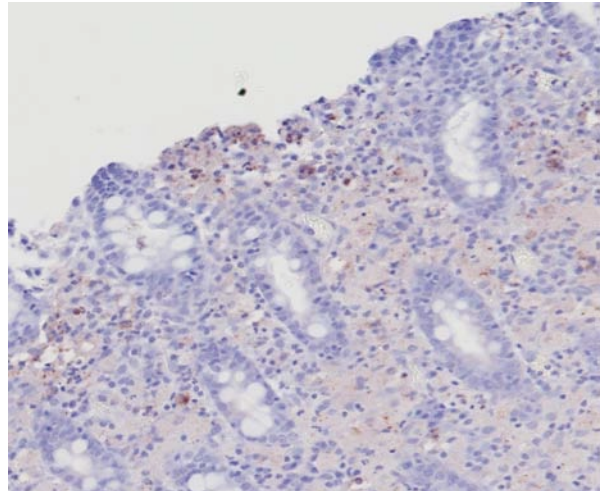
2-3. Colon, dog: The colonic mucosa is moderately thickened, and a thick band of histiocytes expands the mucosa, separating glands, and the submucosa, surrounding and separating lymphoid tissue. (HE 140X)



2-4. Colon, dog: The multifocally eroded mucosa is expanded by moderate numbers of foamy histiocytes and fewer neutrophils which separate mucosal glands. (HE 240X)



2-5. The submucosa is expanded by a thick layer of histiocytes ranging up to 20 µm with abundant granular cytoplasm. The overlying crypts are hyperplastic as shown by numerous mitotic figures (HE 400X)



2-6. Colon, dog: Immunohistochemical stains using polyclonal anti-*Escherichia coli* antibody highlighted numerous bacillary bacteria present free in the upper lamina propria and in the cytoplasm of macrophages. (Photo courtesy of: Pfizer Inc., Global Research and Development, Groton/New London Laboratories, Eastern Point Road MS 8274-1330, Groton, CT., www.pfizer.com)

colon and rectum of dogs. This condition was first described by Van Kruiningen et al. (1965),⁹ after observing chronic hemorrhagic diarrhea in a Boxer colony, which was characterized as a granulomatous colitis with resemblance to Whipple's disease. Subsequent reports led to the use of the term of canine histiocytic ulcerative colitis (CHUC), which has since gained general acceptance.⁶ CHUC has been distinctively recognized in Boxers and the related French bulldogs. Nevertheless, this condition has also been described in a Doberman pinscher, a Mastiff and one Alaskan malamute.⁷

Here, we report the first case of CHUC in a colony Beagle dog.

Grossly, the colon of affected dogs is thickened, folded and perhaps dilated, with focal areas of scarring. On the mucosa, the lesion varies from red, frequently raised areas to patchy or coalescing ulcers. Histologically, it is characterized by a dense inflammatory infiltrate in the mucosa and submucosa, composed mainly by large numbers of foamy, PAS-positive macrophages which contain phagocytized necrotic cellular debris and bacteria. Additionally, there is distortion of the normal

glandular architecture and decreased numbers of goblet cells. Ulceration, which does not progress beyond the submucosa, arises from the superficial epithelial erosion and destruction of the basement membrane.¹ Resultant clinical signs consist of large bowel diarrhea, tenesmus, hematochezia and marked weight loss.⁴

The inflammatory infiltrate of the colonic mucosa and submucosa was further characterized in an immunohistochemical study. German et al (2000) found increased IgG plasma cells, plus PAS, CD3, L1 and MHC class II positive cells in the lamina propria, in conjunction with increased enterocyte MHC II class expression, and decreased goblet cell numbers in the epithelium. These findings are similar to those of human ulcerative colitis and might suggest a similar pathogenesis at a cellular level.³

The exact cause of this condition remains unknown, and a multi-factorial etiology is highly suspected. In an ultrastructural study, it was concluded that CHUC was probably caused by a lipid-rich, ribosome-rich, coccoid to coccobacillary organism that possesses a cell membrane and range from 100 to 500 nm in size. This agent was suspected to be a *Chlamydia*-like organism.¹⁰ In more recent reports, the infectious agent hypothesis was again brought to attention, since dogs completely recover after treatment with enrofloxacin.^{2,4} In one study, there was demonstration of *E. coli* in 100% of the examined colonic samples with a polyclonal antibody, strongly associating this organism to the disease. In the same study, there was a comparison of this entity with granulomatous leptomeningitis of Beagle dogs, and malakoplakia and xanthogranulomatous cholecystitis in man, in which *E. coli* has also been incriminated, together with abnormal lysosomal function.¹¹ In 2009, Mansfield et al. observed remission of CHUC in Boxer dogs in correlation with the eradication of invasive intramucosal *E. coli*, further supporting the causal involvement of *E. coli* in the development of the disease in susceptible Boxer dogs.⁵

JPC Diagnosis: Colon: Colitis, histiocytic, multifocal, moderate, with glandular hyperplasia.

Conference Comment: In addition to reviewing the contributor's excellent summary of canine histiocytic ulcerative colitis (CHUC), conference participants discussed the use of the term "histiocytic" versus "granulomatous," noting that

"histiocytic" is preferred in this condition, as the lesions lack the epithelioid macrophages and multinucleated giant cell macrophages that are characteristic of granulomatous inflammation. The comparison between human inflammatory bowel disease (IBD), which encompasses both Crohn's disease and ulcerative colitis, and CHUC is interesting. Human IBD is thought to be the result of a combination of defects affecting the host's interaction with intestinal microbes as well as intestinal epithelial dysfunction and abnormal immune responses.⁸ Additionally, in humans, Crohn's disease is thought to be associated with a TH-1 response; whereas ulcerative colitis has been suggested to be a TH-2 mediated disease.⁸ This may not be clear-cut, however, as polymorphisms of the IL-23 receptor have been shown to be protective against both Crohn's disease and ulcerative colitis, suggesting there may also be involvement of a TH-17 response in both conditions. Furthermore, anti-TNF therapy has been effective in some human patients with ulcerative colitis, indicating that a TH-1 response may play a role in ulcerative colitis as well.⁸ As the contributor notes, similar to IBD in humans, a multifactorial etiology is suspected in CHUC, although neither has yet to be fully elucidated. Participants noted some slide variation with some slides exhibiting crypt abscesses and/or crypt herniation absent in other slides.

Contributing Institution: Pfizer Inc.
Global Research and Development
Groton/New London Laboratories
Eastern Point Road MS 8274-1330
Groton, CT
Phone: 860-441-4498
www.pfizer.com

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CASE III: 1/10 (JPC 3165170).

Signalment: 8-year-old female spayed German shepherd dog, *Canis familiaris*.

History: The dog developed acute weakness and vomiting. On clinical examination, the dog presented with pale mucous membranes, superficial breathing and abdominal distension. Abdominal radiographs revealed a moderate abdominal effusion. Cytology of abdominal fluid contained elevated numbers of red blood cells and rare mesothelial reactive cells, suggestive of hemoperitoneum. On exploratory laparotomy a primary splenic ruptured mass was observed and splenectomy was performed. The mass was diagnosed as hemangiosarcoma by histology. To prevent metastatic spread, post-operative chemotherapy with a combination protocol of doxorubicin and cyclophosphamide administered every 3 weeks for a total of 6 treatments was administered after suture removal. The cumulative dose of doxorubicin was lower than 240 mg/m². After the sixth treatment the dog was referred for sudden onset of general weakness. Heart failure with atrial fibrillation rapidly followed and the animal died spontaneously.

Gross Pathologic Findings: At necropsy severe dilation of left and right ventricles was observed. The myocardial wall was generally paler than normal with thin, white, longitudinal streaks beneath the epicardium. Mild pulmonary edema and hyperemia of major internal organs were present. The entire heart was collected for histopathology.

Laboratory Results: Complete blood count, liver and renal panels and electrocardiography were performed before each treatment. All parameters were within normal limits for the entire duration of chemotherapy.

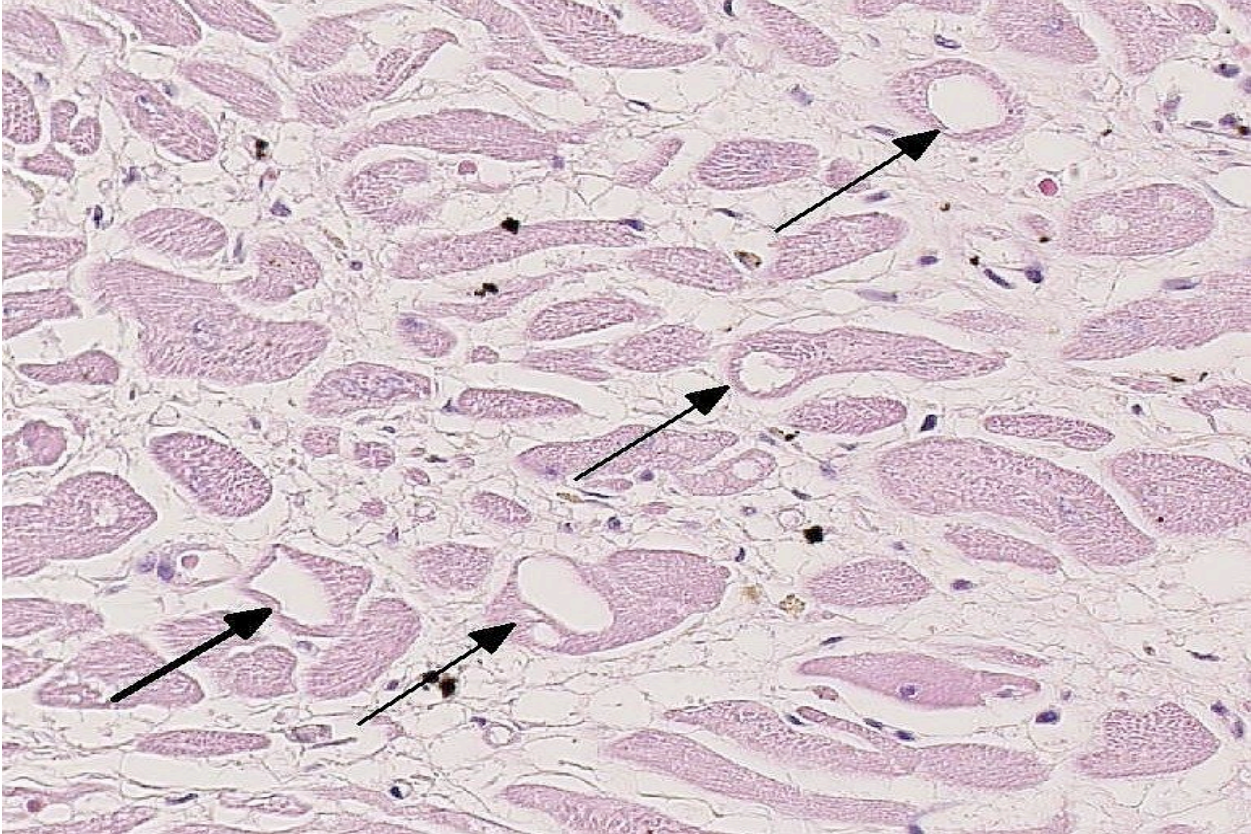
Histopathologic Description: Sections from the ventricular free walls, interventricular septum, and left papillary muscle are provided. All samples provided have similar changes with variable severity: randomly distributed, degenerated cardiomyocytes are present single or in groups admixed with morphologically normal myocytes. Single cells or small groups of cardiomyocytes are characterized by intracytoplasmic, one to multiple, smooth-contoured, empty vacuoles ranging from 4 to 36 µm in diameter (vacuolar degeneration-Adria

cells). In these cells, when visible, the nucleus is displaced to the periphery. Occasionally, scattered myocytes contain a perinuclear area of homogenous, pale eosinophilic cytoplasm, with loss of central cross striations (myofibril loss, myocytolysis). Scattered hypereosinophilic, angulated myocytes and fragmented myofibers are also evident. Cardiomyocytes are multifocally separated by mild interstitial edema and occasional microhemorrhages. Areas of myocardial fibrosis with minimal numbers of lymphocytes and plasma cells may be present in some sections.

Contributor's Morphologic Diagnosis: Moderate, multifocal chronic myocardial vacuolar degeneration and mild chronic interstitial edema and fibrosis.

Contributor's Comment: Myocardial vacuolar degeneration ("Adria" cells), myocytolysis, interstitial edema and fibrosis are considered characteristic findings of doxorubicin-induced cardiomyopathy.^{6,8} Doxorubicin (DOX) is known to be a dose-limiting cardiotoxic compound able to induce an irreversible dilated cardiomyopathy in several species including canine and human.^{1,3,5} Congestive heart failure and death have been documented during the treatment but also months after the completion of chemotherapy in many species. Despite its toxicity, DOX is largely used alone or in combination in order to treat several neoplastic entities including hemangiosarcoma in dogs.⁶ In humans, the incidence of DOX-induced cardiomyopathy is approximately 1.7% and the mortality rate once dilation is initiated reaches 50% of patients.⁸

Toxicity is related to the total cumulative dose administered, as well as to the acute peak concentration levels of the compound. Increased risk of DOX cardiotoxicity has also been correlated with young age, concurrent administration of additional chemotherapy compounds and viral diseases. Retrospective studies of human patients have revealed that more than 4% of patients who receive a cumulative dose of 500-550 mg per square meter of surface area develop congestive heart failure. The incidence rises to more than 18% over 551-600 mg/m².⁸ Based on several clinical studies limiting the cumulative dose to less than 450 mg/m² is the best line of defense against DOX cardiotoxicity. Alternative methods have been the concurrent administration of antioxidants, iron chelators, use of DOX analogues. However, none of



3-1. Heart, dog: Scattered throughout the mildly edematous myocardium, occasional myocytes contain one to multiple discrete clear vacuoles ("adria cells"). (HE 240X)

the approaches have had major success. Recently, combined administration of the hematopoietic cytokines EPO, G-CSF and TPO has prevented DOX cardiomyopathy in animal models.⁸ In this dog, a standard chemotherapy protocol for canine hemangiosarcoma with a cumulative dose of DOX lower than 240 mg/m² (recommended to minimize chronic cardiac toxicity) was used.³ Myocardial vacuolization is described in early and chronic toxicity with a wide range of and from 122 to 265 mg/m² body surface area (BSA) in clinical cases.³ The overall cumulative dose causing fatal cardiomyopathy is therefore controversial, and dogs are generally considered more sensitive to cardiotoxic effects of doxorubicin than humans, where cardiomyopathy and congestive heart failure are reported to occur at a total dose greater than 550mg/m² BSA.²

Prognosis very poor in human patients that develop cardiomyopathy within four weeks after administration of DOX and the majority die within two weeks after onset of symptoms.⁸ Among long survivors after DOX therapy, several develop heart failure six to ten years after conclusion of

chemotherapy. The late-onset cardiotoxic effects of conventional anthracycline therapy highlight the need of lifelong monitoring the cardiac status in human patients. Several techniques (i.e. radionuclide angiography assessment of left ventricular ejection fraction, electrocardiography and echocardiographically derived ejection fraction) have been proven to be poor indicators of early changes and subclinical myocardial injury.^{3,8} Serial endomyocardial biopsies are currently considered the most sensitive and specific indicators of doxorubicin-induced injury.⁸ Biopsies are examined by electron microscopy and graded applying a semiquantitative scoring system¹ based on the percentage of myocytes affected by myofibrillar loss and cytoplasmic vacuolization. Ultramicroscopic markers including myofibril loss, retention of sarcoplasmic reticulum and cytoplasmic vacuolation are utilized to grade injury on a scale of 1 to 3; biopsy samples in which fewer than 5% of cells have typical changes are given a grade 1 while those with changes over 36% are graded 3, the highest severity.⁸ This grading shows a linear correlation with left ventricular function determined by radionuclide angiography and is helpful for

clinical determination of continuation of DOX therapy.⁸

In veterinary medicine, no sensitive predictor tests are currently available to monitor patients treated with doxorubicin. Herman and co-workers (1981) observed that plasma enzymes CPK (creatine phosphokinase), LDH (Lactate Dehydrogenase) and SGOT (Serum Glutamic Oxaloacetic Transaminase) were not reliable indicators of slowly progressive cardiac damage. Similarly to human patients, sequential echocardiograms and EKGs (electrocardiogram) are not considered sensitive predictors for canine cardiomyopathy since no consistent correlation between the severity of rhythm disturbance and the pathologic myocardial changes have been observed.² Myocardial biopsies in dogs, although not routinely performed due to their invasiveness, have been experimentally proven to be a sensitive test to monitor the early doxorubicin-associated cardiotoxicity.⁷

Because DOX cardiotoxicity is dose dependent, it has been used to experimentally induce heart failure in different animal species such as dog, sheep, goats and rodents.⁵ DOX is delivered by intravenous and intracoronary injections at small doses to induce heart failure without systemic toxicity. Experimental DOX heart failure develops via bilateral enlargement, ventricular wall thinning with decreased cardiac output. This model has been utilized to study several treatments for cardiac failure however the model has several limitations: the degree of left ventricular dysfunction varies, is characterized by high incidence of arrhythmias, high cost of multiple intracoronary injections and the irreversible and progressive heart damage.⁵

The mechanism of action of DOX and other antacycline compounds on tumor cells are still a matter of controversy. Suggested mechanisms are: intercalation into the DNA molecule leading to inhibition of transcription, generation of reactive oxygen species leading to lipid peroxidation and DNA damage, DNA binding and alkylation, DNA cross-linking, interference with DNA unwinding and helicase activity, inhibition of topoisomerase II and induction of apoptosis.

Anthracycline compounds including DOX, produce reactive oxygen species (ROS) interacting with mitochondrial enzymes. ROS are produced in vitro by high concentration of DOX that binds to iron forming DOX-iron complexes that bind to DNA and

induce production of partially reduced oxygen compounds. These radicals can damage DNA via strand break formation. However, high concentrations of DOX seem necessary and antioxidant compounds do not diminish DOX cytotoxicity.⁸

The mechanism of DOX-cardiomyopathy remains unclear but seems different from the one underlying DOX's anti-tumour activity. Most studies support the view that increase in oxidative stress evidenced by increases in ROS and lipid peroxidation play a key role along with reduction in antioxidant levels and sulfhydryl groups.^{3,6} Other associated mechanisms proposed have been: inhibition of protein and DNA synthesis, lysosomal and mitochondrial changes, alteration of sarcolemmal Ca²⁺ transport, attenuation of adenylate cyclase, ATPase activities, imbalance in myocardial electrolytes and several others.⁸ Also, DOX downregulates the expression of cardiac-specific genes including contractile proteins (alpha-actinin, myosin light and heavy chains, troponin I, desmin), and sarcoplasmic reticulum proteins. The reduction of myocardial contractility can be directly associated with reduction in muscle proteins. DOX additionally induces apoptosis of endothelial cells and cardiomyocytes contrary to its cystostatic effect in tumor cells. This latest mechanism seems related to p53 activation. However, the role of apoptotic pathways in DOX cardiotoxicity is still controversial.⁸

Transcriptional profiling via genome wide transcriptome analysis has been utilized to determine early cardiac response to DOX in a rat model perfused with DOX leading only to mild cardiac dysfunction.⁹ The main characteristics of cardiomyocyte reprogramming were the repression of transcripts involved in cardiac stress response and stress signaling, modulation of genes with cardiac remodeling capacity and upregulation of energy related pathways. This latest research supports the hypothesis that blunted response to stress and reduced "danger signalling" are prime components of DOX toxicity and can drive to cardiomyocyte damage.⁹

JPC Diagnosis: Heart, cardiomyocytes: Degeneration, multifocal, mild, with intracytoplasmic vacuolation and rare myocardial fibrosis.

Conference Comment: The contributor provides an excellent review of doxorubicin cardiotoxicity. Conference participants discussed the severity of the lesions in this section, favoring the term “mild” to describe both the vacuolar degeneration and fibrosis. This spurred a discussion of the general correlation of clinical cardiac disease and severity of myocardial injury as observed histologically. This correlation is often poor, as a small lesion at a critical site within the heart can lead to death, while even more extensive lesions, if not affecting a critical area, are sometimes asymptomatic. Furthermore, it is not uncommon for animals that die peracutely from cardiac failure to show no detectable microscopic abnormalities.⁵

Contributing Institution: Dipartimento di Patologia Animale, Igiene e Sanita' Pubblica Veterinaria
Sezione di Anatomia Patologica e Patologia Aviare
Facolta' di Medicina Veterinaria
Milano - Italy
<http://www.anapatvet.unimi.it/>

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CASE IV: 09 848 41 (JPC 3164206).

Signalment: Young male dog, *Canis familiaris*.

History: The dog was inoculated experimentally with *Leptospira interrogans* serovar *icterohaemorrhagiae*. The animal was euthanized for human reasons.

Histopathologic Description: The liver is affected by a diffuse and severe lesion which is characterized by a dissociation of hepatocytes and hepatic plates (degeneration with loss of intercellular junction systems). Many hepatocytes have a hypereosinophilic cytoplasm and a karyorrhectic nucleus (individual hepatocyte necrosis). However, a number of hepatocytes are binucleated or show mitotic figures (hepatic proliferation). The sinusoids wall is not visible (endothelial cells lysis) and Kupffer cells show erythrophagocytosis (recent hemorrhages).

In portal tracts, bile ducts are distended by an amphophilic mucinous substance (moderate bile duct mucostasis). The wall of centrilobular veins is infiltrated by a few lymphocytes and neutrophils (discrete lymphocytic and neutrophilic transparietal vasculitis).

Contributor's Morphologic Diagnosis: Liver: Hepatitis, degenerative, diffuse, acute, severe, with hepatocytes dissociation and individual necrosis, moderate bile duct mucostasis, and centrilobular discrete lymphocytic and neutrophilic vasculitis.

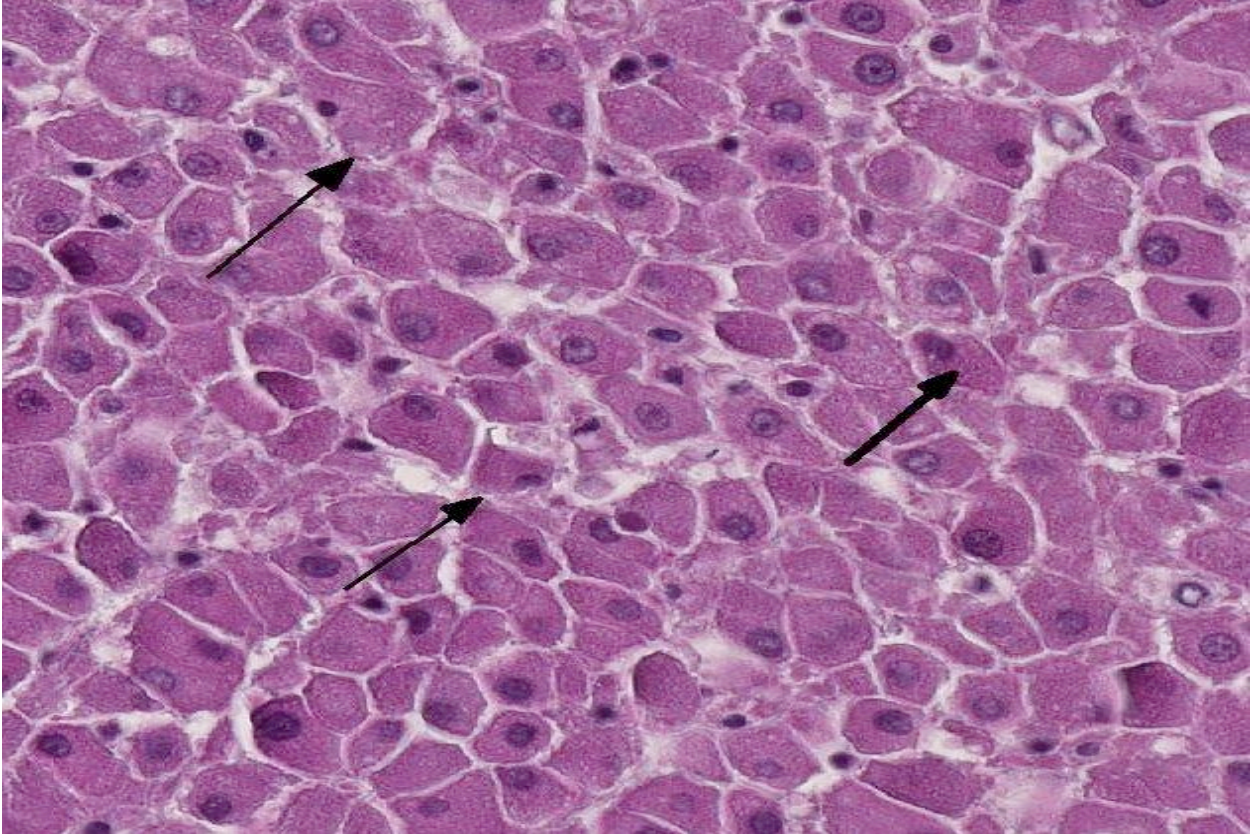
Etiology: *Leptospira interrogans* serovar *icterohaemorrhagiae*.

Special stain: A Warthin-Starry stain was negative.

Contributor's Comment: Leptospirosis is a zoonosis of worldwide distribution, caused by infection with antigenically distinct serovars of the motile spirochete bacterium *Leptospira interrogans sensu lato*.^{2,3,4} Leptospirosis has been identified as one of the emerging infectious diseases.⁴ *Leptospira* organisms are thin, flexible, filamentous bacteria made up of fine spirals with hook-shaped ends. More than 200 different serovars have been identified in the *Leptospira interrogans* complex.³ Canine leptospirosis was first described in 1899. Prior to 1960, serovars *icterohaemorrhagiae* and

canicola were believed to be responsible for most clinical cases of canine leptospirosis. After a bivalent serovar-specific vaccine against *canicola* and *icterohaemorrhagiae* came into widespread use, the incidence of "classic" leptospirosis in dogs appeared to have decreased. However, these vaccines did not induce immunity against other serovars, leading to a relative increase in the incidence of disease attributed to other serovars.³ Leptospirosis can be transmitted directly between hosts in close contact through urine, venereal routes, placental transfer, bites, or ingestion of infected tissues. Recovered dogs can excrete organisms in urine intermittently for months to four years after infection. Indirect transmission happens more often, occurring through exposure of susceptible animals to a contaminated environment (e.g., soil, food or bedding). Water contact is the most common means of spread, and habitats with stagnant or slow moving warm water favor organism survival. Leptospire in contaminated water invade the host through skin wounds or through intact mucous membranes. Once in a susceptible host, leptospire begin to multiply as early as one day after entering the blood vascular space. They invade many organs, including the kidneys, liver, spleen, central nervous system, eyes and genital tract.³

Clinical signs associated with leptospirosis vary and depend on the serovar and the host. In maintenance hosts, leptospirosis generally is characterized by a low serological response, relatively mild acute clinical signs, and a prolonged renal carrier state which may be associated with chronic renal disease. In incidental hosts, leptospirosis can cause severe disease, associated with high titers of agglutinating antibody, and has a short or negligible renal carrier state. The clinical signs observed vary with the susceptibility of the host and with the infecting serovar. In general, young animals are more seriously affected than adults.^{1,2} Clinical signs reported in dogs with leptospirosis include fever, inappetence, vomiting, abdominal pain, diarrhea, polyuria/polydipsia, myalgia, jaundice, epistaxis, hematuria and reproductive failure.^{1,2} Signs of hepatic and renal dysfunction and of coagulation defects usually predominate in dogs with leptospirosis. The severity of clinical signs depends on the age and immunocompetence of the host, the environmental factors affecting the organisms, the serovar involved and its virulence, and the infecting dose. Younger dogs (less than six months) are more severely affected and develop more signs of hepatic



4-1. Liver, dog: Diffusely throughout the section, there is loss of hepatic plate architecture. Hepatocytes are individualized, rounded up, and exhibit single cell necrosis (smudgy nuclei, pyknosis). (HE 256X)

dysfunction. A majority of leptospiral infections in dogs are subclinical. The peracute form is characterized by massive leptospiremia, causing shock and often death with few premonitory signs. Less severe infections are characterized by fever, anorexia, vomiting, dehydration, increased thirst and reluctance to move.³

Renal colonization occurs in most infected animals because the organism replicates and persists in renal tubular epithelial cells, even in the presence of neutralizing antibodies. Acute impairment of renal function may result from decreased glomerular filtration caused by swelling that impairs renal perfusion. Renal function in some dogs that survive acute infections may return to normal within several weeks, or chronic compensated polyuric renal failure may develop.³

The liver is another major organ damaged during leptospirosis. The degree of icterus in both canine and human leptospirosis usually corresponds to the severity of hepatic necrosis. In contrast, the icterus, hemoglobinemia and hemoglobinuria which develop in cattle with leptospirosis result from a specific

hemolytic toxin produced by serovar pomona.

Tissue edema and disseminated intravascular coagulation may occur rapidly and result in acute endothelial injury and hemorrhagic manifestations. *Leptospira* lipopolysaccharides stimulate neutrophil adherence and platelet activation, which may be involved in inflammatory and coagulatory abnormalities.³ Benign meningitis is produced when leptospires invade the CNS; however, it is not as common as in humans. Uveitis is occasionally present. Abortion and infertility resulting from transplacental transmission of leptospires associated with serovar *bataviae* infection have been described. Pulmonary manifestations include labored respiration and coughing. Interstitial pneumonia has been documented as the cause in humans, whereas lung changes in dogs with leptospirosis are associated with pulmonary hemorrhage most likely due to endothelial damage and vasculitis.³

Laboratory abnormalities usually include leukocytosis, thrombocytopenia, increased serum urea and creatinine, electrolyte disturbances,

bilirubinemia and increased serum hepatic enzyme activities. Coagulation parameters may be altered in severely affected animals. Urinalysis abnormalities include bilirubinuria, sometimes glucosuria, proteinuria and increased numbers of granular casts, leukocytes and erythrocytes in the sediment.³

Establishment of a diagnosis is important because animals can serve as reservoirs and pose potential zoonotic risks.¹

The diagnosis of leptospirosis can be accomplished by several techniques. Detection of antibodies using the microscopic agglutination test (MAT) is the most common diagnostic method; however, other methods to detect antibodies, such as immune-fluorescent assays or enzyme-linked immunosorbent assay (ELISA), have been used.^{2,3} Application of silver stains, or immunohistochemical stains to tissue sections are effective for detection of leptospire or leptospiral antigens in the renal tubules and interstitium of the kidney and liver. Low sensitivity is a disadvantage of this diagnostic technique. Leptospire are often present in small numbers in affected tissues, particularly in chronic leptospirosis or in dogs treated with antibiotics.

Serological studies should also be conducted.² Bacteriologic culture of blood, urine, or tissue specimens is the definitive method for the diagnosis of leptospirosis. Leptospiremia occurs early in the clinical course of leptospirosis and is usually of short duration and low level. Therefore, blood is only useful for culture in the first few days of clinical illness and before antibiotic therapy. Leptospire are usually present in the urine of animals with leptospirosis four to ten days after the onset of clinical signs. Culture of leptospire is difficult, time-consuming and requires specialized culture medium. However, isolation of the organism allows definitive identification of the infecting serovar.²

The spectrum of human disease caused by leptospire is extremely wide, ranging from subclinical infection to a severe syndrome of multi-organ infection with high mortality.⁴ Leptospirosis is presumed to be the most widespread zoonosis in the world. The source of infection in humans is usually either direct or indirect contact with the urine of an infected animal. The incidence is significantly higher in warm-climate countries than in temperate regions; this is due mainly to longer survival of leptospire in the environment in warm, humid conditions.⁴

The clinical presentation of leptospirosis is biphasic, with the acute or septicemic phase lasting about a week, followed by the immune phase, characterized by antibody production and excretion of leptospire in the urine. Most of the complications of leptospirosis are associated with localization of leptospire within the tissues during the immune phase and thus occur during the second week of the illness.⁴ A comparison of the prevalence of anti-leptospiral antibodies with the prevalence of clinical signs indicates that subclinical infections are relatively common.²

JPC Diagnosis: Liver: Hepatitis, necrotizing, multifocal, random, with suppurative cholangitis, severe hepatocellular dissociation and single cell necrosis.

Conference Comment: As the contributor states in this interesting review, leptospiruria may persist in an infected animal for months to years. It is believed that down-regulation or differential expression of *Leptospira* antigenic surface proteins and possibly the binding of complement regulatory proteins (i.e. plasma factor H) allow the leptospire to evade the host immune system. Although the virulence factors associated with leptospire have not been fully elucidated, it appears that their toxic components are associated with outer membrane proteins rather than the secretion of specific toxins. Attachment to the host cell is likely mediated by fibronectin-binding protein on the bacterial surface that binds to host extracellular matrix proteins. Infection of the host cell is via receptor-mediated endocytosis. Pathogenic species of *Leptospira* contain sphingomyelinases and other hemolysins, which may play roles in erythrocyte and endothelial cell membrane damage that results in hemolytic anemia, jaundice, hemoglobinuria and hemorrhage observed in acute leptospirosis. Once in the bloodstream, leptospire evade phagocytosis; the mechanism for this is thought to be induction of macrophage apoptosis. Leptospire are cytochemically gram negative bacteria; however, they differ from other gram negative organisms in several ways, including the following: The LPS of leptospire is not as endotoxic as other gram-negative bacteria; and leptospire, unlike other gram negative organisms, activate the host immune response through TLR-2 rather than TLR-4 pathway.

Conference participants discussed the serovars of

Leptospira interrogans that commonly affect domestic animals and humans. The included chart summarizes these serovars and their hosts (both maintenance and incidental), as well as associated

Serovar	Maintenance host	Incidental host	Clinical conditions
Bratislava	Pigs, hedgehogs, horses	Dogs	Reproductive failure, abortions, stillbirths
Canicola	Dogs	Pigs, cattle	Acute nephritis in pups. Chronic renal disease in adult animals
Grippotyphosa	Rodents	Cattle, pigs, horses, dogs	Septicemic disease in young animals; abortion
Hardjo	Cattle, sheep, deer	Humans	Influenza-like illness; occasionally liver or kidney disease
Icterohaemorrhagiae	Rats	Domestic animals, humans	Acute septicemic disease in calves, piglets and lambs; abortions
Pomona	Pigs, cattle	Sheep, horses, dogs	Acute haemolytic disease in calves and lambs; abortions

clinicopathological features. *J. Small Anim. Pract.* 1998;39:231- 236.

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*Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S, FitzPatrick ES. *Veterinary Microbiology and Microbial Disease*. Ames, IA: Wiley; 2011: *Kindle Edition*, location 13471 of 35051.

clinical conditions:⁴

* Adapted from Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S, FitzPatrick ES. *Veterinary Microbiology and Microbial Disease*. 2nd ed.

Contributing Institution: Ecole Nationale Veterinaire D'Alfort
 Unite d'histologie et d'Anatomie Pathologique
 7, avenue du General De Gaulle 94704 Maisons-Alfort cedex FRANCE

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