



WEDNESDAY SLIDE CONFERENCE 2013-2014

Conference 7

30 October 2013

CASE I: CSUVTH Sheep (JPC 4032698).

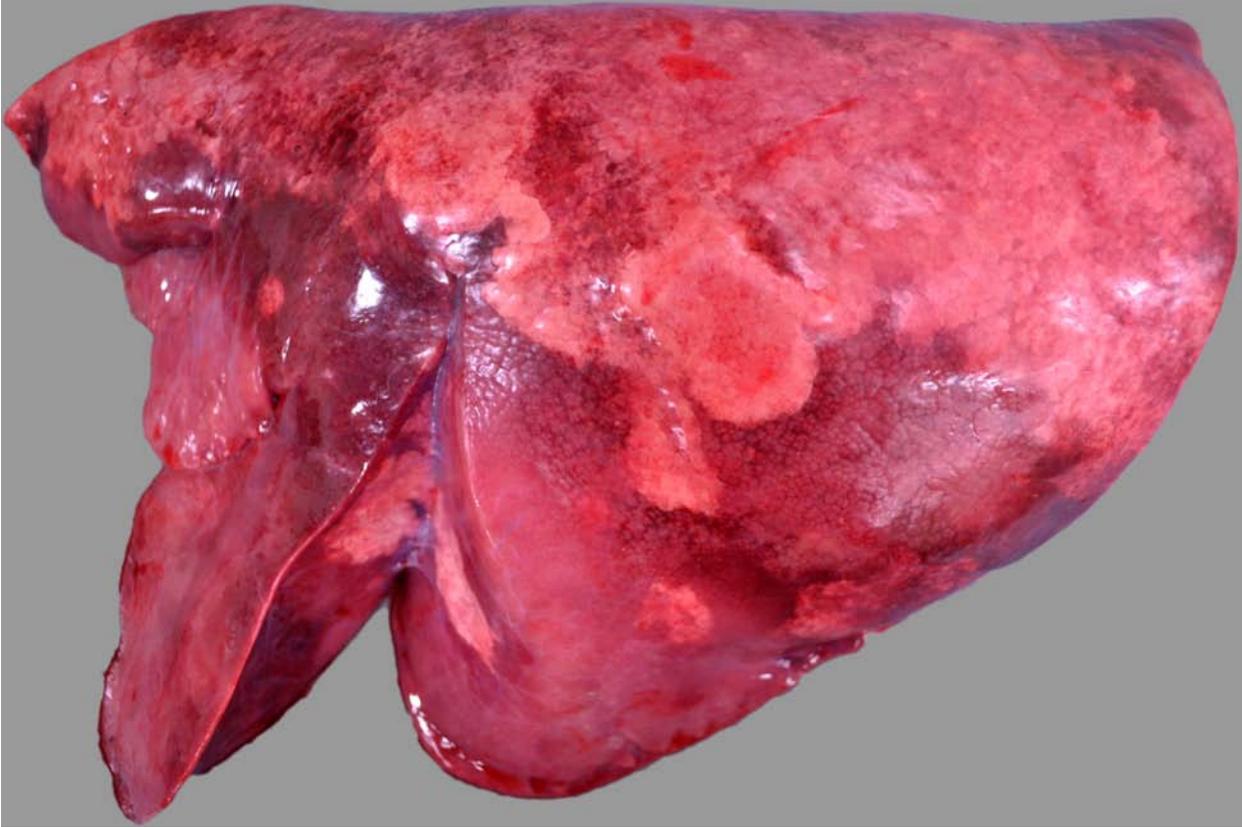
Signalment: 13-month-old Hampshire-cross castrated ram (*Ovis aries*).

History: The animal had a 5-7 day history of progressive ill thrift, and was subsequently euthanized due to poor quality of life.

Gross Pathology: Bilaterally, the cranioventral lung lobes were markedly firm on palpation. Multifocally visible in the immediate subpleural parenchyma of all lung lobes were numerous, coalescing, white, non-raised, nodular foci. On cut section the parenchyma was consolidated, mottled pale tan to dark red to brown, and displayed an increased prominence of cross-sectional profiles of small to medium caliber airways due to circumscription by dense cuffs of moderately firm white tissue.

Laboratory Results: PCR of lung tissue was positive for *Mycoplasma* spp. and was confirmed as *Mycoplasma ovipneumoniae* by genetic sequencing. PCR for Maedi-Visna virus (MVV; Ovine Progressive Pneumonia) was negative. Routine aerobic culture from the lung yielded heavy growth of *Mannheimia haemolytica*.

Histopathologic Description: Lung: Affecting 60-100% of the parenchyma within submitted sections, alveolar lumina are variably filled by large numbers of neutrophils and macrophages admixed with marked amounts of edema, and lesser amounts of fibrin. Frequently, macrophages contain abundant foamy cytoplasm, with rare multi-nucleate giant cells present. Multifocally, moderate numbers of affected alveoli are lined by plump, cuboidal epithelial cells (type II pneumocyte hyperplasia). Diffusely, the lumens of bronchi and bronchioles are also filled with variable numbers of neutrophils admixed with fewer macrophages, sloughed epithelial cells, moderate amounts of fibrin, and rare clusters of coccobacilli. Frequently, the submucosa of bronchi and bronchioles, as well as the adventitia of blood vessels, are expanded by cuffs of lymphocytes and plasma cells up to 10 cell layers thick, occasionally which are arranged in more follicular aggregates. Segmentally, there is hyperplasia of the respiratory epithelium of affected bronchi and bronchioles characterized by crowding and piling up of epithelial cells. Multifocally, interlobular septa are also expanded by ectatic lymphatics, edema, and lesser amounts of fibrin. Within some sections, rare bronchiolar lumens are partially occluded by nodular protrusions of fibrous connective tissue which are



1-1. Lung, sheep: Cranioventral lung lobes were firm, mottled pale tan to dark red to brown and contained numerous white coalescing foci. On cut section the parenchyma displayed an increased prominence of cross-sectional profiles of small to medium caliber airways surrounded dense cuffs of moderately firm white tissue. (Photo courtesy of: Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Dept. of Microbiology, Immunology, and Pathology/Diagnostic Medicine Center, Fort Collins, CO 80524, www.cvmb.colostate.edu)

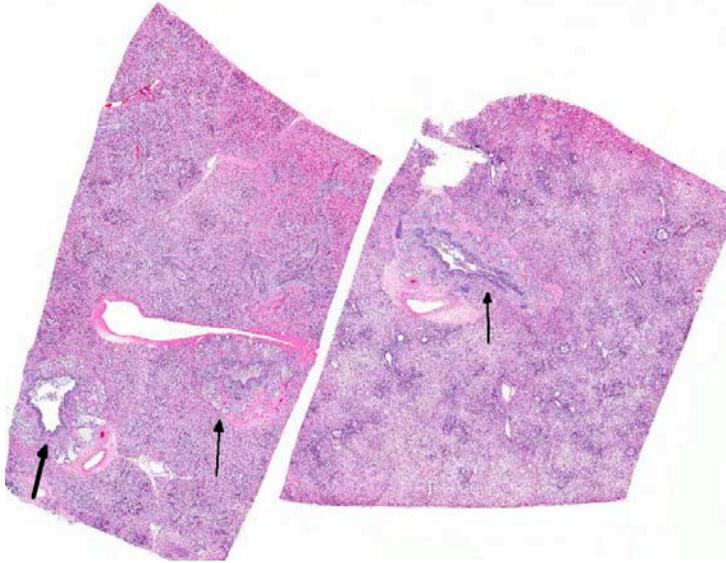
lined by hypertrophied epithelial cells (nodular hyaline casts).

Contributor's Morphologic Diagnosis: Lung; bronchopneumonia, suppurative and histiocytic, chronic-active, diffuse, severe, with bronchial epithelial and type II pneumocyte hyperplasia, and perivascular and peribronchiolar lymphofollicular proliferation.

Contributor's Comment: Numerous *Mycoplasma* spp. have been documented to cause disease in small ruminants including *M. mycoides* ssp. *Mycoides*, large colony type (LC), *M. mycoides* ssp. *capri*, *M. capricolum* ssp. *capricolum*, *M. putrefaciens*, *M. agalactiae*, *M. capricolum* ssp. *capripneumoniae* (the causative agent of contagious caprine pleuropneumonia (CCPP), and *M. ovipneumoniae*.² Similar to cattle, other domestic animal species, and laboratory rodents, these mycoplasmas cause a wide range of pathologic conditions not only including pneumonia/pleuropneumonia, but also

polyarthritis, septicemia/polyserositis, keratitis/conjunctivitis, and mastitis.^{2,5,10} Specifically in sheep, *Mycoplasma ovipneumoniae* is the primary etiologic agent involved in the condition known as “chronic enzootic pneumonia” or “chronic non-progressive pneumonia”, a multi-factorial disease complex which typically affects lambs of 1 year of age or less.^{2,5,10,12} *M. ovipneumoniae* infection is typically subclinical, frequently only causing a mild, non-fatal pneumonia that results in poor growth rates, until it becomes compounded by additional risk factors such as stress, poor air quality, adverse weather conditions, respiratory syncytial or parainfluenza viral infections, and secondary bacterial infections, which combine to cause overt clinical respiratory disease.^{2,5,10,12}

The spectrum of histological lesions present in this animal are a good representation of the pathogenesis of this disease syndrome, as the prominent lymphoid cuffing and bronchial epithelial hyperplasia are consistent with histological findings previously reported for *M.*



1-2. Lung, sheep: The lung is diffusely consolidated, and airways (arrows) are filled with refluxed exudate. (HE 0.63X)

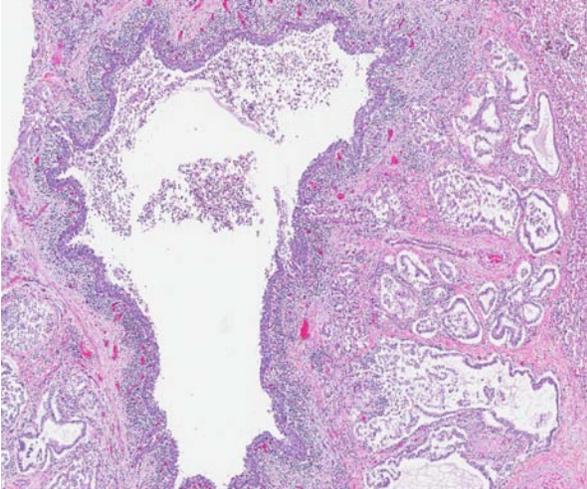
ovipneumoniae, and typify mycoplasmal pneumonias, while the suppurative inflammation is reflective of the secondary *Mannheimia haemolytica* infection and not of *M. ovipneumoniae* infection.^{2,12} Besides *M. haemolytica*, other secondary bacterial infections reported with ovine pulmonary mycoplasmosis include *Pasteurella multocida* and *Bibersteinia trehalosi* (formerly *Pasteurella haemolytica* biotype T).⁵ In addition to domestic sheep, *M. ovipneumoniae* has also been reported to cause similar lesions in Bighorn sheep (*Ovis canadensis canadensis*), as well as predispose them to secondary, fatal *M. haemolytica* pneumonia.^{1,3}

Colonization of the ciliated respiratory epithelium and induction of ciliostasis are shared pathogenic features of *Mycoplasma* spp. pneumonia, factors which are thought, at least in part, to prevent clearance of the organism from the respiratory tract by the innate defense system of the mucociliary escalator.^{4,7} While unique, these features are also characteristic of *Bordetella bronchiseptica* and cilia-associated respiratory bacillus (CAR bacillus), both important bacterial causes of respiratory disease in numerous laboratory and domestic animal species.^{10,11} Specifically, investigations into the ability of *M. ovipneumoniae* to persist in the respiratory tract of sheep have shown that this persistence may be due to a combination of: 1) an initial aberrant immune response to the organism and 2) delayed generation of a protective systemic humoral

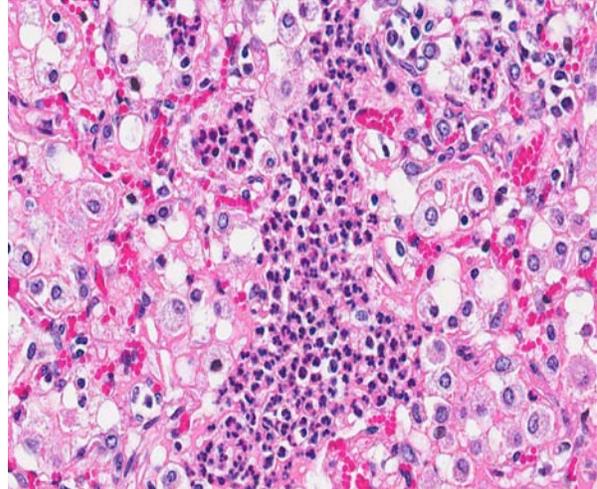
immune response.⁹ Experimental evidence corroborating this includes the presence of ciliary autoantibodies in acutely infected sheep, and the resolution of late clinical disease following systemic generation of antigen-specific IgG antibodies.^{8,9} It is speculated that this delayed development of protective humoral immunity results from a marked variation in antigenicity between organisms, as well as expression of a polysaccharide capsule.^{4,9}

JPC Diagnosis: Lung: Bronchopneumonia, neutrophilic and histiocytic, focally extensive, moderate with peribronchial, peribronchiolar, and perivascular lymphoplasmacytic proliferation.

Conference Comment: A tissue Gram stain revealed moderate numbers of gram-negative coccobacilli within peribronchial glands, interpreted as secondary bacterial colonization. Although none were isolated, conference participants speculated upon the presence of an initiating viral agent, which may have created ideal physiologic conditions for subsequent bacterial infection. Potential etiologic agents include bovine parainfluenza virus 3, bovine respiratory syncytial virus, Maedi-Visna virus, and (less likely) peste des petits ruminants virus. Both bovine parainfluenza virus 3 and bovine respiratory syncytial virus are members of the family *Paramyxoviridae*. Alone, they are unlikely to cause respiratory disease; however, they are commonly associated with both shipping fever and bovine respiratory disease complex, which frequently induce secondary bronchopneumonia due to bacterial agents such as *Mannheimia haemolytica*. Both of these syndromes are characterized by respiratory signs such as nasal discharge, tachypnea, anorexia, fever and general malaise, as exhibited in this case.⁶ Maedi-Visna virus, the cause of ovine progressive pneumonia, is a lentivirus from the family *Retroviridae*, which is closely related to caprine arthritis-encephalitis virus.⁶ Discovered in Iceland, this disease was historically distinguished by clinical signs indicating both respiratory and central nervous system lesions (*maedi* means “dyspnea” and *visna* means “fading away” in Icelandic); however, currently, the most common presentation is a slowly progressive pneumonia, which often



1-3. Lung, sheep: Bronchioles are ectatic and filled with refluxed exudate. Airway epithelium is hyperplastic, and is surrounded by large numbers of lymphocytes and plasma cells. Adjacent submucosal glands are often necrotic. (HE 40X)



1-4. Lung, sheep: Alveoli are filled with viable and degenerate neutrophils and abundant foamy macrophages. Alveolar septa appear congested and surrounded by proteinaceous exudate (fibrin and edema). (HE 400X)

predisposes secondary bacterial infection.² Peste des petits ruminants virus is a morbillivirus which, although not endemic to North America, can also cause respiratory disease in small ruminants.⁶

In this case, PCR analysis implicates *Mycoplasma ovipneumoniae* as the inciting agent. Mycoplasmas, the smallest known bacteria, are obligate parasites that lack a cell wall and have a protein- and lipid-rich plasma membrane. They have a relatively small genome and a propensity for genomic rearrangement, leading to frequent variations in cell surface antigens. These characteristics likely result in an innate ability to evade the host immune response. In addition to ciliostasis, other pathogenic mechanisms of mycoplasmas include alteration of prostaglandin synthesis and induction of lymphocyte apoptosis. Additionally, mycoplasmal membranes contain superantigens, which generate a substantial nonantigen-specific immune response; this is the likely cause of the characteristic peribronchiolar lymphoid cuffing often associated with pulmonary mycoplasmosis.² Superantigens bind the V_B domain of the T-lymphocyte receptor (TCR) with the α -chain of a class II major histocompatibility complex (MHC). This occurs outside of the normal antigen binding site and results in polyclonal T-lymphocyte activation regardless of antigen specificity, as well as massive cytokine release (see 2013-14 WSC conference 1, case 3). In lambs, infection with *M. ovipneumoniae* and *M. arginini* can induce

paroxysmal coughing of such severity as to induce rectal prolapse, known as “coughing syndrome.”⁸

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CASE II: 13-3472 (JPC 4032481).

Signalment: 2.5-year-old intact female Bernese Mountain dog (*Canis familiaris*).

History: The owners had intended to use the bitch for breeding. Multiple breedings never resulted in pregnancy. Ovariohysterectomy was performed in March of 2013. Uterus and ovaries were submitted for histopathology to determine the cause of infertility.

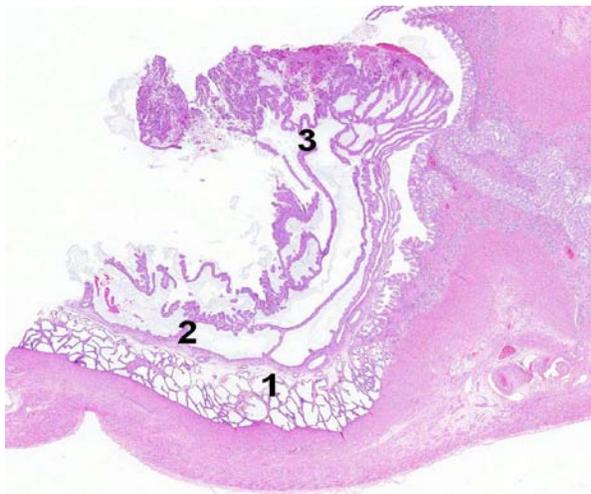
Gross Pathology: At surgery, the midbody of the right uterine horn had a circular 'lump'. Both ovaries had multiple dark nodules.

Macroscopically, the mid-region of the uterine mucosa of both horns was multifocally and segmentally expanded by a few, nodular, firm masses. There was one mass in the right uterine horn (3.0 x 2.5 x 1.5 cm, partially bisected by submitter) and two smaller masses in the left uterine horn (1.8 cm and 1.5 cm diameter), all of which oozed moderate amounts of clear fluid on sectioning.

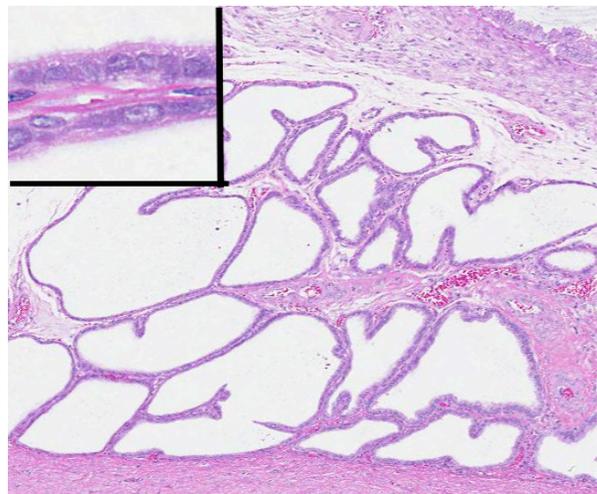
Histopathologic Description: In the affected uterine segments, the endometrium was disrupted by discreet, multilayered masses. Within these focal enlargements, deep endometrial glands were lined by a single layer of cuboidal epithelial cells, contained variable amounts of globular to homogeneous, proteinaceous material, and were

separated by a fine collagenous stroma, reminiscent of the deep glandular layer of the placenta. This layer of dilated glands was covered by a thin band of fibrous tissue, reminiscent of the supraglandular layer of the placenta. Luminal to the band of fibrous tissue was a second segment composed of folds and small villi of columnar endometrial epithelial cells with weakly eosinophilic to vacuolated cytoplasm, reminiscent of the outer layer of the placenta. The third, and most luminal layer was composed of many, large, irregularly sized papillary projections and cysts filled with mucus and protein and lined by pseudostratified, flattened to cuboidal to columnar epithelial-like cells, reminiscent of the placental labyrinth. These cells had moderate to marked anisokaryosis with rare multinucleated cells. The projections and cysts protruded into and partially occluded the uterine lumen. Abundant karyorrhectic and hypereosinophilic debris was scattered among the inner endometrial mucosa. The smooth muscle bundles of the inner and outer myometrium were flanked and mildly separated by increased amounts of collagen bundles.

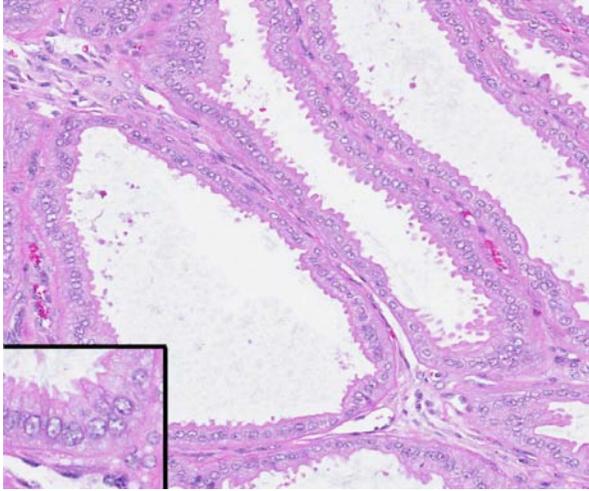
The mucosa distal and proximal to the uterine masses described above contained pools of erythrocytes, and endometrial glands were nested and separated by moderate amounts of collagenous stroma. Luminal epithelium was tall columnar with plump cytoplasm containing numerous cytoplasmic vacuoles.



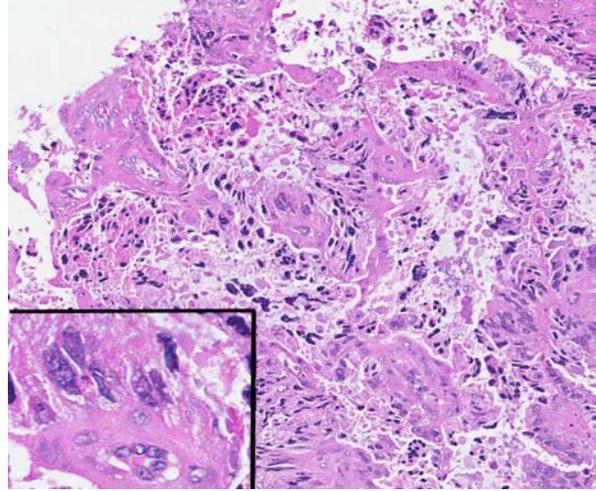
2-1. Uterus, dog: Segmentally, the hyperplastic endometrium exhibits a three-layer appearance reminiscent of placental formation, known as "pseudoplacental endometrial hyperplasia". #1 is the deep glandular region, #2 is a linear strip of dense collagen separating glandular layers, and #3 is a layer of villous proliferation resembling the junctional zone. (HE 4X)



2-2. Uterus, dog: The deep glandular regional consists of cystic glands lined by attenuated cuboidal cells with indistinct cell borders and a moderate amount of eosinophilic granular cytoplasm (inset). (HE 40X)



2-3. Uterus, dog: The innermost layer of the junctional zone is composed of large dilated glands lined by columnar epithelium (inset) which contain abundant basophilic mucin. (HE 40X)



2-4. Uterus, dog: The outermost layer of the junctional zone is composed of papillary projections of endometrial cells, of which over 50% are necrotic. Multinucleated cells are commonly seen in this area (inset). (HE 40X)

Ovaries: In two cross-bisected sections, approximately 40 to 60% of the right and left ovaries were composed of large corpora lutea. The remaining portions of the ovaries had follicles in various developmental stages.

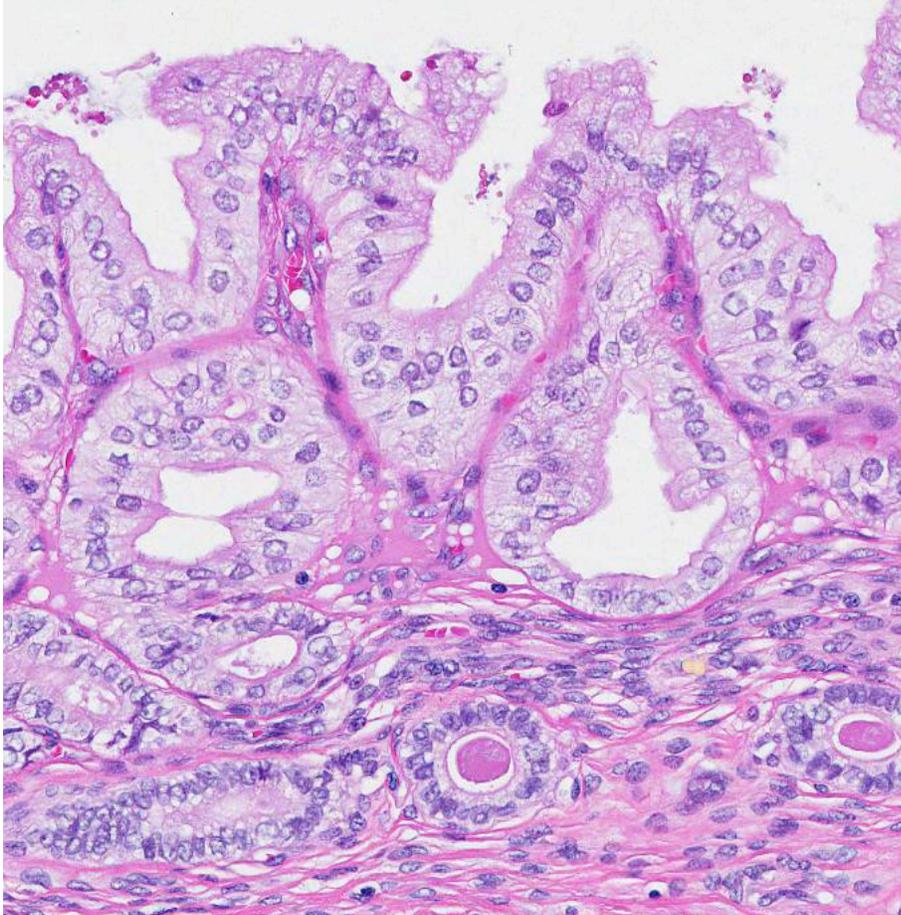
Contributor's Morphologic Diagnosis: 1. Uterus: Segmental endometrial hyperplasia (pseudo-placentational endometrial hyperplasia).
2. Ovary: Multiple ovarian corpora lutea.

Contributor's Comment: Endometrial hyperplasia is a proliferative condition of the uterine mucosal epithelium that is generally considered to be caused by abnormal elevations in either estrogen or progesterone, or both. In the bitch, the normal estrous cycle includes differentiation and proliferation of endometrial glands under estrogen influence during proestrus, and more extensive proliferation and coiling of glands during estrus and metestrus/diestrus under the influence of rising progesterone levels. As progesterone levels fall during late diestrus, the endometrium regresses to a quiescent/atrophic state during anestrus.³

In females of most domestic species (especially mares, sows and cows), the pathologic condition of endometrial hyperplasia is associated with hyperestrogenism.⁸ Endogenous estrogen from cystic follicles or granulosa cell tumors and ingestion of exogenous estrogenic plants have been implicated. In the bitch and the queen, the condition usually referred to as cystic endometrial

hyperplasia (CEH)-pyometra complex has been attributed to diffuse cystic proliferation of endometrial glands under prolonged progesterone influence after estrogen priming. Although some authors have argued that CEH and pyometra are not necessarily linked,¹ it is generally accepted that the same prolonged high progesterone levels that cause endometrial gland proliferation also increase susceptibility of the uterus to infection.³ Besides the CEH-pyometra complex, McKentee described 3 other forms of endometrial hyperplasia in the bitch: 1) estrogen-induced CEH, 2) focal endometrial polyps and 3) endometrial hyperplasia associated with pseudopregnancy; the latter is the subject of this report.

Segmental endometrial hyperplasia, also called pseudo-placentational endometrial hyperplasia (PEH) and deciduoma, is a condition of the canine uterus not always associated with pseudopregnancy.^{2,7} Although the cause of spontaneous cases is unknown, the condition has been induced by intraluminal stimulation of the canine uterus in the luteal phase of the estrous cycle.^{5,6} Clinically it has been associated, along with CEH and endometritis, with infertility and pregnancy loss in the bitch.⁴ The term 'deciduoma' as used by some authors is derived from conditions in primates and rodents and is less accurate in that it implies a neoplastic rather than hyperplastic lesion.⁹



2-5. Uterus, dog: Adjacent to the segmental area of hyperplasia, endometrial cells demonstrate progesterational change. (HE 100X)

PEH is clearly distinguishable from CEH both grossly and histologically.⁹ While CEH is generally a diffuse reaction involving the entire endometrium, PEH is characterized by focal or multifocal masses mimicking placentation sites. Histologically, the masses are characterized by the 3 distinct zones mimicking the normal maternal placenta.² The deepest, outermost zone, also called the stratum spongiosum, is composed of dilated glandular structures. That zone is separated from the highly folded luminal epithelium by a dense connective tissue band. The luminal surface consists of pseudostratified epithelium with scattered necrotic syncytia formation; fetal remnants and membranes are not identified.⁹

McKentee described regression of similar lesions in cases of pseudocystitis,³ presumably as a result of falling progesterone levels. In a study of infertility in bitches, PEH was identified by uterine biopsy at various stages of the estrous

cycle, but hormone levels were not recorded.⁴ In the case of the young bitch in this report, presence of multiple corpora lutea in the ovaries at the time of ovariohysterectomy suggests that the lesions formed or at least were maintained in the presence of high circulating progesterone; whether the lesions would have regressed as CLs regressed is unknown. The fact that the dog had documented infertility, however, suggests the PEH may have been interfering with fertility in this case.

JPC Diagnosis: Uterus, endometrium: Hyperplasia, pseudoplacental, focally extensive.

Conference Comment:

The contributor provides an excellent review of pseudo-placental endometrial hyperplasia (PEH). Some conference participants identified a number of multinucleated syncytia within the luminal epithelium, suggesting the possibility of syncytiotrophoblast formation and true placentation in this case.

Dr. Robert Foster of the University of Guelph was consulted on this possible interpretation. Dr. Foster confirmed that the presence of syncytia in the outermost layer is an expected finding in PEH, and the lack of multinucleated cells within deeper layers argues against the possibility that they are true syncytiotrophoblasts.

In this case, the corpora lutea are considered essentially normal, the cellular atypia seen within the largest nodule is considered to be within acceptable morphologic limits. In the area of the endometrium adjacent to PEH, the progesterational effects (vacuolation of endometrial cells,

tortuosity of glands, and presence of secretory material within deep glands) of these corpora lutea is seen.

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CASE III: H11-0101-A (JPC 4006298).

Signalment: Dog 1: 7-year-old female spayed Cavalier King Charles Spaniel (*Canis familiaris*).
Dog 2: 12-year-old female spayed miniature Poodle (*Canis familiaris*).

History: Four dogs presented to our referral hospital over a seven-week period with clinical signs of severe liver disease (including one or more of: inappetence, lethargy, vomiting, diarrhoea, polyuria and polydipsia). All four dogs had a common history of consuming a cooked commercial camel meat and sweet potato diet as a novel protein for management of suspected allergic skin disease. Two of these dogs subsequently deteriorated despite supportive therapy and were euthanized. Dog 1 was submitted for a full necropsy examination whereas surgical biopsy specimens of liver and pancreas were collected from dog 2 two days prior to euthanasia.

Gross Pathology: Post mortem examination of dog one revealed generalized jaundice and widespread petechial and ecchymotic haemorrhages on mucosal and serosal surfaces. The liver was small with a rough, granular to nodular surface and the pancreas was also irregularly thickened and firm. A necropsy examination was not performed on dog 2, and only limited information on the gross appearance of the liver (or pancreas) was provided by the surgeon, who simply noted that the liver demonstrated “severe liver changes with minimal normal hepatic tissue identified”.

Laboratory Results:

Dog 1

	Day 1	Day 13	Day 18	Day 19	Reference range
Lipase	1500	-	-	2500	13-200 U/L
Amylase	2200	-	-	3500	65-1140 U/L
ALT	1100	1500	-	1455	21-142 U/L
ALP	550	1000	-	900	20-184 U/L
AST	N	290	-	-	10-60 U/L
GGT	13	30	-	-	
Bilirubin	N	36	-	181	0-8 umol/L
Cholesterol	9.6	10	-	10	3.3-6.9 mmol/L
Urea	N	-	-	1.9	3.6-10.0 mmol/L
PT	-	-	19.4	-	5.1-7.9 secs
APTT	-	-	24.8	-	8.6-12.9 secs

Serum biochemistry:

*N = within the reference range

(-) = not performed or result not provided

Dog 2:

	Day 1	Day 5	Day 6	Day 7	Day 8	Reference range
ALT	3120	3200	-	1960	1460	21-142 U/L
ALP	136	633	-	561	531	20-184 U/L
AST	926	956	-	687	577	10-60 U/L
Bilirubin	8	134	-	144	123	0-8 umol/L
Cholesterol	5.3	6.0	-	4.6	4.1	3.3-6.9 mmol/L
Urea	4.1	4.5	-	2.3	3.7	3.6-10.0 mmol/L
PT	-	-	18.5	-	-	5.1-7.9 secs

*N = within the reference range

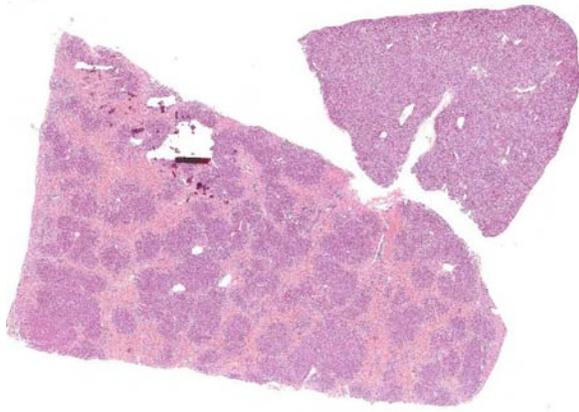
(-) = not performed or result not provided

Toxicology: Indospicine was subsequently detected in the serum or plasma of both dogs as well as in samples of liver and skeletal muscle of dog 1. Detection of indospicine in a sample of the cooked camel meat and sweet potato diet fed to one of the dogs, as well as in samples of camel meat provided by the manufacturer, confirmed the source of the toxin. Aflatoxins B1, B2, G1 & G2 were not detected in either of these samples.

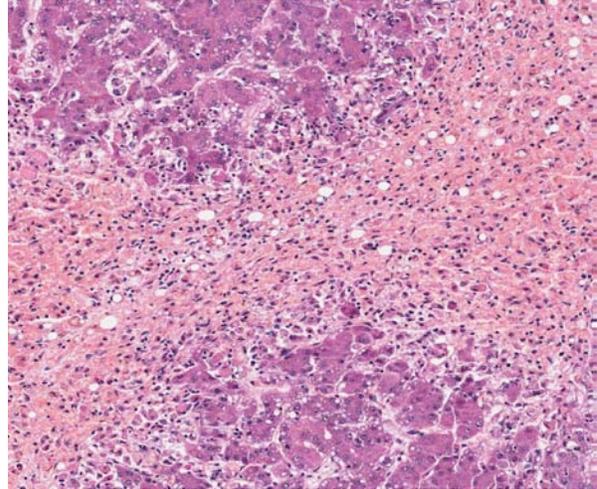
Indospicine was also detected in the serum of the other two dogs that developed clinical signs of hepatic disease, although these dogs subsequently recovered with supportive therapy and have remained well for the duration of follow up. In addition, indospicine was similarly detected in the serum of 15 other dogs known to have been consuming the diet. 3/15 of these dogs had increased serum ALT activity, although with the exception of one dog, none developed clinical signs of liver disease. One dog however subsequently went on to develop clinical signs of severe liver disease, despite withdrawal of the contaminated diet 5 months prior and was euthanized.

Histopathologic Description: A section of liver from each dog is present on the submitted slides. These two sections of liver have been included in order to demonstrate the range of hepatic changes that may be observed as a result of the hepatotoxic effects of indospicine.

Dog 1, liver (larger section of tissue): This section demonstrates extensive disruption of the hepatic



3-1. Liver, dog: There are sections from two different dogs on this slide, suggesting different dosage. A larger section with centrilobular to massive necrosis suggests a higher, more acute dose than the smaller section, in which bridging fibrosis, but little necrosis, suggests a more chronic low-grade exposure to the toxin. (HE 0.63X)



3-2. Liver, dog: Centrilobular hepatocytes are diffusely necrotic, and bands of necrotic hepatocytes bridge between central veins. In some lobules, necrosis extends to midzonal and even portal hepatocytes (massive necrosis). (HE 100X)

architecture by diffuse, centrilobular to massive zonal hepatocyte loss and haemorrhage. Numerous macrophages containing erythrocytes or a fine, golden to coarse dark brown granular cytoplasmic pigment are scattered extensively throughout the areas of hepatocyte loss and haemorrhage, and to a lesser extent the intact hepatic parenchyma. The majority of the cytoplasmic granules stain blue with a Perl's Prussian Blue (haemosiderin). Infiltrating portal triads and scattered throughout the areas of haemorrhage, are frequent clusters of lymphocytes and plasma cells accompanied by fewer neutrophils and macrophages. Within the residual clusters of hepatocytes there are often plugs of bile within distended canaliculi. Bile ducts are often distended by variably cellular casts. The remaining hepatocytes demonstrate moderate anisocytosis, often associated with cytoplasmic macro- and microvesicular vacuolation. There are also scattered individually necrotic hepatocytes. Subcapsular and portal lymphatics are often ectatic. Histochemical staining with Martius Scarlet Blue highlighted the presence of a mild increase in fibrous connective tissue surrounding the portal triads. A thin strand of fibrous connective tissue also dissects between individual hepatocytes in the centrilobular regions.

Dog 2, liver (smaller section of tissue): The architecture of the liver is disrupted by mild to moderate, diffuse bridging fibrosis which

connects central veins. Associated with the fibrosis is a moderate, multifocal, mixed infiltrate of haemosiderophages (confirmed with a Perl's stain for iron), lymphocytes and plasma cells and frequent dilated lymphatics. Portal triads are also expanded by mild to moderate fibrosis, mild biliary hyperplasia and an infiltrate of modest numbers of haemosiderophages, lymphocytes and occasional neutrophils. Associated with the centrilobular and portal fibrosis, there is moderate variation in hepatic lobule size, which is attributable to a combination of hepatocyte degeneration, atrophy and loss particularly surrounding the central veins. Remaining hepatocytes also demonstrate moderate to marked anisocytosis associated with cytoplasmic macrovesicular and microvesicular vacuolation. Occasional hepatocytes are binucleate and some have prominent, multiple nucleoli. There is also occasional single cell necrosis of hepatocytes. The undulating appearance of the capsular surface is attributable to the predominately centrilobular hepatocyte loss, lobular collapse and fibrosis.

Pancreas (sections not included): Examination of the pancreas of both dogs reveals multifocal to coalescing foci of pancreatic fibrosis with degeneration, atrophy and loss of adjacent exocrine pancreatic acini. These changes are accompanied by variably extensive foci of peripancreatic fat necrosis and a neutrophilic infiltrate; minimal and focal in dog 1 but severe and reasonably extensive in dog 2.

Contributor's Morphologic Diagnosis:

Dog 1 (larger tissue section):

1. Liver:
 - a. Severe, diffuse, subacute to chronic, centrilobular to massive hepatic necrosis and loss, with marked haemorrhage and haemosiderosis.
 - b. Mild to moderate, multifocal, chronic, mixed hepatitis.
 - c. Mild, periportal to dissecting, subacute, hepatic fibrosis.
 - d. Mild, multifocal, subacute to chronic vacuolar hepatopathy.

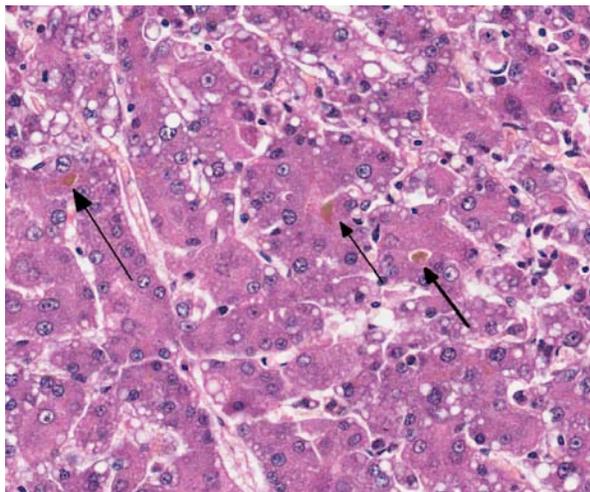
Dog 2: (smaller tissue section)

1. Liver:
 - a. Mild to moderate, chronic, centrilobular to bridging fibrosis, haemorrhage and haemosiderosis.
 - b. Moderate, diffuse subacute to chronic vacuolar hepatopathy.
 - c. Mild to moderate, subacute to chronic, multifocal lymphoplasmacytic hepatitis.

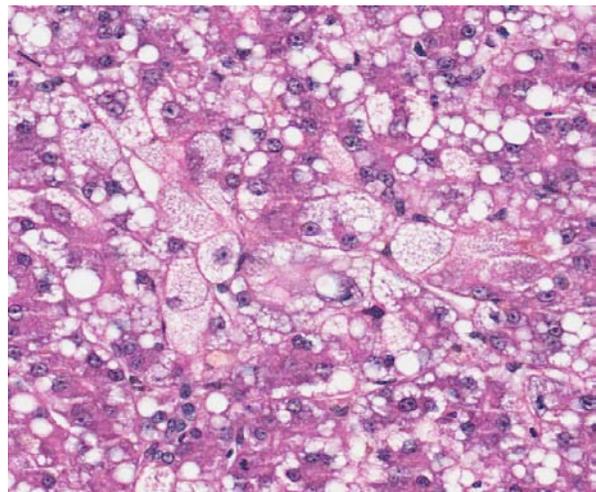
Contributor's Comment: Indospicine (6-amidino-2-amino-hexanoic acid) is a toxic amino acid of plant origin that is found in several species of the plant genus *Indigofera*, many of which are found extensively across the sub-tropical and arid regions of Australia.^{1,7} The toxin was first isolated from the seed and leaf of *I. spicata*,⁹ after this plant species was shown to be hepatotoxic in a range of species including chicks, sheep, cattle,

rabbits and mice.^{4,10,11} Hepatotoxicosis was subsequently induced in rats and mice following oral or parenteral administration of purified indospicine.^{5,8} In addition to being a hepatotoxin, indospicine has also been demonstrated to be teratogenic in cattle, rats, mice and rabbits.¹⁴ Extremely similar in structure to the essential amino acid arginine, indospicine is not metabolized by arginine and is unusual in that it is not incorporated into protein. Rather, it accumulates in body tissues as the free amino acid.¹⁵ Indospicine has been shown to competitively block the incorporation of arginine and subsequently other amino acids into rat liver protein in vitro.^{5,13} Whilst the exact mechanism of toxicity remains unknown, it is thought that these antimetabolite properties may contribute to the hepatotoxicity of indospicine.

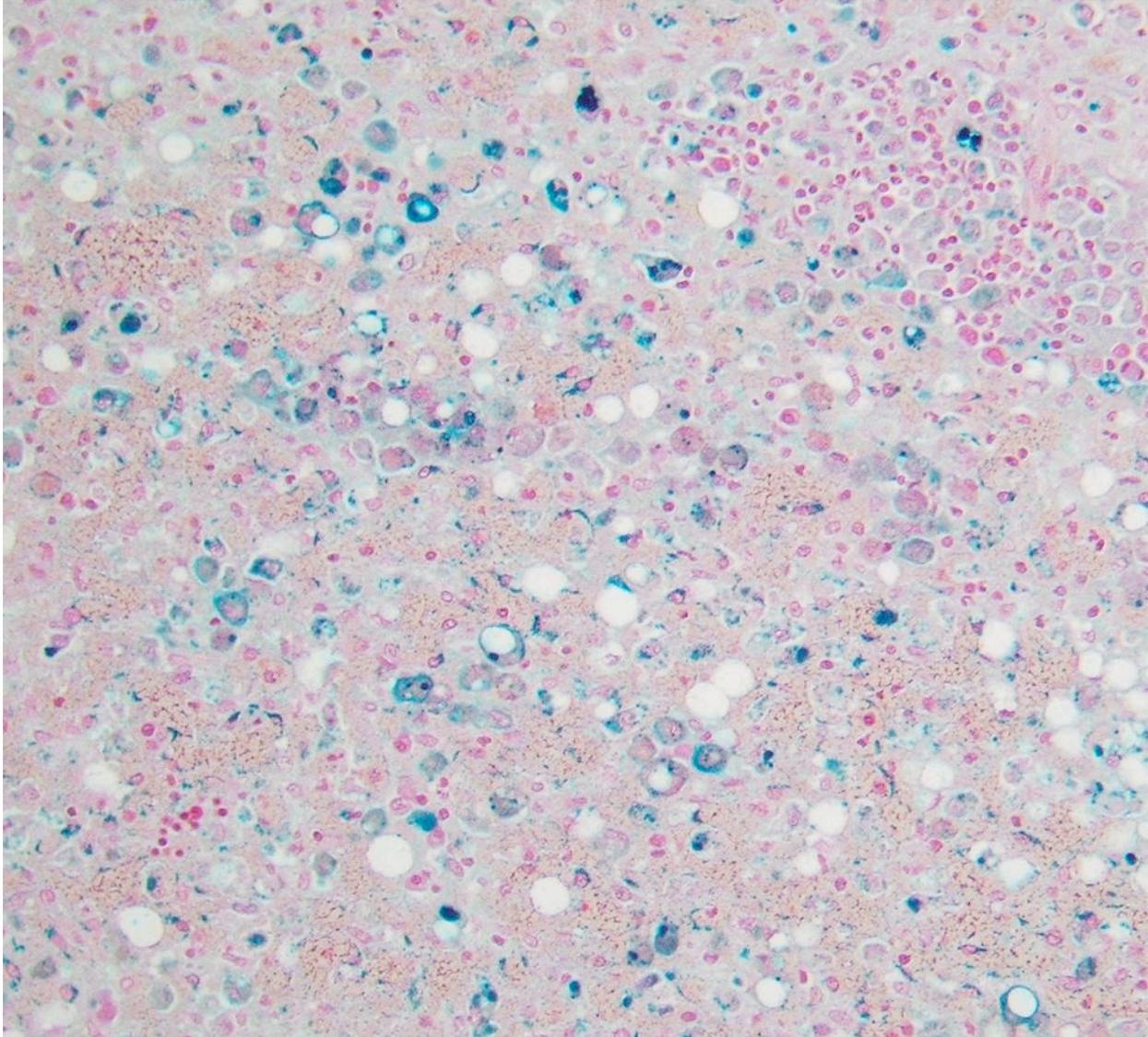
Mammals appear to have a poor ability to metabolize or excrete indospicine, and experimentally levels have been shown to persist in the tissues (including skeletal muscle) of horses, goats, cattle and rabbits for several months following cessation of *Indigofera* spp. intake.⁶ Such indospicine residues have the potential to cause secondary toxicosis in carnivores consuming contaminated meat and severe, sometimes fatal hepatotoxicosis has been reported in dogs consuming contaminated horse and camel meat. There is, however, marked species variability in susceptibility to indospicine hepatotoxicosis with dogs being particularly sensitive.^{6,7} Cattle and sheep are unaffected under normal grazing conditions; however, liver damage



3-3. Liver, dog: Bile canaliculi (arrows) are markedly dilated and filled with bile (cholestasis). Throughout the section, bile ducts are dilated and filled with mucin. (HE 288X)



3-4. Liver, dog: In the second ("more chronic") section, hepatocytes are swollen with accumulation of both large (macrovesicular) and small discrete (microvesicular) fat droplets within the cytoplasm. (HE 320X)



3-5. Liver, dog: Scattered Kupffer cells contain intracytoplasmic hemosiderin granules. (Perl's Prussian Blue, 20X) (Photo courtesy of: Murdoch University, Department of Anatomic Pathology, School of Veterinary and Biomedical Sciences, Faculty of Health Sciences. South Street, Murdoch, Perth, Western Australia, 6150, AUSTRALIA. <http://www.vetbiomed.murdoch.edu.au/>)

has been experimentally induced under conditions of high *Indigofera* spp. intake.⁶ Horses appear resistant to the hepatotoxic effects of indospicine; however, horses grazing *I. linnaei* (Birdsville indigo) may suffer from a neurological disturbance known as Birdsville horse disease,³ presumably due to co-occurring esters of 3-nitropropanoic acid.¹⁷ There are no reports of indospicine or *Indigofera*-associated toxicosis in camels, although the remote location of these animals in the arid regions of Australia generally limits close observation.⁶

The reason behind the species variability and apparently unique susceptibility of dogs to the

hepatotoxic effects of indospicine is similarly unknown. Indospicine is also not a predictable hepatotoxin in dogs, although the characteristic lesions of hepatocellular vacuolation, necrosis, haemorrhage and mononuclear inflammatory cell infiltration primarily seem to target the centrilobular hepatocytes. Note that the cause of the concurrent pancreatic inflammation and fibrosis seen in both of these dogs is unknown,⁷ as it has not been previously reported in cases of indospicine toxicosis in animals, despite the pancreas having the highest levels of indospicine in a variety of tissues tested.^{12,17} The trends seen in naturally occurring cases,^{6,7} as well the results of experimental trials in dogs^{12,17} show a high

degree of variability in the susceptibility of individual dogs to indospicine hepatotoxicosis, with liver failure only occurring in a small proportion of dogs exposed.^{6,7,12,17} These findings have led the suggestion that an idiosyncratic response to the toxin may occur, perhaps in addition to a more consistent toxic effect. The nature of the inflammatory response has also led to postulation that an immune-mediated mechanism may be involved.¹⁶ Whatever the exact mechanism of liver injury, the available evidence suggests that indospicine is not a rapidly necrotizing hepatotoxin. Based on the results of previous experimental studies, prolonged exposure (4 days) is seemingly required for development of clinical disease, with no dogs in short term trials (dosed over four consecutive days) developing signs of liver disease.^{2,7,17}

JPC Diagnosis: 1. Liver: Necrosis, centrilobular to massive, bridging, severe, with marked cholestasis and macro and microvesicular hepatocellular lipidosis.

2. Liver, hepatocytes: Macro and micronodular lipidosis with multifocal hepatocyte necrosis, and loss, mild bridging fibrosis and mild cholestasis.

Conference Comment: Conference participants conducted a comprehensive assessment of the clinical-pathologic findings for this case. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin were markedly elevated in both dogs, while urea and cholesterol were decreased. Additionally, prothombin time (PT) and activated partial thromboplastin time (APTT) were significantly prolonged. These are expected findings in cases of massive hepatic necrosis with secondary cholestasis.²

Although the most striking microscopic lesions in this case are the massive hepatocellular necrosis and loss along with cholestasis and lipidosis, there is also mild bridging fibrosis in dog 2, which was demonstrated with a Masson's stain. This subtle suggestion of chronicity supports the contributor's supposition that indospicine toxicity is likely not a peracute/acute hepatotoxin. Given that indisopicine containing species such as *Indigofera* are not generally found in North America, most conference participants formulated a list of alternative hepatotoxins that could potentially induce similar lesions dogs, including aflatoxicosis, pyrrolizidine alkaloid toxicosis, sago palm toxicity, blue-green algae toxicity,

copper toxicosis, iron overload and amanita toxicity.

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CASE IV: NIAH 2013 2 (JPC 4035422).

Signalment: Four-week-old domestic duck, a crossbreed of wild mallard and domesticated duck (*Anas platyrhynchos*).

History: This domestic duck was inoculated intravenously with H5N1 highly pathogenic avian influenza (HPAI) virus. The bird showed severe neurological symptoms from day 3 postinoculation (PI) and died on day 5 PI.

Gross Pathology: At necropsy, white multiple foci were found in the pancreas. Skin and feathers appeared normal.

Laboratory Results: By virus isolation using eggs, influenza viruses were re-isolated from organs including calami of plucked feathers.

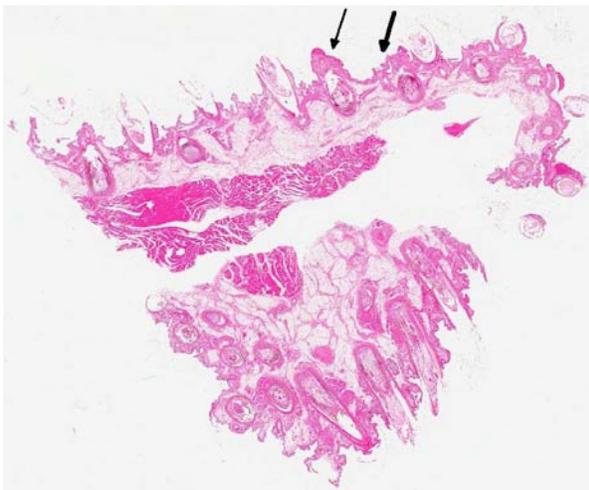
Histopathologic Description: There were many developing feathers in the dermis of the skin. Focal to diffuse epidermal necrosis was observed in some feathers. Lesions were accompanied by mild to severe heterophilic infiltration. The affected feather epidermis rarely had the vesicular formation. Phagocytic cells containing melanin pigments were present in some lesions. The feather pulp of some feathers exhibited heterophilic inflammation, blood congestion, hemorrhage and edematous change. Depending on the section, there were crust formation on the skin epidermis and small aggregates of

lymphocytes around the small vessels of the dermis.

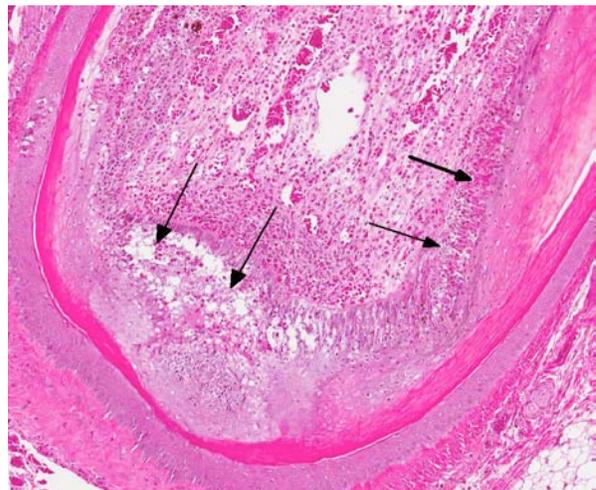
Immunohistochemical analysis to detect type A influenza virus revealed that influenza virus matrix antigens were present in the feather epidermal cells with/without necrotic changes. Few fibroblasts in the feather pulp were also positive for viral antigens. No relation was found between the virus antigen distribution and foci of lymphocytes in the dermis. Other major pathological findings in this case were non-suppurative encephalomyelitis, myocarditis, pancreatic necrosis, myositis, keratitis and focal epithelial necrosis of the beak, tongue and legs.

Contributor's Morphologic Diagnosis: Feathered skin: Epidermal necrosis of feathers with heterophilic infiltration.

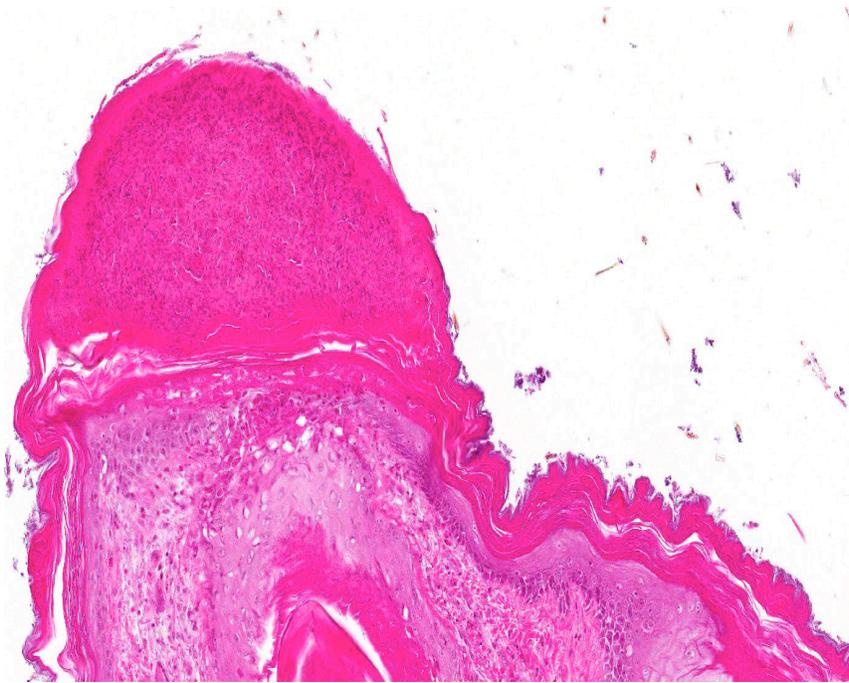
Contributor's Comment: Since 1997, an epidemic of Asian lineage H5N1 subtype HPAI virus has spread from Asia to the Eurasian continent, causing fatal infections in poultry, wild birds, mammals, and humans.¹ Interestingly, this virus can cause clinical symptoms and pathological lesions to waterfowl which have been considered natural reservoirs of avian influenza virus in nature.⁶ However, in contrast to chickens which usually result in fatal outcome after infection, ducks can exhibit asymptomatic clinical course and shed the virus into the environment.² Asymptomatically infected domestic ducks contributed to the viral spread in Southeast Asia.²



4-1. Feathered skin, duck: The epidermis has a serocellular crust and intracorneal pustule (arrows). Multifocal feather follicles are similarly affected by necrotizing folliculitis. (HE 0.63X)



4-2. Feathered skin, duck: There is lytic necrosis of the epithelium of the outer root sheath with vesicle formation. The outer root sheath and pulp are infiltrated by large numbers of heterophils. (HE 40X)



4-3. Feathered skin, duck: There are multiple heterophilic pustules within the epidermis as well as heterophilic infiltration of the underlying dermis. (HE 140X)

Histopathological findings in waterfowl infected with Asian H5N1 HPAI virus are frequently found in the central nervous system, heart and pancreas.⁵ In addition, virus replication in the feather epidermis is one of the characteristic findings in waterfowl infected with H5N1 HPAI virus.⁷ This microscopic feather lesion was reported in domestic ducks, geese and wild swans.^{3,7,8} Even asymptomatic ducks had the feather lesions in the experimental infection.⁷ Spherical virions were observed in the feather epidermis by electron microscopic examination.⁷ These findings raise the possibility that H5N1 HPAI viruses may be released from feathers of infected waterfowl to the environment and that feathers could become potential sources of infection along with their feces and respiratory secretions.

JPC Diagnosis: Skin, feather follicle: Folliculitis, necrotizing and heterophilic, multifocal, moderate, with heterophilic pustular dermatitis and pulpitis.

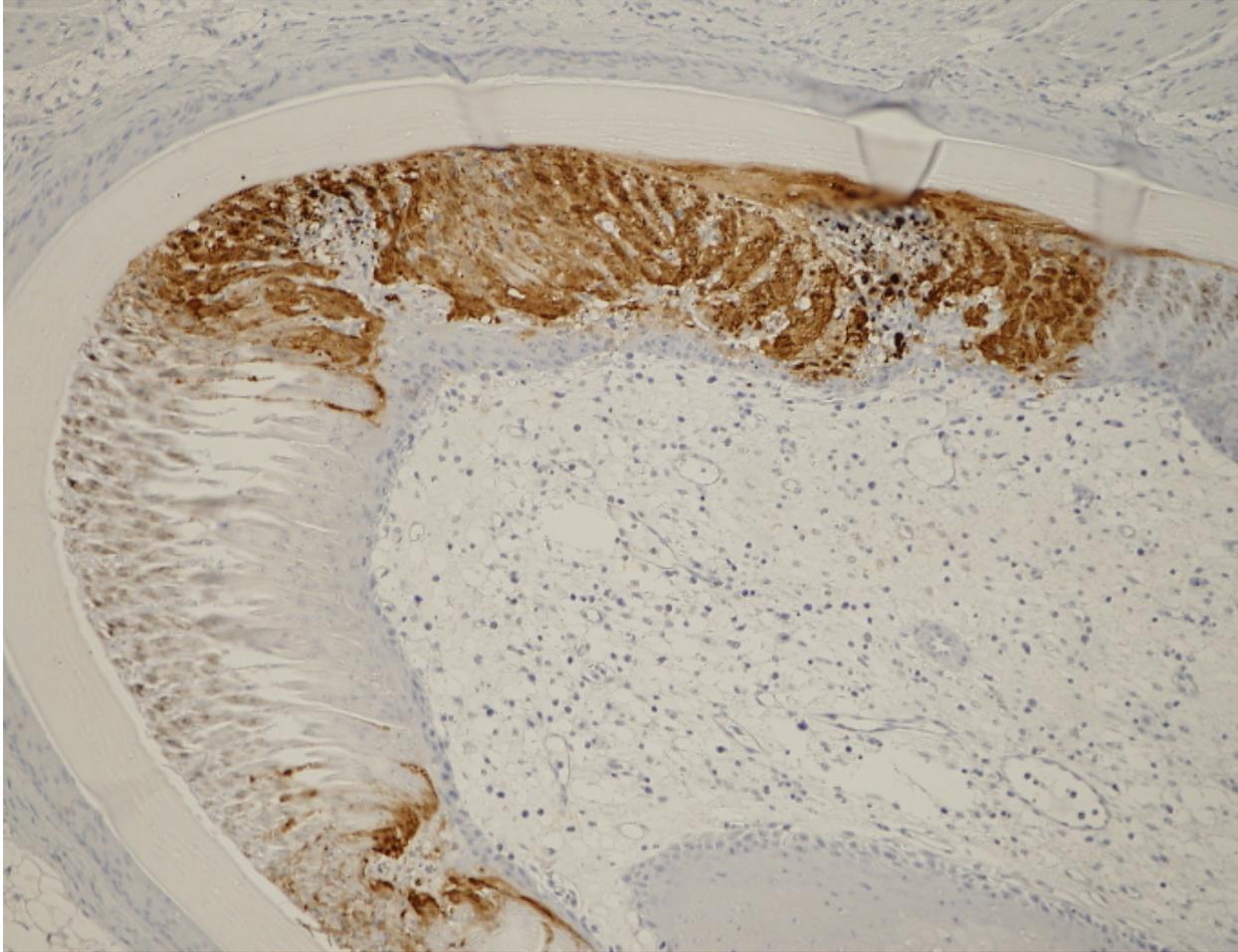
Conference Comment: Highly pathogenic avian influenza virus, a single stranded RNA virus, is a member of the family *Orthomyxoviridae*, which contains three genera: *Influenzavirus A*, *B* and *C*. Influenzaviruses that are pathologic to domestic animals, including avian influenza, make up the

Influenzavirus A genus, although some of these can cross over to humans as well.⁸ Influenza B viruses primarily affect humans, while influenza C viruses, which lack neuraminidase, infect humans. Swine are susceptible to both influenza A and influenza C viruses.⁴ Influenza viruses are sensitive to heat, acid and lipid solvents; thus they are quite labile within the environment.⁴ Currently influenza A viruses are classified into 16 hemagglutinin (H) and 9 neuraminidase (N) types, and further categorized as high or low pathogenicity.¹ The virus strain naming convention provides a great deal of information and consists of the virus type (A, B, C), geographic origin, strain

number, year of isolation and hemagglutinin/neuraminidase subtypes.^{1,4} For example "A/chicken/Scotland/1959 (H5N1)" was the first reported high-pathogenicity H5 avian influenza virus, in Scotland.⁴

The influenza A subtypes most frequently implicated in animal infections include: H5 and H7 in poultry, which can be highly pathogenic; H7N7 and H3N8 (equine influenza viruses 1 and 2), which cause respiratory disease in horses; enzootic H1N1, H1N2 and H3N2, which affect swine; H7N7 and H4N5 in seals; sporadic H10N4 in mink; H1N1, H2N2, H3N2 (historically endemic) and H5N1, H7N3, H7N7 and H9N2 (more recent) in humans; and H3N8 and H3N2 which cause respiratory disease in dogs.⁴

Highly pathogenic avian influenza (HPAI) is confined to subtypes H5 and H7, and is generally introduced into poultry flocks via wild birds (especially ducks).⁴ Low-pathogenicity avian influenza (LPAI), replicates in the gastrointestinal tracts of waterfowl, who shed high concentrations of the virus in feces and have been implicated as an important viral reservoir.³ LPAI infections in domestic birds are typically subclinical (with occasional mild respiratory signs or decreased egg production), however, mutation to highly



4-4. Feather follicle, duck: Feather epithelium shows immunopositive against Type A influenza virus matrix antigens. (IHC HPAI, 80X) (Photo courtesy of: National Institute of Animal Health, Japan, <http://www.naro.affrc.go.jp/org/niah>)

pathogenic avian influenza can occur, with devastating economic affects.⁵ Resulting HPAI viruses can cause severe systemic disease in chickens, with necrosis and inflammation in the skin, viscera and brain. In general, the emergence of new and varied influenza viruses depends on genetic drift (point mutations) as well as genetic shift (genomic segment reassortment).⁴ An important virulence determinant in avian influenza is the amino acid sequence at the hemagglutinin protein cleavage site. Low pathogenicity strains of virus have a single, basic amino acid (arginine) at the cleavage site; insertions, deletions or point mutations resulting in changes to this cleavage site can significantly alter pathogenicity.⁴

In poultry, following binding of hemagglutinin to host cell α 2,3-galactose receptors and the subsequent induction of receptor-mediated endocytosis, avian influenza virus replicates in

(and is shed from) both the respiratory and gastrointestinal tracts. Cell damage occurs secondary to direct virus replication, inflammatory mediators and/or vascular thrombosis and ischemia.⁵ Depending on the host, HPAI can be epitheliotropic, endotheliotropic, neurotropic or pantropic.⁵ The presence of gross findings depends upon the strain and virulence of the virus, and may include ruffled feathers; edema of the comb, wattles, periorbital areas and legs, subcutaneous hemorrhage; multifocal visceral and mucosal hemorrhage and necrosis; pulmonary edema and hemorrhage; pancreatic necrosis; and intestinal lymphoid necrosis.⁵ Microscopic lesions are more frequent than gross lesions and consist primarily of necrosis and inflammation within multiple organs, especially the skin/feather follicles, pancreas, brain, heart, lungs, adrenal glands and primary/secondary lymphoid organs.⁵ Central nervous system involvement can occur

after direct viral spread from the nasal cavity to the brain via olfactory nerves, hematogenous spread, or infection of ependymal cells with subsequent ventriculitis/periventriculitis.⁵ Death can be peracute, or it may follow multi-organ failure; extremely virulent strains of avian HPAI virus can cause up to 75% or even 100% mortality.^{4,5} Similar morbidity and mortality has also been reported in turkeys, quail, guineafowl and pheasants.⁵

Early research demonstrated that HPAI viruses rarely produced fulminant disease in wild birds; however, since 2002 a new Eurasian-African lineage of H5N1 HPAI virus has been reported to cause clinical disease and death in ducks under both natural and experimental conditions.^{3,5,6} As noted by the contributor, lesions in waterfowl infected with HPAI virus frequently occur in the brain, heart and pancreas, with characteristic virus replication in the feather epidermis,^{7,8} as demonstrated in this case.

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