The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 13

9 January 2008

Moderator:

Dr. Bruce Williams, DVM, DACVP

CASE I – 02-3323 (AFIP 3074949).

Signalment: 7-year-old, castrated male, *Mustela putorius furo*, ferret

History: In January of 2000 Eucchus presented with bilaterally symmetric hair loss over the dorsum, at that time both adrenals were at the upper range of normal size and nodular by ultrasound. Hormonal panels supported a clinical diagnosis of adrenal disease (Estradiol 174pmol/L (normal 30-180pmol/L); 17-OH -progesterone 0.85nmol/L (normal 0-0.8 nmol/L); androstenedione 30.7nmol/L (normal 0-15nmol/L). At that time the owner elected medical management with repeated injections of Depo-lupron. Depo-lupron is a GnRH analogue which inhibits production of LH and FSH. In September of 2000, the owner reported that Eucchus' belly would be soaked in urine after he urinated. Eucchus also repeatedly presented with alopecia and flaky skin. Urinary symptoms and alopecia resolved following increasing doses of Depo-lupron. In December of 2001 and again in June of 2002 Eucchus presented with difficulty urinating and was found to have an enlarged bladder. A urinary tract infection and enlarged prostate were diagnosed and Eucchus was started on antibiotics. At this time an insulinoma was also suspected clinically. Several weeks after discontinuing the antibiotics (August 2002), Eucchus again presented with straining to urinate and antibiotics were resumed. While still on antiobiotics, Eucchus presented in October of 2002, for straining to urinate. At this time the left adrenal gland was markedly enlarged (1cm) by ultrasound and surgery was elected. At surgery, the left adrenal gland was removed and 2 periprostatic cysts were identified which communicated with the urinary bladder. Additionally, 2 discrete nodules were noted in the pancreas and were removed. Following surgery, Eucchus became lethargic, dehydrated and anuric and was euthanized.

Biopsy results of the adrenal and pancreas were consistent with an adrenocortical adenocarcinoma and islet cell tumors (presumptive insulinomas), respectively.

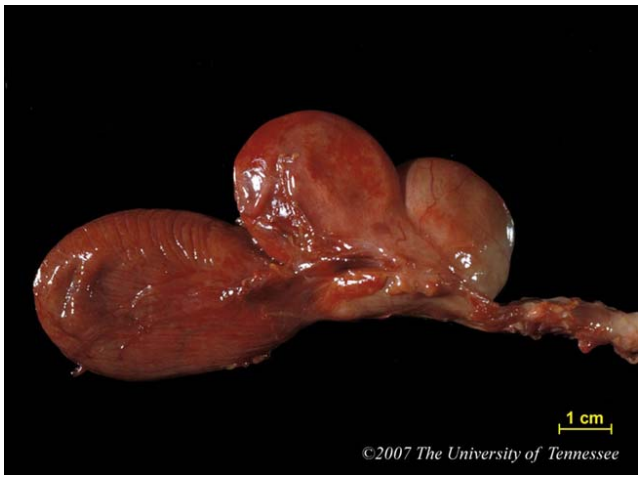
Gross Pathology: At necropsy, Eucchus was found to be obese and the abdomen contained 50ml of serosanguinous fluid. Two 3cm diameter cysts were found surrounding the prostate, just caudal to the **urinary bladder (fig. 1-1)**.

Histopathologic Description: The prostate is markedly expanded by a single large cyst and multiple smaller cysts lined by keratinizing stratified squamous epithelium, and containing variable amounts of keratin. In some sections, there is focal loss of the epithelial lining of one of the cysts with free keratin in the surrounding

stroma. This free keratin is surrounded by a variable infiltrate of neutrophils and macrophages. Throughout the prostate there are decreased amounts of glandular tissue. Remaining glandular structures are lined by a low cuboidal epithelium and rarely contain an eosinophilic secretory product. Scattered clusters of lymphocytes and rarely eosinophils are present in the surrounding fibrous connective tissue stroma.

Contributor's Morphologic Diagnosis: Prostate: Severe glandular atrophy and squamous metaplasia with cyst formation.

Contributor's Comment: Adrenal cortical lesions are the second most common neoplasm of ferrets, after pancreatic islet cell tumors. An increased incidence of proliferative adrenal lesions occurs in ferrets neutered at an early age (2-4months), and is likely due to chronic stimulation of the cells of the zona reticularis by luteinizing hormone.[4] Adrenal gland-associated endocrinopathy (AAE) is associated with the presence of hyperplastic or neoplastic adrenal lesions which produce high levels of estrogenic compounds (estradiol-17b, androstenedione, dehydroepiandrosterone sulfate, 17-hydroxyprogesterone, progesterone). Lesions associated with AAE include bone marrow toxicity[4] and Cushingoid features (thin skin, muscular atrophy, pot-bellied appearance)[1], bilateral symmetrical truncal alopecia, vulvar swelling in sprayed females, reversion to sexual behavior in neutered animals, mammary gland hyperplasia in castrated males, and dysuria in males associated with squamous metaplasia of the prostate and prostatitis.



1-1. Prostate gland, ferret. Caudal to the urinary bladder and surrounding the prostate gland are two cysts measuring up to 3 cm in diameter. Photograph courtesy of the University of Tennessee, College of Veterinary Medicine, Department of Pathobiology; 2407 River Drive, Room A201; Knoxville, TN 37996-4542

The squamous metaplasia in the prostate of this ferret is likely due to increased levels of circulating estrogenic compounds. Six cases of prostatic squamous metaplasia with concurrent prostatitis have been reported in male ferrets with proliferative adrenocortical lesions. As in dogs, the prostatitis has been attributed to the presence of keratin.[2] The absence of significant prostatitis in this case may be unusual. The observed prostatic atrophy is likely a result of castration at a young age and failure of the prostate to develop normally.[3]

In dogs, squamous metaplasia of the prostatic glandular epithelium has been associated with estrogen-producing Sertoli cell tumors, or exogenous administration of estrogens. In such cases, squamous metaplasia affects the prostatic urethra, uterus masculinus and prostatic ducts. Similar changes have been reported in swine. In cats, exogenous estrogen results in prostatic enlargement due to epithelial hyperplasia and cystic dilation of the glands; squamous metaplasia and cornification, however, only occur in the urethral epithelium.[3] Enlargement of the prostate is most commonly associated with constipation, and less commonly stranguria.[3]

AFIP Diagnosis: 1. Prostate: Prostatic cysts, multiple, ferret (*Mustela putorius furo*), carnivore.
2. Prostate, glands: Squamous metaplasia, multifocal, mild, with prostatitis and keratinizing cysts.

Conference Comment: Squamous metaplasia of the prostate with keratinizing prostatic cysts is a common sequel in male ferrets diagnosed with adrenal-associated endocrinopathy.[1,8] Chronic elevation of circulating luteinizing hormone (LH), resulting from early neutering, is required for metaplastic transformation.[1] Elevated circulating LH acts on the zona reticularis[4], stimulating cellular proliferation as well as the production of high levels of circulating estrogenic compounds, including estradiol-17b, and rostenedione, dehydroepiandrosterone, 17-hydroxyprogesterone, and progesterone.[2]

Luteinizing hormone receptors (LHRs) are usually present on ovarian thecal cells, granulosa cells, luteal cells, and testicular Leydig cells.[1] LHRs have also been identified in the adrenal gland of fetal (but not adult) mice, and low levels of LHR mRNA has been detected in the adrenal cortex of normal intact ferrets, indicating the presence of non-functional receptors.[1]

Tumors in the ferret adrenal gland include nodular hyperplasia, adrenocortical adenoma, and adrenocortical carci-

noma.[1,8] In the case of the latter, metastasis usually occurs late in the disease, and early complete removal of neoplastic adrenals carries a fair prognosis.[8] In contrast to other species, plasma concentrations of cortisol are only infrequently elevated in ferrets with AAE.[1,8]

Squamous metaplasia of glandular epithelium due to hyperestrogenism, has been documented in men, mice, dogs, and sheep.[1,2,3,6] Experimental induction of prostatic squamous metaplasia in the mouse model has revealed proliferation of basal cells with keratinization following injections with estrogen. In affected ferrets, squamous metaplasia of prostatic epithelium is followed by cyst formation and purulent inflammation as a result of keratin production[2] and may ultimately result in dysuria and post-renal azotemia.

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CASE II – 07-A5 (AFIP 3064916).

Signalment: 130-140-day-old, laying hens

History: Thirty hens were submitted for post mortem examination with a history of respiratory distress and high mortality from a flock of 25,000. During a high wind storm the roofs of 2 houses were damaged; numerous birds were outside and intermixing of birds from both houses occurred.

Gross Pathology: There was severe, catarrhal, hemorrhagic tracheitis. Caseous casts were present in the tracheas of several birds. Four had caseous plugs lodged in the larynx.

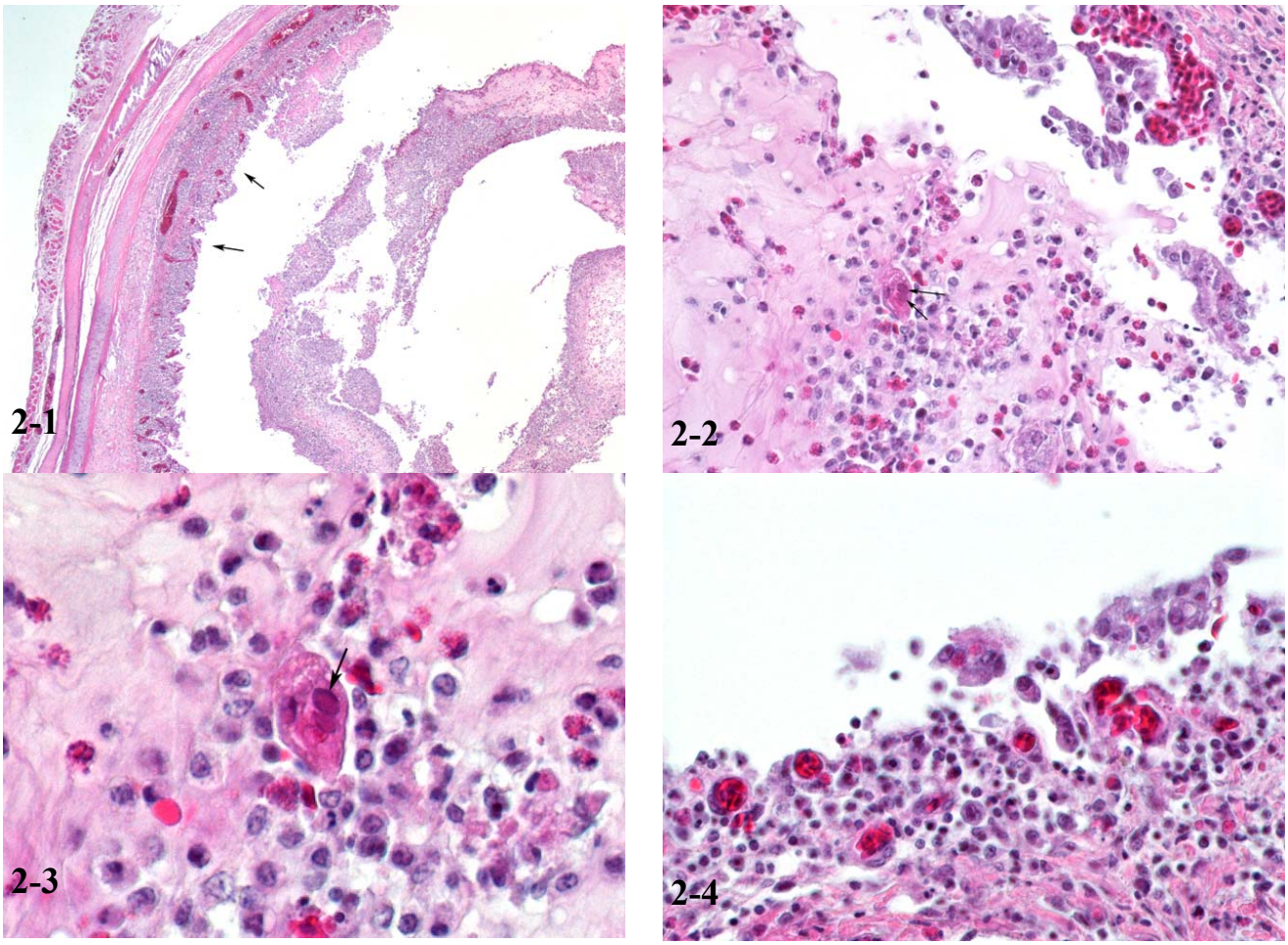
Laboratory Results: No significant bacterial pathogens were cultured.

PCR for Newcastle's Disease Virus (NDV) and Avian Influenza virus (AI) were negative. PCR for Infectious Laryngotracheitis virus (ILT) was positive.

Histopathologic Description: Trachea: The lumen contains large amounts of necrotic cellular **debris (fig. 2-1)**, fibrin and mats of bacterial colonies. The mucosa has diffuse erosion to ulceration of pseudostratified epithelium and formation of large, angular multinucleate **syncytia (fig. 2-2)** within the mucosa and within sloughed luminal debris. Within syncytia, many nuclei contain large, eosinophilic nuclear **inclusion bodies (fig. 2-3)** that marginate chromatin. The submucosa is moderately expanded by congested blood vessels and dense lymphocytic **infiltrates (fig. 2-4)**.

Contributor's Morphologic Diagnosis: Diffuse, severe, necrotizing and catarrhal tracheitis with syncytia formation and nuclear inclusions (Infectious Laryngotracheitis)

Contributor's Comment: Infectious laryngotracheitis (ILT) is a disease primarily of chickens caused by *Gallid herpesvirus 1*, an alphaherpesvirus. The disease was first described in 1925 and was the first major avian viral disease for which an effective vaccine was developed. It has a worldwide distribution, causing the most characteristic signs in adult laying hens. Natural routes of infection are upper respiratory and ocular, although oral transmission can occur. Viral replication is limited to respiratory tis-



2-1. Trachea, laying hen. The tracheal epithelium is effaced and replaced by abundant necrotic debris, fibrin, edema and hemorrhage (Arrows) admixed with colonies of bacterium. (H&E 40X).
2-2. Trachea, Laying hen. The tracheal epithelium is multifocally ulcerated and replaced by clumped epithelium (syncytia) (arrows).
2-3. Trachea, laying hen. Expanding the nuclei of sloughed epithelial cells and syncytial cells, marginating the chromatin are eosinophilic inclusion bodies (arrows).
2-4. Trachea, laying hen. Admixed within the necrosis and sloughed epithelium are moderate numbers of lymphocytes, plasma cells, heterophils and occasional macrophages.

Photomicrographs courtesy of the Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040

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sues. The trigeminal ganglion is the principle site of latency. LTV can persist as latent infections in recovered birds, and virus can be re-excreted in birds under stress. The virus may also persist as endemic infections in backyard and fancier chicken flocks.

Clinical signs vary from severe epizootic forms (as in this case) to mild endemic forms. Coughing, gasping and expectoration of blood-stained mucus characterize severe forms; mild forms show unthriftiness, decreased egg production nasal discharge and hemorrhagic conjunctivitis. Severe forms have high morbidity (90 - 100%); mortality usually ranges from 10 - 20%. Endemic forms have low morbidity and mortality. Although antigenically homogeneous, different virus strains with differing virulence have recently been differentiated by PCR-RFLP techniques.[5] Differential diagnoses for respiratory disease in chickens include the diphtheritic form of avian pox,

NDV, AI, infectious bronchitis, fowl adenovirus infections and aspergillosis.

The most consistent gross lesions of ILT are in the larynx and trachea. In mild cases, the only lesions may be conjunctivitis, sinusitis and mucoid tracheitis. In severe forms, diphtheritic changes can be striking, consisting of mucoid casts along the entire length of the trachea. Mucoid plugs in the larynx (as seen in this case) are also common. In some cases, hemorrhage predominates. Histologically, early lesions consist of loss of goblet cells and mononuclear inflammation. As the lesions progress, respiratory epithelial cells lose cilia, enlarge and form multinucleate syncytia. Nuclear inclusion bodies are present only in early stages (1 - 5 days). Confirmatory diagnostic procedures include viral isolation on embryonated chicken eggs, serology and PCR. Control of the disease in laying flocks is generally by vaccination, whereas tight biosecurity and sh orting growing cycle will often make vaccination of broiler flocks unnecessary. Vaccines are usually modified live virus, and mixing of flock with different immunity levels can cause disease outbreaks. In this case, birds from a non-vaccinated house were mixed with vaccinated birds, causing a disease outbreak. Newer deletion mutant vaccines are underdevelopment that will not only provide a safer ILT vaccine but show promise as vector vaccines for other avian infectious diseases, such as AI.[1]

AFIP Diagnosis: Trachea: Tracheitis, necrotizing, subacute, diffuse, moderate, with epithelial syncytia, intranuclear inclusion bodies, and intraluminal serocellular coagulum, chicken (*Gallus domesticus*), avian.

Conference Comment: The contributor gives an excellent overview of Infectious Laryngotracheitis (ILT). Chickens and pheasants are the only natural hosts, although isolation from peafowl and experimental infection of turkeys has been described.[1]

Ultrastructural features are those of a typical herpes virion and include a DNA- containing core within a 100nm icosahedral capsid surrounded by a variably sized proteinaceous tegument layer and an outer envelope with incorporated viral glycoproteins.[1] The viral glycoproteins appear as fine spikes projecting from the surface of the envelope.[2] Viral particle sizes vary between 200-350nm depending on the amount of incorporated tegument protein.[1]

Tegument proteins are common in enveloped viruses and are usually a combination of essential and non-essential proteins that are released shortly after viral entry into the cell. These proteins may aid in suppression of the immune response, suppression of host mRNA transcription, or transcribing/translating viral genes. Formation of tegument proteins is generally done late in the viral infectious cycle, following replication of viral genes.[3]

Viral replication of ILT is similar to that of other alphaherpes virus.[1,2] Within the infected cell nucleus, viral

Table 2-1. Alphaherpesviruses[4]

- Porcine herpesvirus-1: Pseudorabies, Aujeszky's disease
- Canine herpesvirus-1: Canine herpes
- Feline herpesvirus-1: Feline viral rhinotracheitis (FVR)

Bovine:
- BHV1: Infectious bovine rhinotracheitis
Infectious pustular vulvovaginitis
Infectious balanoposthitis
- BHV2: Bovine mammillitis virus/ Pseudo-lumpy skin disease
- BHV5 : Bovine herpesvirus encephalitis (no inclusion bodies)

Equine:
- EHV1: Equine herpesviral abortion, rhinopneumonitis, neurologic disease
- EHV3: Equine coital exanthema
- EHV4: Equine rhinopneumonitis, abortion

Avian:
- Avian HV1: Infectious laryngotracheitis
- Avian HV2 (Gallid herpesvirus-2): Marek's disease
- Anatid HV1: Duck plague

Nonhuman primate:
- Herpes virus simiae (Cercopithecine herpesvirus 1; B virus): Herpes B
- Herpesvirus tamarinus (Cebid herpesvirus 1; Herpes T): localized disease in
squirrel monkeys; generalized disease in marmosets, tamarins, owl monkeys
- Herpesvirus simplex, type 1: oral lesions in humans, apes, monkeys
- Herpesvirus simplex, type 2: genital lesions in humans, apes, monkeys
- Simian varicella: Simian varicella in macaques, African green monkeys,
Patas monkeys
- Herpesvirus papio 2: Oral and genital lesions in baboons

capsids are formed and filled with viral DNA. These nucleocapsids are then enveloped by the inner nuclear membrane and deenveloped by the outer nuclear membrane when transported into the cytoplasm.[1] Within the cytoplasm the capsids associate with an electron dense tegument and are enveloped by a second budding event in the trans-Golgi region. The mature virus particles are then released by exocytosis.[1]

A list of common veterinary alpha-herpesviral infections is included in table 2-1.[4]

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CASE III – W401/06 (AFIP 3034592).

Signalment: 10-12-month-old lambs (breed unknown), sex unknown, *Ovis aries*

History: Abattoir liver specimens derived from 260 lambs from New South Wales. Fifty percent of the livers were condemned at the abattoir. Lambs had no previous history of illness.

Gross Pathology: Liver had retained their shape, but were small, yellow and the capsular surface was markedly granular in appearance. On cut section, nodular regeneration was apparent throughout.

Histopathologic Description: The capsular surface of the liver was undulated. The normal acinar architecture of the liver was replaced by extensive nodular regeneration and segmental collapse and condensation. The portal triads showed increased mature biliary ductular profiles and there was a moderate mononuclear, primarily lymphocytic infiltration, which extended into the surrounding sinusoids. A moderate degree of fibrosis was present, radiating from the portal triads into the periphery of the regenerative nodules.

Periportal hepatocytes appeared large, and many appeared to be entrapped by collapsed stroma. The nuclei varied in sizes and shape, but were predominantly large and vesicular with dispersed chromatin. Other nuclear profiles included dark nuclei with smudged nuclear chromatin, fibrillary nuclear chromatin, and irregularly shaped nuclei.

There was a minimal degree of fatty change affecting hepatocytes within regenerative nodules. (Note: The degree of fatty change present may vary between submitted slides). The majority of nuclei within these nodules were unremarkable. Occasional apoptotic hepatocytes were scattered throughout the parenchyma.

Contributor's Morphologic Diagnoses: Chronic active hepatopathy with marked nodular regeneration, hepatic magalocytosis and karyomegaly, liver sheep.

Contributor's Comment: The histological changes present are indicative of a two-phase process. Previously there has been extensive loss of hepatocytes, resulting in condensation of parenchyma and liver shrinkage accompanied by nodular regeneration. Currently, residual periportal hepatocytes are undergoing degenerative changes with megalocytosis, karyomegaly and apoptosis.

Anecdotal history suggested that these lambs had been supplemented prior to slaughter with lupin grain. In addition, it is likely that these animals had been recently grazing lupin stubble.

Lupinosis is a sporadic disease reported primarily in Australia, New Zealand, South Africa and Europe.[4] It is a mycotoxic liver disease caused by infection of *Lupinus* spp with the fungus *Diaporthe toxica* (formerly *Phomopsis leptostromiformis*). In southern regions of Australia, *Lupinus* spp (primarily *L. cosentini*) are commonly used

as fodder, either as stubble or as grain.[4] The fungus produces the toxic agents phomopsin A and B with A being two to three times more toxic than B.[3] These toxins bind to tubulin and interfere with the ability of hepatocytes to form microtubules and therefore undergo mitosis. The result is hepatic atrophy and fibrosis. Hepatocytes typically swell and have large vesicular nuclei.

Lupinosis is typically a sub-acute to chronic disease, and can affect other species including cattle, donkeys, goats, horses and pigs.[1] Clinically sheep show non-specific neurological signs and frequently die from misadventure or from copper poisoning. Other organ systems can exhibit cytotoxic effects, including adrenal glands, pancreas, kidneys, rumen and skeletal and cardiac musculature.[1]

Lupinus spp themselves also contain quinolizidine alkaloids that can cause teratogenic abnormalities such as crooked calf disease (due to angyrine) and neurotoxic signs.[5]

Although the history and pathological changes present in this case are suggestive of lupinosis, other causes of toxic hepatophy including pyrrolizidine alkaloids can not be excluded.

AFIP Diagnosis: 1. Liver: Nodular regeneration, diffuse, with megalocytosis, biliary reduplication, and moderate portal bridging fibrosis, breed unspecified (*Ovis aries*), ovine.
2. Liver: Hepatitis, lymphocytic, subacute, multifocal, mild.

Conference Comment: Conference participants suggested a differential diagnosis of lupin toxicosis, pyrrolizzidine alkaloid toxicosis and aflatoxicosis as potential causes of the changes noted in the distributed slides.

Phomopsis leptostromiformis, a fungus that grows on lupine (*Lupinus* sp.) plants, produces a toxic metabolite, phomopsin. Affected livers exhibit multifocal necrosis and remaining hepatocytes undergoing mitotic arrest in metaphase, resulting in a marked increase in mitotic figures.[2] Chronic affected livers are smaller than normal as a result of necrosis, inability to regenerate due to mitotic inhibition, and progressive fibrosis.[5] Nodular regeneration may occur with sporadic ingestion of the toxin.[5]

Following ingestion, pyrrolizidine alkaloids are converted to pyrrole esters by hepatic cytochrome p450 enzymes, which react with cytosolic and nuclear proteins and nucleic acids to inhibit DNA synthesis and mitosis in hepatocytes.[5] Megalocytosis, a characteristic finding in pyrollizidine alkaloid toxicosis, occurs when some hepatocytes are able to replicate their DNA yet are unable to divide.[5]

Aflatoxins are also metabolized by the hepatic mixed-function oxidase system to toxic and non-toxic metabolites.[5] The most potent of these is the 8,9-epoxide metabolite of aflatoxin B1, which binds to adenine in nucleic acids, resulting in very similar microscopic findings to animals metabolizing pyrollizzidine alkaloids.[5]

Chronic inconsistent ingestion of any of these toxic principles can result in end-stage liver disease over time. The characteristic micro- and macronodular regeneration seen in end-stage livers can have numerous causes other than toxicity:[2]

1. Chronic toxicity (therapeutic agents or naturally occurring toxins)
2. Chronic cholangitis and/or obstruction
3. Chronic congestion (right side heart failure)
4. Inherited disorders of metal metabolism (copper or iron)
5. Chronic hepatitis
6. Idiopathic

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CASE IV - BB425/06 (AFIP 3032272).

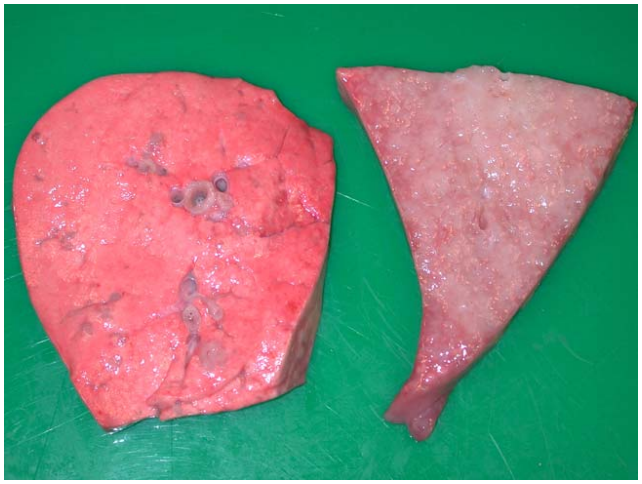
Signalment: Adult (age unknown), female, Greyface sheep (*Ovis aries*)

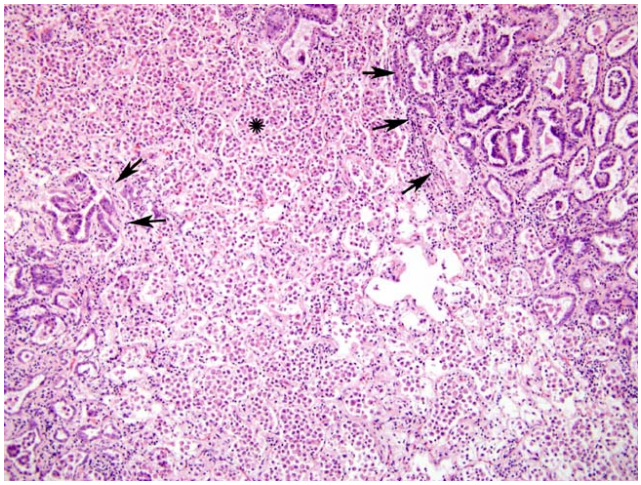
History: A mature Greyface ewe was culled due to prolonged respiratory distress. At necropsy, the only significant findings were in the lungs.

Gross Pathology: The lungs were almost diffusely much firmer and heavier than normal and failed to collapse. There were moderate to large amounts of frothy fluid in the tracheal and bronchial lumens. The cranioventral lung lobes and the caudal portions of the caudal lung lobes were expanded by fairly well demarcated, firm, ill-defined masses which were grey to pale purple. On cut section, the parenchyma in these areas was consolidated, firm and pale **grey (fig. 4-1)**. Both sections are from this sheep but the section on the left is relatively unaffected while the parenchyma in the section on the right is virtually replaced by slightly nodular, pale grey **homogeneous tissue (fig. 4-1)**. Affected areas also exuded frothy fluid, especially on cut section.

Histopathologic Description: Most of the normal lung parenchyma is replaced by multiple variably sized, nodular proliferations of well-differentiated cuboidal to low columnar **epithelial cells (fig. 4-2)**. The nodules are non-encapsulated, infiltrative and often coalesce with each other. The epithelial cells form tubuloacinar structures with occasional papillary projections, supported by a fibrovascular stroma. Some of the tubuloacinar structures contain pink, proteinaceous material. Individual cells have variably distinct cell borders with centrally to basally located hypochromatic nuclei, moderate amounts of faintly granular eosinophilic cytoplasm and indistinct nucleoli. Nuclear pleomorphism is mild and mitotic figures rare. Rare nodules are centrally necrotic or infiltrated by necrotic and viable neutrophils and cellular debris. In many areas, the surrounding alveolar spaces are flooded by very large numbers of alveolar and epithelioid macrophages, with fewer neutrophils, lymphocytes and plasma cells and occasional multinucleated giant cells. The macrophages are very plump with homogeneous, pink cytoplasm which sometimes causes peripheralization of the nucleus. Many bronchioles and bronchi are cuffed by large, discrete aggregates of large numbers of lymphocytes forming lymphoid follicles, many with distinct germinal centers. The bronchiolar and bronchial lining cells are variably hyperplastic or attenuated.

Contributor's Morphologic Diagnosis: 1. Ovine pulmonary adenocarcinoma (OPA)
2. Severe, diffuse, histiocytic interstitial pneumonia with marked BALT hyperplasia





4-1. Greyface sheep. On cut section, the right lung is variably consolidated, firm and pale in comparison to the lung on the left, also from the same animal. Photograph courtesy of the Veterinary Pathology Unit, Easter Bush Veterinary Centre, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, EH25 9RG, United Kingdom
4-2. Lung, Greyface sheep. Multifocally, markedly expanding and replacing the pulmonary architecture are nodular coalescing proliferations of epithelial cells which form variably sized tubules and acini (arrows). Adjacent less affected alveoli are expanded by high numbers of alveolar macrophages, lymphocytes, plasma cells and neutrophils (star). (H&E 100X)

Contributor's Comment: OPA has a number of synonyms, including sheep pulmonary adenomatosis and jaagsiekte (Afrikaans for "driving sickness"). It is a naturally occurring, transmissible disease characterized by the development of pulmonary neoplasia and caused by an exogenous betaretrovirus called jaagsiekte sheep retrovirus (JSRV).[6] First recognized in South Africa, it now occurs worldwide, with the exception of the Antipodes. The incidence in the UK has been recorded to be as high as 30% and it can result in mortality rates of up to 50% in affected flocks.[4]

In this case, the gross changes were typical of the classical form of the disease. An atypical form occurs (although has apparently not been reported in Scotland) whereby the nodules are more discrete, harder and much drier. Lesions generally only occur in the lungs although metastasis to lymph nodes can arise and is one of the main features helping to classify the lung lesions as truly neoplastic, rather than simply proliferative. Extrathoracic metastases have also been reported.[4] Electron microscopy has confirmed that the alveolar proliferations are composed of type II pneumocytes, while those arising in bronchioles are composed of Clara cells. Both cell types are secretory, accounting for the copious amounts of frothy fluid produced, which tends to flood the respiratory passages in the classical form. This excessive fluid accumulation is absent from the atypical form.[6] Microscopically, the neoplasm is classified as a bronchioloalveolar carcinoma. The histological findings in this case were typical and tend to be identical between the two forms.[4]

The lung tropism of the JSRV and resultant neoplastic transformation of pulmonary epithelial cells is apparently unique in the retrovirus group. The exact mechanism of neoplastic transformation is the subject of much current research. Two genes appear to be important to this tropism and viral infectivity, the env gene and the long terminal repeat (LTR) gene. The env gene permits viral entry of cells because it encodes the viral glycoprotein which allows interaction with cell receptors. Thus, the virus can only infect cells which specifically express its receptor, although pulmonary epithelial cells are not the only cells to do so.[4] The LTR gene is integrated into the cellular genome after viral entry and induces viral expression by interacting with cellular transcription factors. It can activate proto-oncogenes via insertional mutagenesis, whereby provirus is inserted near a proto-oncogene and drives its overexpression.

Of these two genes, the env gene is gaining favor as the more likely oncogenic agent since it has been shown to function as an oncogene, at least experimentally in mammalian fibroblast and epithelial cell lines. The mechanism of cell transformation is unclear although it is believed to involve the cytoplasmic tail of the envelope transmembrane protein as well as two downstream cell signaling pathways, H/N-Ras-MEK-MAPK and Akt-mTOR.[2] The insertional mutagenesis theory seems less likely since the random nature and the infrequency of insertion in the desired place within the genome would not be efficient enough for proto-oncogene activation.[4]

The sheep genome also contains 15-20 copies of endogenous retrovirus which is very similar to the exogenous JSRV. The main difference lies in the LTR region of the genome such that the endogenous form of the virus does not have the same transcriptional efficiency in pulmonary epithelial cells as the exogenous, tumor-inducing form. The existence of the endogenous virus may explain the lack of an antibody response in OPA infected sheep, since the endogenous elements may promote immunotolerance during fetal development.[4]

There was marked BALT hyperplasia in this case, for which there could have been two main reasons. Firstly, it can occur in the atypical form of OPA. We felt this was less likely since the concomitant fibrosis and marked lymphoplasmacytic inflammation usually seen in the atypical form were not present.[6] Secondly, the possibility of concurrent maedi was considered since combined infections have been frequently recognized; no further testing was performed to confirm or refute this possibility.[5] There was also quite severe histiocytic inflammation. The widespread and marked infiltrate of plump macrophages is commonly found around neoplastic nodules; they are believed to be induced by the excessive surfactant protein production but their exact role in the pathogenesis is still unclear. Recent work suggests they reflect a cellular immune response to the presence of neoplastic cells. The apparent ineffectiveness of this response is believed to be due to putative immunosuppressive properties of the excess surfactant protein.[7]

AFIP Diagnosis: 1. Lung: Carcinoma, Greyface sheep (*Ovis aries*), ovine.
2. Lung: Lymphofollicular hyperplasia, diffuse, moderate.
3. Lung: Pneumonia, interstitial, multifocal, mild.

Conference Comment: The contributor gives an excellent overview of retroviral-induced ovine pulmonary adenocarcinoma (OPA). This section also exhibits the typical histologic finding of abundant macrophages located at the periphery of the neoplasm in OPAs which are presumably attracted by the abundant surfactant secreted by the neoplasm. We agree with the contributor that

there is likely at least one other disease process occurring in addition to OPA in the distributed section; ovine lentivirus pneumonia and a concomitant bacterial superinfection were also discussed in conference.

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