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Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
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**CONFERENCE 25
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The Armed Forces Institute of Pathology
Washington, DC 20306-6000

CASE I – 01-3983 (AFIP 2787168)

Signalment: Ten-year-old, spayed female, Tibetan Terrier

History: The dog was gagging and coughing several weeks prior to a scheduled vaccination appointment. At the appointment, thoracic radiographs revealed an interstitial pneumonia, and the dog was started on antibiotics. Several weeks later she re-presented to a veterinarian and was lethargic, sore and irritable with subcutaneous hemorrhages in distal limbs. At that time, the PCV was 30%, platelet count 7000, and there was a bloody discharge from the right nostril. The attending veterinarian initiated dexamethasone therapy. Two days later the PCV was 19%, platelet count was 72,000, WBC was 42,000, PT > 100 (N: 6-12), and PTT > 100 (N: 10-25). The dog went into respiratory distress that afternoon and died.

Gross Pathology: The dog was in excellent body condition. The lungs were diffusely consolidated and firm, with red and tan mottling.

Laboratory Results: Post-mortem culture of tissue samples of lung were negative for bacteria.

Contributor's Morphologic Diagnosis: Lung: metastatic adenocarcinoma, Tibetan Terrier, canine.

Contributor's Comment: Multifocally filling alveolar capillaries, peribronchial vessels and lymphatics are neoplastic emboli consisting of ribbons, papillary structures and occasional acini. The structures are typically 1-2 cells wide and are not adherent to the endothelial surfaces. There is minimal to mild distention of alveolar capillaries and moderate distention of peribronchial vessels and lymphatics by neoplastic cells. The neoplastic cells are polygonal with well-defined cell

borders, moderate amounts of eosinophilic cytoplasm, and high nuclear to cytoplasmic ratio. Nuclei are large (10-15 um), ovoid, with marginated basophilic chromatin and 1-2 prominent magenta nucleoli. There is moderate anisokaryosis, and mitotic figures are 3-6 per hpf (40X). The cells are uniformly oriented and some appear to be ciliated. Few larger neoplastic foci within peribronchial vessels contain wispy basophilic mucinous material. There is mild multifocal hemorrhage and edema within alveolar spaces.

Of all the tissues examined, the lungs were most severely affected, with scattered intravascular neoplastic foci also observed in the brain, kidney, bladder, and liver. No extra-vascular neoplastic foci were observed and no primary neoplastic focus was found at necropsy. While the exact source of the neoplasm was not determined, common metastatic canine adenocarcinomas include mammary, hepatocellular, apocrine gland of the anal sac, and less commonly thyroid and pancreas. Immunohistochemical staining performed at the Connecticut Veterinary Diagnostic Laboratory demonstrated that the tumor cells were strongly positive for cytokeratin and negative for vimentin. Further characterization of the tumor origin may be possible by ultrastructural analysis.

We speculate that the worsening anemia was due to microangiopathic hemolysis from trauma as erythrocytes traveled through the vessels of the lungs and other tissues. As the erythrocytes travel through these tissues they may be either overtly hemolyzed or damaged, becoming poikilocytes that are latter removed by splenic macrophages. Mechanisms associated with thrombocytopenia and neoplasia include decreased platelet production, increased consumption as in disseminated intravascular coagulopathy (DIC), immune-mediated platelet destruction, or a reduction in hematopoietic growth factors. Prolongation of clotting times (PT, PTT) and the responsive of the thrombocytopenia to steroids are most consistent with immune-mediated platelet destruction and DIC in this case.

AFIP Diagnosis: Lung: Adenocarcinoma, metastatic, Tibetan Terrier, canine.

Conference Comment: Like the contributor, conference participants favored the interpretation of metastatic adenocarcinoma over primary pulmonary adenocarcinoma. The same interpretation was favored by the Department of Pulmonary and Mediastinal Pathology at the AFIP. In humans, immunohistochemistry using cytokeratin 7, expressed in glandular and transitional epithelium; cytokeratin 20, expressed in gastric and intestinal epithelium, urothelium and Merkel cells; thyroid transcription factor-1, expressed in pulmonary and thyroid epithelial cells; and thyroglobulin are often used in conjunction when attempting to determine the site of origin of pulmonary neoplasms.

Recent studies have described similar tissue specific distributions of cytokeratins 7 and 20 in normal feline and canine tissues. The expression of each

are by and large preserved when the tissues undergo malignant transformation and may provide important information when attempting to determine the site of origin.

In some slides, pulmonary arterial walls are variably thickened. Pathologists from the Department of Pulmonary and Mediastinal Pathology commented that this change resembles hypertensive arteriopathy as seen in humans and could be related to the intravascular carcinoma.

Contributor: University of Connecticut, Department of Pathobiology and Veterinary Science, Storrs, CT

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CASE II – 11853/00 (AFIP 2790603)

Signalment: Ten-week-old, male, Landrace, pig (*Sus scrofa domestica*)

History: This pig had been experimentally infected with porcine circovirus type 2 (PCV2) at 5 weeks of age. Five weeks later it developed red to black macules and papules on the perineal region, lower hind-limbs trunk and ears, became lethargic and was euthanized.

Gross Pathology: There were multiple red to black macules and papules on the perineal region, lower hindlimbs, trunk and ears. The internal and external iliac,

mesenteric, prescapular, parotid and renal lymph nodes were enlarged, oedematous and hemorrhagic. The kidneys were enlarged with multiple cortical petechiae. Pelvic and perirenal oedema was seen. Large hemorrhagic foci were present in the testes.

Laboratory Results: None.

Contributor's Morphologic Diagnoses: 1. Kidney: glomerulonephritis, global, exudative, severe, with multifocal interstitial plasmacytic and histiocytic infiltrates. 2. Kidney: vasculitis, necrotizing and leukocytoclastic, severe, pig, porcine dermatitis and nephropathy syndrome.

Contributor's Comment: There is a severe generalized exudative glomerulonephritis. Bowman's spaces are filled with serum, fibrin and occasional erythrocytes. Many glomeruli are necrotic and infiltrated by neutrophils. The lumina of many tubules contain hyaline casts and tubular epithelial cells frequently contain large numbers of hyaline droplets. Some tubular epithelial cells are necrotic. Mild multifocal interstitial plasmacytic to granulomatous nephritis is present. There is a severe necrotizing, leukocytoclastic vasculitis involving vessels at all levels of the kidney. Fibrinoid necrosis and perivascular infiltrates of neutrophils and macrophages are present in affected vessels.

Porcine dermatitis and nephropathy syndrome (PDNS) was first described in the UK in 1973 and has subsequently been reported from several other countries including Canada, Spain, Chile, Italy, The Netherlands, South Africa and the USA. Necrotizing and hemorrhagic skin lesions over the perineum and hindlimbs, and enlarged pale kidneys with cortical petechiae are characteristic gross lesions. Microscopic lesions comprise systemic necrotizing vasculitis, glomerulonephritis and frequently contain hyaline casts.

etiopathogenesis of PDNS is unknown, recent studies using in-situ hybridization, virus isolation, immunohistochemistry, and PCR suggest their involvement. Similarities in lesions observed in PDNS and those observed in postweaning multisystemic wasting syndrome (e.g. lymphoid depletion, granulomatous inflammation of lymphoid tissue, interstitial pneumonia, and syncytial cells) and PRRS (e.g. interstitial pneumonia, lymphadenopathy, and perivascular mononuclear cuffing) further suggest involvement of these two viruses. It is proposed that additional, as yet unknown, predisposing factors may play a significant role in the onset of this disease.

The same studies have failed to demonstrate virus or viral antigen in endothelial cells. This further supports immune complex deposition with subsequent humoral or cell mediated injury as the cause of the vascular lesion.

Contributor: Veterinary Sciences Division, Stormont, Belfast BT4 3SD, Northern Ireland

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CASE III – 300/01 (AFIP 2790950)

Signalment: 3-year-old, female, gray seal (*Halichoerus grypus*)

History: The animal was one of 10 gray seals of a private zoo. All animals were bred in captivity and housed together in freshwater pens. Seals were fed with

fresh fish of different species twice a day. In a two-week period, two adult females died post partum due to a *Streptococcus dysgalactiae* septicaemia. This seal and another three week-old juvenile male showed severe central nervous signs of two days duration. Animals were euthanized humanely, due to poor prognosis.

Gross Pathology: The distal nasal conchae were covered with numerous adults and nymphs of mites of two to four millimeters length. The nasal mucosa, mainly the caudo-medial conchae, displayed moderate amounts of adhering mucinous whitish to reddish material, and multifocal hemorrhages. The lungs showed an acute moderate diffuse alveolar edema and emphysema.

Laboratory Results: Virologic and microbiologic investigation of brain, lungs, kidney, liver, spleen, intestine revealed no specific pathogens. The toxicological investigation of the blood for about 2600 known toxins by HPLC gave negative results.

Parasitologically, adults and nymphs of the mite species were determined as *Halarachne halichoeri*.

Contributor's Morphologic Diagnosis: Nasal cavity: Rhinitis, granulomatous, chronic, diffuse, moderate to severe, with multifocal erosions and ulcerations, hemorrhages and numerous acarids in different developmental stages on the conchal mucosal epithelium.

Etiology: Parasitic mites of the species *Halarachne halichoeri*.

Contributor's Comment: Histological examination of the nasal conchae reveals multifocal arthropods, two to four mm in length, in different developmental stages on the surface of the conchal epithelium. Focally, gravid arthropod females (in few slides) are apparent. The mucosal epithelium is multifocally covered with moderate amounts of mucous, containing cellular debris, neutrophils and erythrocytes. In locations of parasitic attachment, multifocal epithelial erosions or ulcerations (not on all sections) are seen. The subepithelial connective tissue reveals a multifocal, moderate to severe, coalescing infiltration with macrophages, lymphocytes and plasma cells as well as a minimal subepithelial infiltration with neutrophils and eosinophils and a moderate subepithelial edema. The mucosal epithelium shows hyperplasia, and a moderate diffuse intraepithelial infiltration with neutrophils. Focally, bone tissue shows irregular reversal lines, suggestive of an increased moderate remodelling of conchal bone tissue.

In the past, infection with nasal mites has been reported in different species of wildlife and captive seals. Endoparasitic mites of pinnipeds, e.g. seals, walruses and sea lions, belong to the family Halarachnidae, with the viviparous genera *Halarachne* and *Orthohalarachne*. Adult mites of this family of acarids occur on the

surface of mucous membranes of the oropharynx, bronchi and lungs. Larval stages are habitants in the oropharynx. The parasites are feeding on the mucosal epithelia.

The acarid life cycle includes three stages. The first, free, hexapodid larvae, a second octopodid proto-nymph and a third deuto-nymph larval stage, followed by the development into the adult stage. Distribution of the parasitic acarids and infection of the host is restricted to the hexapodid larval stage, according to the high mobility and resistance to exogenous factors of this larval stage. Proto- and deuto-nymphs have a short larval stage span, they do not feed and have a low activity level. These factors are interpreted as possible adaptations to the parasitic life circle of these species.

The expired CO₂ of the upper respiratory tract of the final host seems to be the attractant for the hexapodid larvae. Direct naso-nasal contact and sneezing are the possible ways of infection. Numbers of larvae are much higher than numbers of adults (up to 99% of the total mite population, depending on the mite species).

The occurrence of parasites of the genus *Halarachne* is limited to the oro-nasal cavity. Different species of the genus *Orthohalarachne* may occur together in one host, occupying different microhabitats. Adults and larvae of *Orthohalarachne attenuata* and larvae of *Orthohalarachne diminuta* reside in the oro-pharyngeal epithelia, and adults of *Orthohalarachne diminuta* occur in the bronchi and lungs. The impact of parasitic infection is variable, depending on the intensity of acaridal infection. Alterations range from moderate chronic non-purulent rhinitis to severe chronic purulent inflammation of the conchal mucosa. Epithelial desquamation, focal conchal hemorrhages and edema as well as intra-pulmonary haemorrhages, leading to an impairment of respiration followed by lesions in the lungs and secondary alveolar emphysema may occur. Intensive infection predisposes the host to more serious diseases, or even kills the host. A transmission from animal to humans with following ocular discharge has been reported so far for one case.

AFIP Diagnosis: Nasal turbinate: Rhinitis, lymphoplasmacytic, exudative and proliferative, diffuse, moderate, with many acarids, gray seal (*Halichoerus grypus*), pinniped.

Conference Comment: The contributor has provided a complete review of this entity.

Contributor: Institut für Veterinar-Pathologie, Giessen, Germany

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CASE IV – 4-75-01 (AFIP 2808175)

Signalment: Aborted fetus, ovine

History: Abortions in this flock began 4-5 weeks prior to anticipated lambing. Yearling ewes were most affected, with an abortion rate of about ten percent. Abortions continued into lambing with stillborn and perinatal mortalities. The flock was vaccinated for "vibrio" in the fall at breeding. Six weeks before scheduled lambing the flock was given tetracycline pellets.

Gross Pathology: None submitted.

Laboratory Results: Cultures of abomasal fluid and liver reveal *Campylobacter fetus* subsp. *fetus* (*intestinalis*). Cultures were negative for Brucella and smears were negative for Chlamydia.

Contributor's Morphologic Diagnoses: 1. Liver: Hepatitis, necrotizing and suppurative, acute, multifocal and coalescing, marked.
2. Placenta: Placentitis, acute, multifocal and coalescing, moderate.

Contributor's Comment: Both *Campylobacter fetus* and *Campylobacter jejuni* are causes of abortion storms in ewes. Up to 70% of susceptible ewes may abort. Ovine campylobacteriosis is characterized by late-term abortions as well as

stillbirths and weak lambs with high mortality shortly following birth. Infertility is not a feature, unlike *C. fetus* subsp. *venerealis* infection in cattle. Transmission is via ingestion; *C. fetus* is an intestinal inhabitant. Abortion occurs in the last six weeks of gestation. Placental lesions are nonspecific, and consist of multifocal necrosis at the periphery of cotyledons as well as complete cotyledonary necrosis. The chorioallantois is hyperemic and edematous. Fetal changes may include ascites and pleural effusion, subcutaneous edema, and a characteristic umbilicated hepatic necrosis ranging in size from 0.5 to 2.0 cm diameter.

Diagnosis is made on the basis of darkfield examination of wet mount preparations from fresh tissue-abomasal fluid, liver, or placenta. Campylobacter organisms have a curved or "gullwing" appearance and a characteristic darting movement. Microaerophilic culture (10% CO₂) on antibiotic media and visualization of small gram-negative curved coccobacilli confirms the diagnosis. The fluorescent antibody test is another means of identification.

AFIP Diagnoses: 1. Liver: Hepatitis, necrotizing, suppurative, random, multifocal, moderate, breed not specified, ovine.
2. Chorioallantois: Placentitis, lymphoplasmacytic and neutrophilic, diffuse, mild.

Conference Comment: The differential diagnosis discussed in conference for late term abortions and stillbirths in ewes included both bacterial and protozoal etiologies. *Brucella ovis* (gram-negative coccobacillus) infection is characterized by vasculitis of chorionic vessels, intercotyledonary edema, and variable cotyledonary necrosis. A brown exudate is often present. *Chlamydophila abortus*, a gram-negative coccobacillus, is present as an infectious non-proliferative stage (elementary body) and a non-infectious proliferative stage (reticulate body). The placental lesion is similar to that caused by *B. ovis* with vasculitis, irregular areas of intercotyledonary edema, and generally more severe inflammation and necrosis of cotyledons. Organisms are best-visualized distending chorionic cells using Ziehl-Neelsen or Gimenez stains. In contrast, *Coxiella burnettii* (gram-negative) infection results in more prominent intercotyledonary inflammation and thickening. The inflammation is suppurative and histologically can be observed extending into cotyledons. Vasculitis is not usually a prominent feature. Like *C. abortus*, organisms distend the cytoplasm of hypertrophic chorionic epithelial cells. Gross lesions secondary to *Toxoplasma gondii* infection are characterized by intercotyledonary edema with miliary foci of inflammation, necrosis, and mineralization of the cotyledons. Histologically, chorionic epithelial cells are hyperplastic and hypertrophic with rare intraepithelial organisms.

Contributor: Montana Veterinary Diagnostic Laboratory, Bozeman, MT

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