The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
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CONFERENCE 4
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Conference Moderator: Dr. Yvonne Schulman
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Armed Forces Institute of Pathology
Washington, DC 20306-6000

CASE I – 219176 (AFIP 2788705)

Signalment: Adult, female, Sulfur Crested Cockatoo (*Cacatua* sp.)

History: The bird presented with a three month history of progressive ataxia with intention tremor. After initial diagnostic procedures were performed the bird was euthanatized.

Gross Pathology: There was mild variation in thickness of cerebellar folia with multifocal loss of definition between adjacent folia. All other necropsy findings were within normal limits.

Laboratory Results: Complete blood count revealed mild leukocytosis (13,850/mm^3; normal 5,000-10,000). Blood chemistry profile revealed elevated creatine kinase (517 U/L; normal 50-300) and decreased albumin: globulin ratio (0.6; normal 1.5-4.5). Protein electrophoresis was within normal limits. *Chlamydophila psittaci* titers were negative.

Contributor's Morphological Diagnosis: Cerebellar primitive neuroectodermal tumor

Contributor's Comment: Cerebellar folia are variably expanded by a non-uniform infiltrate of large, angular to plump spindloid mononuclear cells. In highly cellular regions, infiltrative cells are arranged in dense sheets, with scattered foci of necrosis and frequent vacuolated macrophages. In less cellular areas, cells are arranged in perivascular cords and palisades that fill Virchow-Robins spaces. Few rosettes surround pools of bright eosinophilic, proteinaceous material. Rare pseudorosettes are present. Neoplastic cells often obscure both cerebellar white matter and cortex (most notably the granular and Purkinje cell layers), and occasionally expand the meningeal surface. Cells contain irregularly indented, roughly oval, heterochromatic nuclei, occasional indistinct nucleoli and small

amounts of indistinctly vacuolated, pale eosinophilic cytoplasm with indistinct cell borders. There is moderate anisocytosis and anisokaryosis, and 1-3 mitotic forms per high power field. In some sections, narrow cords of neoplastic cells infiltrate the caudal cerebral cortex and white matter, midbrain, base of the optic lobe, and the brainstem. Infiltrative cords are centered on small central blood vessels and occasional rosettes and pseudorosettes are present.

Immunohistochemical staining performed at the AFIP revealed diffuse positive staining for synaptophysin. Neoplastic cells were negative for glial fibrillary acidic protein, neurofilament protein and neuron-specific enolase. Results are consistent with primitive neuroectodermal tumor.

Primitive neuroectodermal tumors (PNET's) of cerebellar origin (medulloblastomas, or cerebellar neuroblastomas) are believed to arise from the external germinal layer of the cerebellum. There is ongoing controversy as to the exact histogenesis of these tumors and their relation to other PNET's such as neuroblastoma has not been definitively determined. Medulloblastomas may exhibit variable differentiation, including neuronal, ependymal, glial and possibly mesenchymal forms.

Medulloblastomas are the most common intracranial neuroblastic neoplasms of humans. Most are diagnosed prior to the age of 20 years, although few become clinically apparent in adulthood or late in life. Medulloblastomas are typically considered highly malignant and commonly present as solid masses with permeative perimeters. Neoplastic cells often exit the cerebellar parenchyma, cover the pial surface and then re-enter the neuropil along Virchow-Robins spaces (similar infiltrative behavior is seen in the submitted case). Dissemination via the CSF is common. Clinical signs may arise secondary to direct interference with neural pathways (usually cerebellar) and occasionally are attributed to hydrocephalus secondary to obstruction of CSF flow.

Canine medulloblastomas routinely express vimentin, while expression of other immunohistochemical markers (including synaptophysin, neurofilament proteins and glial fibrillary acidic protein) is variable. The intermediate filament protein nestin is consistently positive in human tumors but appears to lack cross-species reactivity.

Development of medulloblastoma in humans has been linked in various studies to mutations in the transcription and growth-promoting beta-catenin genes and apoptosis-promoting Bax gene, and to reduced or altered expression of the cell-growth regulatory membrane protein Patched 1 (Ptc1). The molecular basis of tumorigenesis in animals is not presently known.

Medulloblastomas are occasionally reported in dogs and cattle, and are rarely seen in pigs and cats. Primary central nervous system tumors are rare in birds, although a wide variety have been reported, including astrocytoma, high-grade astrocytoma (glioblastoma), oligodendroglioma, choroid plexus papilloma, meningioma and neuroblastoma/ganglioneuroma.

AFIP Diagnoses: Cerebellum: Medulloblastoma (cerebellar primitive neuroectodermal tumor), Sulfur Crested Cockatoo, avian.

Conference Comment: The contributor has provided a concise review of medulloblastomas (cerebellar primitive neuroectodermal tumors). Conference participants included high-grade astrocytoma and medulloblastoma in their differential diagnosis. Embryonal features (small blue cells with scant cytoplasm), the arrangement of neoplastic cells in patternless sheets, rosettes and palisades, extension along the pia, and expansion of Virchow-Robins space are discriminating features. The rosettes and synaptophysin positivity indicate neuronal differentiation.

Contributor: Department of Pathology, Angell Memorial Animal Hospital, Boston, MA 02130

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CASE II – PA 3809 (AFIP 2787959)

Signalment: 4-month-old, female, beef-cross calf (*Bos taurus*), bovine

History: This calf was received for research use. Skin lesions developed approximately one week after arrival.

Gross Pathology: Multifocal and coalescing, 4-6 cm, circular, dry, crusty & scaly areas of alopecia were present on the face and muzzle.

Laboratory Results: A pure culture of *Trichophyton mentagrophytes* was grown from one of the cutaneous lesions

Contributor's Morphologic Diagnosis: 1. Acanthosis, diffuse, moderate
2. Orthokeratotic hyperkeratosis, diffuse, mild-moderate
3. Follicular keratosis, multifocal, mild-moderate
4. Folliculitis and perifolliculitis, subacute, patchy, mild-moderate, with focal areas of follicular expansion and early pustule formation
5. Multifocal, faintly visible (H&E), mildly refractile structures consistent with mats of fungal hyphae infiltrating hair shafts

Overall Diagnostic Impression: Gross & microscopic findings consistent with classical bovine dermatomycosis

Contributor's Comment: Both gross and microscopic findings are consistent with classical bovine dermatomycosis, often referred to as ringworm. Organisms were much more distinctly visible with a periodic acid Schiff (PAS) stain. The diagnosis was also confirmed via culture results (*Trichophyton mentagrophytes*).

Although cattle of any age may be affected, significant lesions generally develop only in younger calves. Seasonality is commonly noted, with infections being more common in winter and spring. Proximity of confinement and possibly nutrition seem to be more important in the spread of the disease than environmental factors such as temperature and sunlight.

Lesions of dermatomycosis generally begin to appear 1 - 2 weeks after exposure, and by 24 days are well established. The lesions typically expand in size for 4 - 8 weeks before beginning to resolve as animals develop an immune response. As dermatophytes are strict aerobes, fungi die out under the crust in the center of most lesions, leaving only the periphery active. It is this mode of growth that produces the centrifugal progression and the characteristic ring form of the lesion. Dissemination occurs by animals rubbing against contaminated objects such as posts, fences and feed holders. Although causative fungi rarely grow saprophytically, the spores remain viable in the environment for long periods and are relatively resistant to chemical disinfectants. Grooming commonly spreads infection to new areas of the body. Spores can also exist on the skin of some

cattle without causing lesions, and "carrier" animals of this type may act as important sources of infection.

The clinical course is generally uneventful, although infected areas around the eyes or mouth may affect the calves' ability to forage and slow their rate of growth. Secondary bacterial infection of active lesions may occur but is uncommon. Infected animals do pose a threat of transmission to other cattle and humans.

Trichophyton verrucosum is the most common dermatophyte seen in cattle in the U.S., although *Trichophyton mentagrophytes* can cause occasional infection, as in this case.

A successful vaccine (Ringvac bovis LTF-130, Alpharma, Oslo Norway) has been developed, which elicits a protective response from the cellular branch of the immune system.

AFIP Diagnoses: Haired skin: Folliculitis and dermatitis, eosinophilic, lymphoplasmacytic and histiocytic, chronic, focally extensive, moderate, with hyperkeratosis, and many endothrix hyphae, beef-cross calf (*Bos taurus*), bovine.

Conference Comment: Dermatophytes usually infect superficial keratinized structures (ie. skin, hair, feathers, hooves, claws) and are classified into one of three genera: *Microsporum*, *Trichophyton*, and *Epidermophyton*. Infectious conidia typically gain entry via a defect in the stratum corneum. Germination results in the development of mycelium with colonization of hyphae along the outer root sheath and invasion into hair shafts. Invasion is assisted by release of hyphal keratinase, collagenase, and elastase.

The endothrix hyphae, associated inflammation, and characteristic gross lesions (accompanying 2x2) led conference participants to consider *Trichophyton* sp. as the primary differential. *T. verrucosum* was considered most likely based upon reported incidence. In this case, culture of the organism was necessary in arriving at the diagnosis of the less commonly reported *T. mentagrophytes*.

Contributor: University of Pittsburgh, Division of Laboratory Animal Resources, Pittsburgh, PA 15261

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CASE III – 99.1554 (AFIP 2791477)

Signalment: 8-week-old, female, pigeon, avian

History: Seven young pigeons (6 to 8 weeks of age) from a loft producing 15,000 breeders per year, were submitted for necropsy to the Department of Pathology of the National Veterinary School in Nantes. The reported clinical signs, which occurred 3 weeks post-fledging, were not specific, and included diarrhea, lethargy, dehydration, rapid weight loss and death within 2 to 4 days after the first signs. Mortality rates varied from 5% to 40% among flocks in the loft (80 animals per flock for a total of 10 flocks). Both males and females were affected, but squabs and mature pigeons remained healthy.

Gross Pathology: No gross lesions in the submitted bird. Four of the seven birds had macroscopic lesions, ie. diffuse acute necrotizing bursitis (4/7), multifocal acute necrotizing enteritis (4/7), multifocal caseous bronchopneumonia (2/7) and multifocal necrotizing hepatitis and splenitis (1/7).

Laboratory Results: None

Contributor's Morphologic Diagnoses: 1. Bursa of Fabricius, mild lymphoid depletion, with multifocal moderate lymphocytic pyknosis and presence of numerous cytoplasmic "botryoid" inclusion bodies in follicular cells (epithelial cells and macrophages).
2. Thymus, mild cortical thymocytic depletion, with presence of rare sparse cytoplasmic "botryoid" inclusion bodies in epithelial cells and macrophages.

Etiologic diagnosis: Pigeon circovirus infection

Contributor's Comment: Pigeon circovirus (PiCV) infection is an emerging disease recognized for the first time in the United States in 1990. A subsequent retrospective study demonstrated circovirus by transmission electron microscopy in tissues from pigeons as early as 1986 in Canada and 1989 in Australia. PiCV was later reported in several European countries. Three other circoviruses are known to be causal agents of spontaneous animal diseases: chicken anemia virus (CAV)

infection in fowl, psittacine beak and feather disease (PBFD) virus in psittacine birds, and porcine circovirus (PCV2) which causes postweaning multisystemic wasting syndrome in pigs. Circoviruses are tiny non-enveloped icosahedral viruses (15 to 24 nm in diameter) with a single-stranded circular DNA of 1.76 to 2.31 kilobases.

Specific diagnosis of PiCV is based on histopathological findings. Typical basophilic inclusion bodies (5 to 25 um in diameter) are found in the cytoplasm of mononuclear phagocytic cells, exclusively in primary and secondary lymphoid organs. In affected animals, the bursa of Fabricius invariably contained follicular cells (epithelial cells and/or macrophages) with inclusion bodies. In the present sample, Feulgen staining confirmed the DNA content of inclusion bodies (magenta staining) and improved their visualisation in histological sections. Ultrastructural examination showed that inclusion bodies were composed of small icosahedral viral particles (13 to 18 nm in diameter), which were densely packed and regularly arranged in non-membrane-bound electron-dense paracrystalline arrays.

AFIP Diagnosis: 1. Bursa of Fabricius: Lymphocytolysis, diffuse, moderate, with many botryoid intracytoplasmic inclusions, etiology consistent with pigeon circovirus, pigeon, avian.
2. Thymus: Botryoid intracytoplasmic inclusions, few.

Conference Comment: Pigeon circovirus, like other reported avian circoviruses, affects young birds and has a tropism for lymphoid cells resulting in lymphocytolysis. Within bursal lobules there is variable loss of distinction between the lymphoepithelialis and lymphoreticularis. Clinical disease, secondary to immunosuppression, is a result of subsequent bacterial, viral, fungal, or parasitic infections.

Labeling of DNA fragments with the TUNEL assay suggests apoptosis has a role in the pathogenesis. It is unclear if apoptosis is the direct result of circoviral infection or some other process unrelated to viral replication. Apoptosis has been suggested to be of major importance in Marek's disease, infectious bursal disease, Newcastle's disease, and chicken anemia virus disease.

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CASE IV – 99-003586 (AFIP 2679933)

Signalment: 9-year-old, female, Labrador retriever, canine

History: One week history of anorexia & lethargy progressing to dyspnea, stiff gait, pyrexia and death.

Gross Pathology: Lungs were diffusely dark red, wet & heavy. Terminal bronchi contained yellow fluid with small clumps of white-tan material. The left ventricular free wall and interventricular septum of the heart were slightly thickened and there was an indentation at the apex. The stomach was empty. The small intestine contained a small amount of tan pasty material & the colon was empty. The gall bladder was mildly distended with bile. The urinary bladder was empty.

Laboratory Results: Serum chemistry parameters that were markedly elevated were amylase, urea nitrogen, phosphorus and creatinine. There was strong staining of glomerular capillary loops for *Borrelia burgdorferi* by immunohistochemical methods.

Contributor's Morphologic Diagnosis: 1. Chronic membranous glomerulopathy with capsular fibrosis, global, diffuse, severe.
2. Pyelonephritis, moderate, multifocal.

Contributor's Comment: The cause of death in this case was renal failure. Two major changes were seen in kidneys. Membranous glomerulopathy is most commonly caused by deposition of immune complexes in the glomerular capillary basement membranes. *Borrelia burgdorferi* (Lyme disease agent) is one possible cause among numerous others. The capsular fibrosis (determined by blue staining with Masson's trichrome stain) indicated that this condition had been going on for some time. The pyelonephritis in this case may or may not have been related to the

glomerulonephritis. Whether this reaction was centered on blood vessels or tubules could not be determined from the sections examined. Most other lesions could be related to uremia and its effects (e.g., myocardial changes), although in this case the myocardial lesions could also have been due to *B. burgdorferi* infection.

AFIP Diagnosis: 1. Kidney: Glomerulonephritis, membranoproliferative, global, diffuse, severe, with multifocal plasmacytic and lymphocytic interstitial nephritis, scattered tubular necrosis, and fibrin thrombi, Labrador retriever, canine.
2. Kidney, inner medulla: Nephritis, interstitial, neutrophilic, acute, multifocal, moderate

Conference Comment: *Borrelia burgdorferi* is a Gram-negative spirochete, vectored by ticks, and responsible for Lyme disease. Lesions associated with Lyme disease in dogs include polyarthritis, lymphadenopathy, myocarditis, and renal disease. The renal lesion is invariably fatal and considered histologically distinctive.

Histologically, acute tubular necrosis, interstitial lymphoplasmacytic inflammation, glomerulonephritis, and a lack of histologic features associated with end-stage kidneys (significant tubular atrophy or interstitial fibrosis) characterize Lyme nephritis. The glomerulonephritis is most commonly described as membranoproliferative; although, membranous has also been reported.

In this case, along with thickened glomerular capillary walls, conference participants identified an increase in glomerular cellularity consistent with membranoproliferative glomerulonephritis. Conference participants also commented on the lack of striking tubular changes that are generally characteristic of Lyme nephritis and the presence of a concomitant inner medullary interstitial nephritis. Because of these inconsistencies with the reported literature, most preferred the general interpretation of immune-mediated membranoproliferative glomerulonephritis rather than the more specific diagnosis of Lyme nephritis.

This case was reviewed in consultation with Dr. Sharda Sabnis, Division of Nephropathology, Department of Genitourinary Pathology, AFIP. In her review, Dr. Sabnis concurred with the interpretation of membranoproliferative glomerulonephritis. Further, she noted deposits within glomerular capillary lumina that stained deep magenta with Masson's trichrome and strongly for IgM immunoglobulins by immunohistochemistry. Dr. Sabnis related that the distribution and staining characteristics of these deposits are highly suggestive of cryoglobulin. In addition, fibrin deposits were demonstrated with the PTAH stain.

Contributor: University of Minnesota, Department of Veterinary Diagnostic Medicine, St. Paul, MN 55108

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