The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
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CONFERENCE 8
6 November 2002

Conference Moderator: Dr. Linda Johnson, Diplomate, ACVP
Weston, CT 06883

CASE I – Case 2 (AFIP 2839936)

Signalment: 2-year-old male Wistar rat

History: This is a control rat on a 2-year study and it was sacrificed moribund at the end of the study. Clinical observations included bilateral foot sores, lacrimation, labored respiration and decreased motor activity.

Gross Pathology: Enlargement of multiple lymph nodes, enlarged pale spleen and liver, pale thyroid, red pituitary mass, bilateral hind limb foot sores, bilateral enlarged adrenals with red discoloration.

Laboratory Results: None.

Contributor's Morphologic Diagnoses: 1. Malignant lymphoma, liver, spleen and mesenteric lymph node
2. Hemangiosarcoma, mesenteric lymph node

Contributor's Comment: This male rat, a control animal, was sacrificed moribund during the last week (on day 735) of a 2-year carcinogenicity study. Malignant lymphoma was present in multiple organs, including, liver, lung, kidney, bone marrow, thymus, multiple lymph nodes, spleen, heart, adrenal and pancreas. The mesenteric lymph node also contained a hemangiosarcoma consisting of numerous irregular vascular spaces, lined by pleomorphic endothelial cells and separated by collagenous stroma. In some sections, the mesenteric node is completely replaced by the hemangiosarcoma, while in others it is a large area expanding within the node.

Bacterial colonies were present in some sections, e.g. some slides of spleen and mesenteric node. This is thought to be due to bacteremia resulting from the hindlimb footsores. Although no hematological evaluations were present to support this, production of neutrophils and other phagocytic cells was probably reduced because the bone marrow and spleen were infiltrated by malignant lymphoma.

AFIP Diagnoses: 1. Liver; spleen; mesenteric lymph node; and mesenteric adipose tissue: Malignant lymphoma, Wistar rat, rodent.

2. Mesenteric lymph node: Hemangioma.
3. Mesenteric lymph node: Lymphadenitis, subacute, multifocal, mild, with fibrin thrombi and bacterial colonies.

Conference Comment: Conference participants discussed the suitability of subcategorizing this lymphoma; and there are various classification schemes. Frith *et al.* propose the following subcategories: 1) Follicular center cell, 2) Plasma cell, 3) Immunoblastic, 4) Lymphoblastic (Lymphocytic), and 5) Large granular lymphocyte lymphoma (leukemia). Also, in this case, there is evidence of neoplastic cells within vessels, which is suggestive of leukemia.

Hemangiomas of the mesenteric lymph node are frequently reported in the Wistar. Primary neoplasms of the lymph nodes are uncommon in rats; however, in this case, blood-filled, vascular spaces are lined by a single layer of well-differentiated, neoplastic, endothelial cells that have indistinct cell borders, small amounts of eosinophilic fibrillar cytoplasm, oval nuclei, finely stippled chromatin, and less than one mitotic figure per ten high power fields. Because of the degree of differentiation of the neoplastic cells and low mitotic rate, conference participants interpreted the mass lesion to be benign.

Contributor: Novartis Pharmaceutical Corp., One Health Plaza, East Hanover, NJ 07936

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CASE II - CP02-1505-03 (AFIP 2840045)

Signalment: Two female FVB/NJ retired breeders, 6-months-old.

History: Submitted for routine health screen.

Gross Pathology: The mice were in good body condition with adequate body fat stores. There were no lesions identified on the gross examination.

Laboratory Results: Cultures of the liver and spleen were negative. Complete blood counts and differentials were within normal limits on both animals.

Contributor's Morphologic Diagnosis: Diffuse retinal degeneration type 1

Contributor's Comment: Sections of the eye are examined. In the retina, there is diffuse loss of the outer nuclear layer and the photoreceptor layer outer segment which is composed of the rods and cones. The lens, choroid, iris, and cornea are unremarkable. In some sections, the harderian gland remains attached to the globe.

Retinal degeneration in FVB/NJ mice is classified as retinal degeneration-1 (*rd1*). It is identical with the rodless retina described by Keeler in 1966. Retinal degeneration-1 (*Pde6b*^{rd1}) is the result of decreased activity of the beta subunit of cGMP-phosphodiesterase which is caused by a murine viral insert and a second nonsense mutation in exon 7 of the *Pde6b* gene.[1] This mutation is found in several common inbred mouse strains, including, BPD/J, BUB/BnJ, C3H/HJ, C3H/HeJSx, C3H/HeOuJ, C3h/HeSnJ, C3HheB/FeJ, C3HfB/Bi, CBA/J, DA/JuSn, FL/1Re, FL/4Re, FVB/NJ, WBReJ*Kit*^{w}/+, WC/ReJ-+/+, and WC/ReJ-*Mgf*-^{Sl}/+. This list is taken from the JAX laboratory product list and is by no means complete. Mice with an ancestry of C3H and FVB strains are likely to be affected because these two strains commonly carry the mutation.[2] Due to the wide spread use of FVB mice in the generation of mutant animals, it is important that the veterinary pathologist is aware of these lesions and they are not interpreted incorrectly.

Retinal development is not complete in the mouse until shortly before the eyes open at postpartum day 14. Mice homozygous for *Pde6b*^{rd1} will show signs of degeneration of the photoreceptor layer as early as day 17. The loss of the photoreceptor layer is the result of apoptosis that begins with the loss of rods initially followed by apoptosis of cones. The retinal vasculature is slowly affected with a 35% decrease in vascular profiles by 1 month of age.[2] These mice are easily typed by phenotype based on attenuated vessels and pigment patches in the fundus and by genotype with PCR for *Dde I*.[1] By two months of age, vessels are difficult to identify on the fundic exam. The vascular change is thought to contribute to the loss of retinal ganglion cells and nerve fibers in older *Pde6b*^{rd1} mice. The human condition that resembles this lesion is called autosomal recessive retinitis pigmentosa. Various mutations in the catalytic domain of the human homolog of *Pde6b* have been identified in patients with this condition.

AFIP Diagnosis: Eye, retina, photoreceptor layer, outer nuclear and outer plexiform layers: Degeneration and loss, diffuse, severe, FVB/NJ strain, mouse, rodent.

Conference Comment: Mice and rats are well suited for scotopic (dark-adapted) vision, in which rods are the predominant photoreceptors. Rods contain the visual pigment, rhodopsin, which is composed of retinal (vitamin A aldehyde) bound to an opsin protein. Rhodopsin absorbs light and initiates the visual stimulus through a photochemical process that results in signal transduction to the bipolar cells of the outer plexiform layer, and then to ganglion cells of the inner plexiform layer. Axons of the latter collectively form the optic nerve and transmit the visual signal to the brain. Presently, there are 16 known genetic mutations that produce retinal photoreceptor degeneration in one or more strains of mice. The differential diagnosis also includes light-induced retinal degeneration, which varies markedly by mouse strain and the associated differences in uveal pigmentation.

Contributor: Department of Pathology, St. Jude's Children's Research Hospital, 332 N. Lauderdale St., Memphis, TN 38105

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CASE III – LRL Case #1 (AFIP 2840862)

Signalment: 1-week-old, straight-run, specific-pathogen-free White Leghorn (Line 22) layer chick, avian, *Gallus gallus domesticus*

History: 8 days after oral administration of an infectious agent.

AFIP LRL Case # 1 2, 5 or 7	1 green 4	2 5	3 6

Explanation: Section 1 with green dye from untreated control and sections 2 to 6 from birds treated with 1 X 10^{-3} to 1 X 10^{-7} dilutions of inoculum, in that order. Numbers 2, 5

or 7 on the label represent individual replicates, necessary to obtain the requisite number of slides for submission.

Gross Pathology: Atrophy of the bursa of Fabricius correlated with decreased absolute and relative weights in birds given the three lower dilutions.

Laboratory Results: Fluorescent RT-PCR (Taqman) assay of bursas for viral RNA and ELISA of serum antibody titers from infected birds confirmed infection by the infectious bursal disease virus (IBDV).

Contributor's Morphologic Diagnosis: Atrophy, marked, bursa of Fabricius due to IBDV infection.

Contributor's Comment: IBDV, ubiquitous in commercial poultry operations, is a double stranded RNA *Birnavirus* which causes lesions in the bursa of Fabricius as well as other lymphoid structures, leading to immunosuppression and death. A spectrum of morphologic changes from acute to chronic has been described in IBD or Gumboro disease (Cheville 1967; Helmboldt and Garner 1965).

Microscopic examination revealed marked bursal atrophy characterized by decreased height and width of the bursal plicae. This change occurred in all IBDV-infected chickens given 1 X 10^{-3} to 1 X 10^{-5} virus dilutions. Within affected bursal plica, lymphoid follicles were depleted of lymphocytes and had a granulomatous and/or glandular appearance. In addition, there was proliferation of the endodermal epithelial cells demarcating the cortex and medulla and presence of multiple mucinous cysts. The interstitial tissue had slight to moderate fibroplasia and minimal to moderate subacute inflammation. The latter consisted largely of normal lymphocytes, plasma cells and few heterophils. Although lymphocytes appeared to repopulate bursal follicles of infected birds, healthy follicles were only found in birds given 1 X 10^{-6} and 1 X 10^{-7} virus dilutions. The interstitial alterations together with the glandular change and multiple cysts increased in severity and frequency with increasing doses of IBDV.

The specimens submitted document subchronic lesions in birds experimentally administered graded levels of a highly virulent virus in a laboratory setting. No birds died during the early, acute phase of the infection. The outcome of IBDV infection would be different under field conditions because of intercurrent disease(s) or infection with the highly pathogenic (vvIBDV) strain (Lasher and Shane 1994).

AFIP Diagnosis: Cloacal bursa: Lymphoid depletion, diffuse, mild to severe, White Leghorn (*Gallus gallus domesticus*), avian.

Conference Comment: Infectious bursal disease is an acute, highly contagious, immunosuppressive disease caused by serotype 1 Infectious Bursal Disease Virus (IBDV) in predominantly young, 3-6 week-old chickens. IBDV is a member of the family *Birnavirus*, genus *Avibirnavirus.* Additional genera include *Aquabirnavirus*, which causes infectious pancreatic necrosis in fish, and *Entomobirnavirus*, which causes disease in insects. The primary target of IBDV is immature B-lymphocytes, and the cloacal bursa is most severely affected. With very virulent strains, the cecal tonsils,

thymus, spleen and bone marrow are also affected. Transmission occurs via direct contact, fomites, or airborne dissemination. Opportunistic infections contribute to the morbidity and mortality. On gross exam, the bursa is greatly enlarged due to edema, hyperemia, and hemorrhage by 3-4 days post-infection. The bursa then atrophies to one-third normal size by 8 days post-infection.

The slides contain multiple bursal specimens from different chickens, as noted by the contributor. Therefore, the severity of lymphoid depletion, interfollicular fibroplasia, epithelial hyperplasia, and the number of mucinous cysts are variable among the different specimens. A mild heterophilic bursitis is evident in some sections.

Contributor: Greenfield Laboratories, Eli Lilly and Company, 2001 West Main Street, Greenfield, IN 46140

References:

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CASE IV - 02-A-66 (AFIP 2840861)

Signalment: One-year-old, male, rhesus macaque, *Macaca mulatta*, nonhuman primate

History: This animal was found dead in an outdoor corral.

Gross Pathology: This juvenile rhesus macaque was in poor flesh and approximately 8% dehydrated. There were no visible stores of subcutaneous or visceral fat. Scant thymic tissue was apparent in the mediastinum. The small intestine was mildly dilated and the serosal vessels were congested and purple. The enteric contents were hemorrhagic and the mucosa was eroded, red-tan, irregular and tattered. The duodenum and cranial jejunum were more severely affected. The cecal and colonic mucosal surfaces contained multiple 1.0 – 3.0 mm raised, white foci with hemorrhagic borders. The mesenteric lymph nodes were markedly enlarged five to ten times normal size and were edematous on sectioned surface.

Laboratory Results: *Yersinia pseudotuberculosis* and *Campylobacter fetus ss. jejuni* were isolated from the small intestine at necropsy.

Contributor's Morphologic Diagnoses: 1. Small intestine: Enteritis, necrotizing, erosive, acute, diffuse, severe with villous loss and blunting, micro-abscessation, hemorrhage, congestion, edema, fibrin thrombi, lymphangiectasis, lymphangitis with colonies of gram-negative coccobacilli, rhesus macaque (*Macaca mulatta*), nonhuman primate.
2. Mesentery: Atrophy, adipose tissue, diffuse, moderate.
3. Mesentery: Mesenteritis, acute, multifocal, moderate, with congestion, edema, lymphangiectasis and lymphangitis.

Contributor's Comment: Small intestine: The mucosal architecture is markedly altered by extensive foci of necrosis that destroy and blunt villi. Within the lamina propria of denuded remnants of villi, frequently extending to the necks of crypts and multifocally fingering down into the submucosa and lymphoid follicles are large colonies of myriad gram-negative coccobacilli. Moderate numbers of neutrophils admixed with eosinophilic cellular and karyorrhectic debris, fibrin and hemorrhage surround these colonies and often exude into the lumen. Lacteals and submucosal lymphatics are markedly ectatic and contain high numbers of neutrophils, fewer erythrocytes and accumulations of fibrin. There is rare crypt abscessation and necrosis. The submucosa is variably expanded by hemorrhage, fibrin, edema and an inflammatory infiltrate composed predominantly of neutrophils and fewer macrophages, lymphocytes and plasma cells. There is transmural congestion with mildly hypertrophied endothelial cells and few fibrin thrombi. Similar changes described for the submucosa are present in the mesentery and there is diffuse atrophy of adipose tissue.
Note: Not all sections contain lymphoid follicles or mesentery.

The enteric lesions are consistent with yersiniosis. *Yersinia* sp. are gram-negative, coccobacilli that are facultative anaerobes in the Enterobacteriaceae family. *Yersinia enterocolitica* and *Y. pseudotuberculosis* are enteroinvasive bacteria and cause diarrhea, dehydration, anorexia, and weight loss. Septicemia may result. Illness may also be peracute. Abortion and stillbirth have been reported.[1]

Transmission is via the fecal-oral route. *Yersinia* cross the intestinal epithelium primarily via the M cells and colonize the mucosal-associated lymphoid tissue. The invasin protein of *Yersinia* binds with beta 1 integrins of the host cell which are involved in the adherence of epithelial cells to the extracellular matrix. Other *Yersinia* surface proteins such as Ail, PsaA, and YadA also confer invasiveness but at a greatly reduced level.[3] The consequent changes in the host cell cytoskeleton allows for uptake of the bacteria in a macropinocytic vacuole. The bacteria migrate through the cytoplasm upon dissolution of the vacuolar membrane. Once the bacteria access the lymphoid tissue they resist phagocytosis by secreting the effectors YopH, T, and E that disrupt the cytoskeleton of macrophages. This permits extracellular survival in the gut-associated lymphoid tissue and the mesenteric lymph nodes thereby allowing local spread to villous epithelial cells and systemic dissemination.[3] Chromosomal and plasmid genes determine virulence. Bacterial chromosomal encoded genes are required for the

internalization of the bacteria by intestinal epithelial cells. Plasmid encoded proteins are necessary for antiphagocytic activity.[2]

Gross lesions may include multifocal necrosis or abscessation in the spleen and liver, mesenteric lymphadenopathy and ulcerative enterocolitis. The characteristic microscopic lesion is ulcerative enterocolitis with necrosis, an inflammatory infiltrate composed primarily of neutrophils with fewer mononuclear cells, and colonies of gram-negative coccobacilli. Other lesions include multifocal acute and necrotizing hepatitis, splenitis, and lymphadenitis.

AFIP Diagnoses: 1. Small intestine: Enteritis, necrohemorrhagic, acute, transmural and diffuse, marked, with lymphangitis and large colonies of coccobacilli, Rhesus macaque (*Macaca mulatta*), nonhuman primate.
2. Mesentery: Peritonitis, acute, diffuse, severe, with lymphangitis.

Conference Comment: The contributor has provided a concise summary of yersiniosis in nonhuman primates. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* cause similar lesions that must be differentiated by bacterial culture. The differential diagnosis for necrohemorrhagic enterocolitis in nonhuman primates also includes *Salmonella* sp., *Camplyobacter* sp., *Shigella* sp. and *Entamoeba histolytica*. In addition to *Yersinia*, other bacteria that form large colonies include *Actinomyces* sp., *Arcanobacter* sp., *Clostridium* sp., *Staphylococcus* sp., and *Streptococcus* sp.

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