The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2002-2003

CONFERENCE 17
12 February 2003

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CASE I – 1614-1 (AFIP 2850109)

Signalment: 5-month-old outbred female Swiss-Webster mouse

History: Sentinel mouse in a laboratory colony housed on dirty bedding from other mouse cages, part of an infectious disease surveillance program. Euthanized due to lethargy and unthriftiness.

Gross Pathology: Stomach distended approximately three times by gas, fluid, and partially digested food. Kidneys shrunken, pale, and pitted. Multifocal hemorrhagic necrosis and thrombosis of the ovaries bilaterally.

Laboratory Results: Negative for all murine infectious pathogens tested in the colony surveillance program by serology, respiratory and intestinal cultures, fecal examination, anal tape, and skin scrapings.

Contributor's Morphologic Diagnosis: Kidney: Chronic nephropathy characterized by membranous glomerulopathy, lymphoplasmacytic adventitial vasculitis and perivasculitis, tubular degeneration, ectasia, and regeneration with protein, hemoglobin, cellular, waxy, and granular casts, and lymphoplasmacytic and histiocytic interstitial nephritis, severe.

Contributor's Comment: This case is consistent with the previously reported syndrome of gastric dilatation and chronic nephropathy in mice exposed to dirty bedding [1]. Although the mean age of affected mice in the published report was 10 months, similar lesions are sometimes found in animals as young as 3 or 4 months of age. The kidney disease appears immune-mediated and is presumably the result of chronic high antigen exposure, although there may be more than one inciting process.

Membranous glomerulopathy is suggestive of antigen-antibody deposition and/or complement activation with attack of glomerular vessels or basement membranes. Congo Red stain was negative for amyloid in glomeruli and tubules, and no infectious agents were identified with Gram, Giemsa, or silver stains -- not submitted). In larger vessels, disease is centered on the tunica adventitia. Indeed, polarizing microscopy demonstrates infiltration of inflammatory cells between adventitial connective tissue fibers, with marked serosal disruption in established lesions. A similar adventitial- and peri-vasultitis, with thrombosis and hemorrhagic necrosis was present in the ovaries (not submitted). The reason for gastric distention, the result of delayed emptying, is not clear but in the above study was strongly correlated with serum creatinine levels [1]. Amidated serum gastrin was increased in mice in contact with dirty bedding, whether or not there was grossly apparent gastric distention. Other suggested causes for delayed gastric emptying include uremia, LPS hyper-responsiveness, dysregulated gastrointestinal hormone levels, and autonomic nervous dysfunction.

Although antigen exposure occurs across cutaneous and/or mucosal surfaces, the skin, lungs, and gastrointestinal tracts of these mice rarely demonstrate significant microscopic lesions. Curiously, the vasculitis appears most often to affect a restricted set of abdominal organs including kidneys, female reproductive tract, and mesentery. Tissue tropism is well documented in various human and veterinary systemic vasculititides, including polyarteritis nodosa. Although certain histologic features present in the kidneys of these mice are reminiscent of chronic progressive nephropathy of rats (old rat nephropathy), disease in the former progresses much more quickly and is not known to be influenced by diet. Because all of the sentinel mice in our surveillance program are females, we have no information on gender influence on disease progression.

Renal perivascular mononuclear cell infiltrates are commonly seen in otherwise healthy laboratory mice, and are believed by many to be a response to environmental antigens. Likewise, female reproductive tract vascular thrombosis and/or hemorrhage is a common "spontaneous" lesion of mice. The severe disease seen in mice exposed to dirty bedding may be an extreme manifestation of the normal murine responses to environmental antigens.

AFIP Diagnosis: Kidney: Glomerulonephropathy, chronic, diffuse, severe, characterized by interstitial lymphoplasmacytic nephritis; tubular ectasia, proteinosis, and loss; and glomerular sclerosis, Swiss-Webster mouse, rodent.

Conference Comment: The contributor has summarized a recently recognized association between gastric dilatation and chronic nephropathy in mice exposed to high levels of environmental antigens. A report by Garcia, et al, suggests that the primary glomerular injury may be attributed to immune complex deposition involving IgA. Such a pathogenesis would be consistent with exposure of mucous membranes to excessive amounts of foreign environmental antigens.

The pathogenesis of membranous glomerulonephritis involves deposition of antigen-antibody complexes along the subepithelial basement membrane. Subsequent

activation of complement, and infiltration and activation of leukocytes leads to a cascade of events resulting in glomerular injury. Ultrastructurally, there is diffuse thickening of the glomerular capillary basement membrane and accumulation of electron-dense, immunoglobulin-containing deposits along the subepithelial basement membrane.

As mice age, they can also develop a nephropathy with similarity to the basic features of chronic progressive glomerulonephropathy/nephrosis in aging rats. Key features include thickening of glomerular basement membranes and Bowman's capsule, tubular ectasia, proteinaceous casts, and variable degrees of interstitial fibrosis, mononuclear cell infiltration and thickened proximal tubule basement membranes.

Conference participants discussed membranous glomerulonephritis as a major cause of the nephrotic syndrome, whose four components are generalized edema, proteinuria, hypoproteinemia, and hyperlipidemia. The prolonged, severe, protein-losing nephropathy of nephrotic syndrome results in early loss of low molecular weight proteins (albumin); and later, loss of higher molecular weight proteins (antithrombin III) into the glomerular filtrate which are ultimately excreted in urine. Loss of antithrombin III produces a hypercoagulable state, which leads to a secondary (acquired) increased risk of thrombosis. The reported thrombosis of the ovarian vasculature in this case may be linked to loss of antithrombin III into the urine.

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CASE II - B-15032 (AFIP 2838985)

Signalment: 2-year-old male, *Macaca fascicularis* (Cynomolgus monkey).

History: Intermittent unilateral epistaxis (right side). “Puffy” or swollen right eyelid (see clinical image). Eventual nasal discharge (clear) and stertor, rare mouth-breathing after exertion. Mass visible within nasopharynx on pharyngoscopic examination.

Gross Pathology: The upper eyelid of the right eye appeared slightly elevated; the lower eyelid depressed. A reddish glistening nasal mass obstructed the lower half of the nasopharynx bilaterally and protruded into the right orbit.

Laboratory Results: Not available

Contributor's Morphologic Diagnosis: Nasal adenocarcinoma

Contributor's Comment: The clinical image demonstrates slight displacement of the globe of the right eye. The gross image shows the mass on the floor of the nasopharynx bilaterally. The arrow indicates the nasal septum (both images provided to AFIP).

The histologic section is coronal, through the nasopharynx. A large mass is broadly attached to the nasal septum bilaterally and to the floor of the nasopharynx. It extends along the turbinates; the remaining nasal septal cartilage is slightly displaced to one side. The mass is composed of cuboidal to low columnar cells with scant eosinophilic or more abundant vacuolated cytoplasm, a high nucleus:cytoplasm ratio, and round to ovoid nuclei occasionally with a distinct nucleolus. Mitotic figures are infrequent. The cells are arranged in tubuloacini and also in sheets. Where the cells are arranged in sheets, cells are more spindle-shaped to stellate; such cells nonetheless form a morphologic continuum with the cells arranged in acini, which are in some places contiguous with the nasal epithelial cells bordering the mass. Where tubuloacini are present, they frequently contain pale eosinophilic secretion. The vacuolation of cells, especially where they form sheets, creates a microcystic pattern that mimics formation of tubuloacini. Dense fibrous connective tissue bands extend from remaining turbinate bones. There are areas of superficial ulceration, hemorrhage, and necrosis within the mass, however, these are not pronounced.

In posterior sections that have hard palate, the mass extends through the bone of the hard palate and bulges into the oropharynx, although it does not breach the epithelium of the palate. Where it extends into the bone, osteoclasts are prominent within the adjacent fibrous connective tissue.

Carcinomas of the nasal cavities and sinuses in domestic animals include squamous cell carcinomas, adenocarcinomas, undifferentiated (anaplastic) carcinomas, transitional carcinomas, adenoid cystic carcinomas, and mucoid cystic carcinomas. The presence of numerous glandular structures, and the apparent continuity of these cells with the cells forming sheets support the diagnosis of nasal adenocarcinoma. The sheets of cells with formation of microcysts are suggestive of other tumor types, so a diagnosis of anaplastic carcinoma could also be supported. Transitional carcinomas (no relationship to transitional cells of the bladder) are so-named because they are thought to be a transition between squamous cell carcinoma and anaplastic carcinomas; these tumors consist of thick bands of cells with indistinct cell borders. There may be microcysts within the epithelial layers, and where this mass forms sheets it resembles such a tumor. However, the bands of epithelial cells in transitional carcinomas rest upon a distinct basement membrane, which does not appear to be

present in this case. Adenoid cystic carcinomas are rare tumors with a multilobulated appearance and a striking cribriform pattern. Mucoepidermoid carcinomas contain mucin-filled acini as well as areas of squamous differentiation not considered to be present in this tumor. The WHO classification system recognizes squamous cell carcinomas, transitional carcinomas, adenocarcinomas, and spindle cell carcinomas. The latter are uncommon tumors considered to be a type of squamous cell carcinoma lacking keratinization. Similarly, transitional carcinomas are considered to be an intermediate between squamous cell carcinomas and anaplastic carcinomas.

Nasal adenocarcinomas occur in clusters (i.e. endemically) in various species and countries (e.g. sheep from Europe and the USA, pigs from Brazil, India, and China), sometimes in multiple species on an individual farm, suggesting a viral etiology. Type C retroviruses have been associated with lesions from cattle, sheep, and goats, though causation has not been established. Nasal adenocarcinomas do not appear to have been reported in Cynomolgus monkeys (MEDLINE keywords nasal carcinoma AND monkey).

AFIP Diagnosis: Nasal cavity: Adenocarcinoma, Cynomolgus macaque (*Macaca fascicularis*), nonhuman primate.

Conference Comment: This case generated considerable discussion among conference participants. Based on H&E-stained sections, most participants favored a diagnosis of olfactory neuroblastoma. Despite the presence of numerous, well-differentiated gland-like structures, a significant spindle-cell component suggested neural origin. However, the results of subsequent immunohistochemical analysis are most consistent with the contributor's interpretation of nasal adenocarcinoma. Glandular structures throughout the neoplasm are strongly positive for cytokeratin, and negative for neuron specific enolase (NSE), neurofilament protein (NFP), and synaptophysin. Bodian's stain also failed to reveal neural fibers typical of olfactory neuroblastoma. According to the World Health Organization, the immunoprofile of olfactory neuroblastoma in animals does not demonstrate a consistent pattern. Upon review of the H&E stained slide and immunohistochemical results, the Department of Otorhinolaryngic-Head and Neck Pathology also favored a diagnosis of nasal adenocarcinoma.

The contributor has provided a concise summary of nasal carcinomas of domestic animals. Olfactory neuroblastoma is a rare malignant neuroendocrine neoplasm that purportedly arises from olfactory epithelium. There are many synonyms, including esthesioneuroblastoma. The ultrastructural feature of neuronal cell processes that contain dense core granules and microtubules is the most specific criterion in the diagnosis of olfactory neuroblastoma. Helpful morphologic features are the fibrillary cytoplasmic background (nerve fibers) and the presence of Flexner Wintersteiner-type or Homer Wright-type rosettes. In humans, microscopic grading (I to IV) subdivides this neoplasm, primarily based on the degree of epithelial versus neuronal differentiation. Biological behavior includes wide metastasis, with invasion of the cranial cavity and brain.

We are grateful to the Department of Otorhinolaryngic-Head and Neck Pathology for their assistance with this case.

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CASE III – LRL Case #2 (AFIP 2840851)

Signalment: Adult dog, gender and breed unspecified

History: Two dogs accompanied owners on a mushroom-collecting outing. Both dogs developed acute gastrointestinal illness (vomiting, diarrhea) followed by onset of fatal liver and renal failure

Gross Pathology: Not Available

Laboratory Results: Not Available

Contributor's Morphologic Diagnosis: Liver; marked, diffuse, centrilobular hepatocellular necrosis with diffuse sinusoidal congestion

Contributor's Comment: While no ill effects were present in the owners of these dogs as a result of collecting and eating wild mushrooms, it is probable that these dogs ingested poisonous mushrooms during the expedition. *Amanita phalloides* (death cap mushroom) are common in the area of the expedition. The timing and pattern of the clinical signs and the histologic finding of extensive centrilobular liver necrosis in these dogs are consistent with Amanita mushroom toxicity. Enteritis and proximal renal tubular lesions occur in cases of serious or fatal poisoning and were observed in one of these two dogs (slides not submitted of the intestine or kidney).

Amanita phalloides contains two well-characterized toxins. Phalloidin interacts with actin in gastrointestinal smooth muscle and is responsible for the gastrointestinal signs usually seen within 10-12 hours post ingestion. Of the amatoxins, the most potent is alpha-amanitin. This toxin is readily distributed to hepatocytes via enterohepatic circulation and interferes with protein synthesis by binding to RNA polymerase II. Onset of liver disease is usually delayed and occurs 2-3 days after ingestion.

AFIP Diagnosis: Liver, hepatocytes: Degeneration, necrosis, and loss, centrilobular to midzonal, acute, diffuse, severe, with congestion and hemorrhage, breed not specified, dog, canine.

Conference Comment: The centrilobular to midzonal pattern of necrosis, while most suggestive of a toxic etiology, is not diagnostic for any particular intoxication. In the absence of a history of exposure to mushrooms, a differential diagnosis that includes, but is not limited to acetominophin, blue-green algae (*Microcystis* sp.), cocklebur (*Xanthium* sp.), cycad palm (*Cycad* sp.), aflatoxin, and phenol toxicosis would have to be considered.

The phallotoxins include phalloidin, phalloin and phallasin. The amatoxins include alpha-, beta-, gamma-, and epsilon-amanitin, amanin, amanullin and proamanullin. The mechanism of action of amatoxins - inhibition of messenger RNA synthesis, and therefore protein synthesis – primarily affects metabolically active cells (hepatocytes, gastrointestinal crypt epithelium), and results in cell death by necrosis. Elevations of cytosolic leakage enzymes (aspartate aminotransferarse [AST], alanine aminotransferase [ALT]), inducible enzymes (alkaline phosphatase), and bilirubin are common in mushroom toxicities. Poisonings often include a latent period of 6-48 hours. Diagnosis of *Amanita* mushroom toxicity is based on history, clinical signs, identification of mushrooms in gastric contents or in the animal's environment, and detection of amatoxins in urine, serum and gastrointestinal contents. Important sequelae of liver failure include coagulopathy, hepatic encephalopathy, coma, and death in the terminal stages.

The sensitivity of the centrilobular zone to toxic insult was discussed. Predisposing factors include high levels of cytochrome P450 involved in metabolism of compounds to toxic metabolites, low oxygen tension, and reduced levels of glutathione relative to periportal regions of the hepatic lobule. The correlation between the classical architecture of the hepatic lobule and the more functionally-based unit, the hepatic acinus, was also discussed.

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CASE IV - I02-2408 (AFIP 2840050)

Signalment: 3-year-old, female, Dwarf, rabbit (*Oryctolagus cuniculus*)

History: After spending eight weeks in France, the rabbit showed conjunctivitis and swollen eyelids. Due to these findings, clinicians suspected myxomatosis, euthanised the animal and sent it for pathological examination.

Gross Pathology: Swollen eyelids due to subcutaneous edema, mucopurulent conjunctivitis, discharge around eyes and nose, thickened skin of the ears.

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Skin, ear: Subacute severe intraepidermal vesicular dermatitis with intracytoplasmic inclusion bodies and myxedema (myxomatosis)

Contributor's Comment: Myxoma virus is a poxvirus (genus Leporipoxvirus) of which two subtypes have been described: the South American type (found in the forest rabbit *Sylvilagus brasiliensis*) and the Californian type (found in the brush rabbit *Sylvilagus bachmani*). Myxoma virus is spread indirectly by biting arthropods, but can also be transmitted directly by respiratory droplets. In the European rabbit (*Oryctolagus cuniculus*), myxoma virus causes the lethal disease myxomatosis characterized by systemic spread of the virus and the development of secondary skin lesions (myxomas).

Microscopically, an unusual feature of the histopathology of the myxoma virus-infected skin lesions is the proliferation of cells in the walls of small blood vessels and the appearance of large stellate "myxoma cells". At the molecular level, M11L, a membrane-associated protein expressed by myxoma virus, has been found to co-localize with host cell mitochondria and to inhibit apoptosis of infected leukocytes.

A nice example of host-virus co-evolution is the release of myxoma virus for 'biological control' of the wild European rabbit population in Australia. This subsequently led to the selection for rabbits with resistance to myxomatosis and attenuated strains of the myxoma virus. Though little is known about the mechanism of resistance in the rabbit, an enhanced innate immune response is suspected that would allow the rabbit to increase an effective cellular immune response.

AFIP Diagnosis: Haired skin, pinna: Atypical mesenchymal proliferation, myxomatous, diffuse, moderate, with epithelial hyperplasia and ballooning degeneration, and eosinophilic intracytoplasmic inclusion bodies (myxomatosis), Dwarf rabbit, (*Oryctolagus cuniculus*), lagomorph.

Conference Comment: Like all poxviruses, myxoma virus is a double stranded DNA virus that is characteristically brick shaped, replicates in the host cell cytoplasm, and has a complex virion structure. Other important members of the leporipoxvirus genus include rabbit (Shope's) fibroma virus, hare fibroma virus, and squirrel fibroma virus. All three are characterized by single to multiple, cutaneous fibromas on the distal limbs, feet, head, ears, or genitalia. Microscopically, they resemble each other, with eosinophilic, intracytoplasmic inclusion bodies in the epidermis and underlying stroma.

Innate antiviral immunity is largely dependent on secretion of interferons by virus-infected cells. Interferons bind to receptors on nearby cells, activating macrophages and natural killer cells that exert phagocytotic and cytolytic effects against virus-infected cells. However, cell mediated immunity is the most important component of the immune response to viral infection. Viral antigens, expressed on an infected cell's surface in association with MHC class I molecules, are recognized by class I MHC-restricted cytotoxic T cells that lyse the cell, thereby limiting viral replication. Antigen presenting cells, such as dendritic cells, macrophages, B lymphocytes, Langerhans cells of the skin, and endothelial cells can also present viral antigens in association with MHC class II molecules to class II MHC-restricted helper T cells. Myxoma virus evades the immune system through expression of several virulence factors that modulate the immune response. Some virulence factors down regulate expression of MHC class I molecules on the surface of infected cells, thereby preventing recognition by cytotoxic T cells. Other virulence factors bind interferon-gamma, which down regulates the expression of MHC class II molecules, preventing recognition by helper T cells. Binding of interferon-gamma also decreases macrophage activation.

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*Sponsored by the American Veterinary Medical Association, the American College of Veterinary Pathologists and the C. L. Davis Foundation.