The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2005-2006

CONFERENCE 3
28 September 2005

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Division of Veterinary Resources
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CASE I –AFIP #2 (AFIP 2983842)

Signalment: Female, adult, BALB/c mouse

History: Lungs submitted from dead mice, 9 days post injection with vaccinia virus.

Gross Pathology: Lung lobes are non-collapsed and spongy.

Laboratory Results: None

Histopathologic Description: Multifocal individual mostly medium to small diameter bronchioles are lined by multiple piled layers of swollen epithelial cells which occasionally occlude the airway lumen. Epithelial cells are expanded by large, clear, indiscrete cytoplasmic vacuoles and most cells contain variably sized, but generally large, brightly eosinophilic, glassy, intracytoplasmic inclusion bodies. Scattered small numbers of lymphocytes and macrophages fill immediately adjacent alveolar spaces.

Contributor's Morphologic Diagnosis: Bronchiolitis, proliferative, multifocal, with intraepithelial, intracytoplasmic inclusion bodies

Contributor's Comment: Histologic lesions in the lung (and in sections of nasal cavity) are consistent with *Orthopoxvirus* infection. These are large DNA viruses of the poxvirus family to which vaccinia, variola, monkeypox, cowpox, ectromelia virus and others belong. Orthopoxviruses share antigenic cross-reactivity, but each is a distinct species.
Most information on *Orthopoxvirus* infections in mice is based on reports of ectromelia virus infection, specifically (1). These viruses are not highly contagious and can be experimentally transmitted via a number of routes, but the primary means of natural transmission is through cutaneous trauma, requiring direct animal contact. Other studies have indicated that transmission was facilitated by handling of animals.

Young mice suckling immune dams were protected by maternal antibodies from disease, but not from infection. The hypothetical model of infection (based on ectromelia virus infection; mousepox), involves invasion through skin, local replication, release to regional lymph nodes, primary viremia and replication in the spleen and liver. Between 3-4 days post exposure, secondary viremia ensues leading to replication in the skin, lungs, kidney, intestine and other organs. There is increasing evolution of lesions days 7-11 post exposure, including cutaneous rash.

The disease scenario differs markedly between mouse genotypes with susceptible strains such as C3H, A, DBA, SWR and BALB/c often dying acutely with minimal lesions or opportunity for virus excretion. Other mouse strains develop illness and progress to develop cutaneous lesions and virus shedding. Still others, such as B6 mice, are resistant to disease, but allow virus replication and excretion. In most cases, liver necrosis is present and is sometimes the only lesion noted. Pathognomonic viral inclusion bodies in respiratory epithelium, along with the history of vaccinia virus vaccination allow for definitive diagnosis in this case.

AFIP Diagnosis: Lung: Bronchitis and bronchiolitis, acute, diffuse, moderate, with epithelial ballooning degeneration, eosinophilic cytoplasmic inclusion bodies and alveolar fibrin and edema, BALB/c mouse, murine.

Conference Comment: The contributor provides a detailed synopsis of Orthopoxvirus infections in mice. Although this case was caused by experimental infection with vaccinia virus it shares many characteristics with mousepox caused by ectromelia virus.

Ectromelia virus was discovered in England in 1930 and has been extensively studied as a model of the pathogenesis of exanthematous diseases. The study of ectromelia virus led to the concept of a primary and secondary viremia as well as the role of cell mediated immunity, particularly T-lymphocytes and macrophages, in the recovery from infection. [2]

Mousepox has a restricted host range and causes severe disease with a high mortality rate (50-100%). Voles are believed to be the natural host.
There are two recognized forms of mousepox: the rapidly fatal form with few, if any, cutaneous lesions and the chronic form with ulceration of the skin, particularly on the snout, feet and tail often resulting in loss of limbs or tail.

The typical gross findings in mice include conjunctivitis, alopecia, crusting and erythema of the skin and dry gangrene of the extremities and tail. The liver and spleen swell and develop multifocal hemorrhages and pinpoint white foci. Lymph nodes and Peyer's patches may be enlarged and hemorrhagic.

Typical light microscopic findings include intracytoplasmic eosinophilic A-type (Marchal) inclusion bodies within the epidermis, pancreas and intestine. Early in the disease there is epidermal hyperplasia, hypertrophy, spongiosis and ballooning degeneration with inclusion bodies. Later, the epidermis becomes necrotic and ulcerated. The liver develops multiple foci of coagulative necrosis, hepatocellular syncytia and vacuolar degeneration with minimal inflammation. There is splenic necrosis involving both lymphoid follicles and red pulp. Additionally, there may be intestinal mucosal erosions and bone marrow degeneration. The combination of hepatic and splenic necrosis along with cutaneous lesions and characteristic eosinophilic intracytoplasmic inclusion bodies is pathognomonic.[1]

Other important members of the Orthopox genus include (3):
Variola virus (smallpox in humans)
Monkeypox virus (lesions in nonhuman primates vary from mild to severe depending on the species, age and immune status, and range from cutaneous lesions to disseminated visceral infection; fatal infections in humans)
Cowpox virus (self-limiting lesions on the udder in cattle; fatal pneumonia in elephants and felids)
Buffalopox virus
Camelpox virus

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ADDENDUM Conference 3, Case I:

15 May 07
An astute pathologist pointed out to us recently that vaccinia virus does not cause the type inclusion bodies as seen in this case. Cowpox and ectromelia cause large eosinophilic intracytoplasmic inclusions called "A-type" or Marchal-Downie-Bollinger inclusions like the ones present in this conference case. The other poxviruses that do this are raccoonpox and fowlpox[1,2]; to our knowledge neither has been reported to infect mice. Rabbitpox virus does not cause these type inclusions (D. Nichols, personal communication), and rabbitpox virus is classified as a strain of vaccinia virus. Fenner states that eosinophilic A-type inclusion bodies are produced by cowpox virus but not by vaccinia virus and that the only kind of inclusions that vaccinia might infrequently

produce are the B-type (also known as Guarnieri bodies), which are small, irregular, weakly staining, and basophilic to slightly eosinophilic.[1,3] We speculate that the tissue in this conference case came from an experiment involving injection with cowpox and not vaccinia. Perhaps there was some miscommunication about the experimental history between the researcher and the contributing diagnostic laboratory. We wish to thank Dr. Don Nichols for identifying this discrepancy and contributing to this addendum.

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3. Fenner F: Chapter 1. ***In***: Recombinant Poxviruses, ed. Binns MM, Smith GL, p. 13. CRC Press, 1992

CASE II – AFIP 1 (AFIP 2985003)

Signalment: One year old male ICR mouse (*Mus musculus*).

History: The mouse was submitted to necropsy because of observed jaundice. The mouse died shortly before necropsy was performed.

Gross Pathology: Liver (All lobes): Enlarged, soft, with nodular surface, mottled dark red and yellow.
Spleen: Splenomegaly, minimal.
Seminal Vesicle (Right), Prostate Gland: Discoloration, green/yellow, multifocal, moderate.

Laboratory Results: The liver was PCR positive for *Helicobacter hepaticus*. This animal came from an SPF colony, seronegative for a panel of 18 murine viral pathogens.

Histopathologic Description: There is multifocal, marked bile ductule hyperplasia, accompanied by a mixed inflammatory infiltrate of lymphocytes, plasma cells and neutrophils. The inflammation extends from the portal zones across the limiting plate into the hepatic parenchyma, with associated piecemeal necrosis of hepatocytes. Hepatocytes frequently exhibit multifocal, marked cytomegaly. Enlarged hepatocytes typically have karyomegaly, with frequent cytoplasmic invaginations into the nucleus. On most sections there are small foci of cellular alteration, either vacuolated or clear cell foci, or both.

Contributor's Morphologic Diagnosis: Liver: Cholangiohepatitis, chronic, suppurative, multifocal, marked, with marked bile duct hyperplasia, piecemeal necrosis and marked hepatocytomegaly and karyomegaly.

Warthin Starry silver stain: Multiple elongate, curved bacilli are seen localized between hepatocytes (in bile canaliculi) (Figure). An unusually high number of spiral bacteria are seen in the liver of this case. Often the organisms are rare and thus difficult to find.

Additional lesions, not included on sections:
Prostate, seminal vesicle: Abscesses, multiple, well-developed, severe, with myriad intralesional Gram negative bacilli.
Kidney: Pyelonephritis, chronic, suppurative, multifocal, moderate.

Contributor's Comment: The hepatitis and spiral bacilli seen on silver stains are characteristic of *Helicobacter hepaticus* infection. Some of the hepatocellular anisocytosis may be attributable to the advanced age of the mouse.
Helicobacter spp. are significant pathogens of a number of species (reviewed in1), including mice. *Helicobacter* spp. colonize the cecum and colon of rodents and some are associated with liver disease in susceptible strains of mice and rats. Colony acquired infections are persistent, with long term shedding of the bacteria in the feces. Endemic infections are common. *Helicobacter hepaticus* was the first murine helicobacter identified, associated with hepatitis and hepatocellular neoplasia in mice on a long-term toxicology study (2, 3). *Helicobacter*-associated disease is dependent on age, sex (male>female), genetics and immune status of the host, and on bacterial virulence factors. Known *Helicobacter hepaticus* susceptible strains include A, C3H, SCID and other immunocompromised mice. *Helicobacter hepaticus* also causes proliferative typhlocolitis and rectal prolapse in immunocompromised mice. Additional helicobacters known to infect mice include. *H. bilis, H. rodentium, H. ganmani, H. typhlonius, H. muridarum, H. muricola* and *H. rappini (Flexispira rappini)* (1).

Mice are likely infected with helicobacters through ingestion of contaminated feces, and *Helicobacter* spp. are readily transmitted by contaminated bedding (4). *Helicobacter hepaticus* will persist indefinitely in the bile canaliculi. The pathogenesis of hepatitis and hepatocellular tumors is still under investigation, but hepatotoxins and immune mechanisms are likely.

While the typical lesions and presence of intralesional spiral bacilli on silver stains are strong evidence for *Helicobacter* infection, definitive diagnosis requires culture and/or PCR (1). Diagnostic specimens can be lesional tissue, cecal scrapings or fecal pellets.

Although this mouse was not of the recognized *Helicobacter hepaticus*- susceptible strains, the advanced age and concomitant urogenital tract infection may have conferred susceptibility to *Helicobacter*-induced hepatitis.

AFIP Diagnosis: Liver: Cholangiohepatitis, chronic-active, portal and random, moderate, with multifocal marked biliary hyperplasia, piecemeal hepatocellular necrosis, and nodular hyperplasia, ICR mouse, rodent.

Conference Comment: *Helicobacter* spp. are Gram negative microaerophilic curved to spiral motile bacilli. There are at least twenty species definitively identified in animals, many which are nonpathogenic.

Helicobacter hepaticus and *H. bilis* are most commonly associated with disease in mice. Typical clinical findings associated with infection include: wasting; mucoid, hemorrhagic and sticky feces; and rectal prolapse. Typical necropsy findings include typhlitis and colitis. Microscopic lesions include colonic and cecal crypt hyperplasia, variable leukocyte infiltration in the lamina propria of the colon and cecum and, infrequently, randomly scattered foci of hepatocellular necrosis with mixed leukocyte infiltrates (4).

In susceptible mice, *H. hepaticus* causes acute focal non-suppurative necrotizing hepatitis that progresses over the course of several months. Features include oval cell hypertrophy and hyperplasia, increased hepatocellular mitoses, prominent ductule formation and lymphoplasmacytic cholangitis and hepatitis. As the lesions progress, the liver becomes fibrotic and develops extensive bile duct hyperplasia, hepatocytomegaly and peribiliary lymphoid nodule formation (4). In this case, there was marked hepatocytomegaly and karyomegaly noted.

Mouse Hepatitis Virus Infection (MHV), caused by a coronavirus, was considered in the differential diagnosis; however, MHV infection results in virus-induced syncytia formation and acute hepatic necrosis but does not typically result in biliary hyperplasia (4).

H. hepaticus is significant to the research community for several reasons besides the effects of acute infection: it causes an increase in hepatocellular tumors in certain strains of mice and promotes experimental chemical hepatic carcinogenesis thereby confounding research efforts (4).

Contributor: Charles River Laboratories, www.criver.com

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CASE III – 2003Abbott20 (AFIP 2986815)

Signalment: An approximately 13-week-old, female, Sprague Dawley rat

History: This rat is one of ten female rats given a fibrate derivative in the diet for approximately 35 consecutive days. Control rats received the basal diet only. There were no clinical signs of toxicity, but body weight gain and food consumption were decreased in the treatment group. This rat survived to the scheduled necropsy.

Gross Pathology: Absolute and relative liver weights were increased, and hepatomegaly was noted in most of the animals in the treatment group.

Laboratory Results: Increased alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase activities. Creatine kinase (CK) levels were not evaluated.

Histopathologic Description: Sections of skeletal muscle are from the gastrocnemius muscle. Some sections also include soleus muscle. There are multifocal areas of myofiber degeneration characterized by loss of striations, hyaline change, increased eosinophilia, presence of angular fibers and phagocytosis. Infiltration of primarily macrophages is observed associated with degenerate fibers. Evidence of regeneration (nuclear hypertrophy, internalization of nuclei, sarcoplasmic basophilia) is also present. The deep portions of the intermediate gastrocnemius and the soleus muscles are the most severely affected. Immunohistochemistry was performed against myosin from type 1 (slow, oxidative) skeletal muscle fibers or against myosin from type 2 (fast, glycolytic) fibers. Areas of densest type 1 muscle fiber concentration coincided with zones in this rat having the most prominent skeletal muscle degeneration evident in H&E stained sections. Areas lacking type 1 muscle fibers also lacked evidence of degenerative change in H&E sections.

Contributor's Morphologic Diagnoses: Skeletal muscle, caudal thigh: Degeneration, myofiber, multifocal, mild to moderate, with histiocytic cell infiltration and myofiber regeneration.

Liver: Hypertrophy, hepatocellular, centrilobular, moderate (tissue not provided)

Contributor's Comment: Fibrates are marketed as lipid-lowering agents in humans, indicated for the treatment of hypercholesterolemia, mixed dyslipidemia and Types IV and V hypertriglyceridemia. Fibrates and other types of lipid-lowering agents such as statins have been reported to cause myopathy in human patients characterized by myalgia, weakness, CK elevation and rarely rhabdomyolysis. The etiopathogenesis of these myopathic changes is poorly understood; as these various agents have very different chemical structures yet cause an indistinguishable myopathic syndrome in humans[1]. Fibrate-induced myopathy in the rat has been previously reported[2,3]. Interestingly, fibrates appear to selectively damage type 1 fibers in the rat[3]. Conversely,

reports on effects of statins and fibrates in humans and statins in rats indicate that type 2 fibers are primarily affected[1,4].

Alterations in cholesterol content of sarcoplasmic membranes appear to be an important factor in the development of myofiber damage by lipid-lowering agents. Membrane fluidity varies inversely to cholesterol content and increases in fluidity can result in significant sarcolemmal changes[1]. Other mechanisms that have been implicated include deficiencies in selenoprotein synthesis, organic anion transporters, muscle energy metabolism, changes in intracellular ubiquinone concentrations and mitochondrial injury[4].

In addition, fibrates are classical inducers of peroxisome proliferation and cytochrome P450 4A1 enzymes. This appears to be mediated by peroxisome proliferator activated receptor alpha (PPAR α) resulting in hepatocellular hypertrophy evident histologically. This type of change is rodent-specific and generally reflective of an adaptive response to the drug[5].

AFIP Diagnosis: Skeletal muscle: Degeneration and necrosis, multifocal, moderate, with histiocytic myositis, Sprague Dawley rat, rodent.

Conference Comment: Conference attendees discussed the histologic features of myocyte degeneration, regeneration and necrosis. Slides are of muscle in cross-section making the evaluation of regeneration more difficult. Regenerative skeletal muscle fibers have reduced myofiber diameter, slightly basophilic sarcoplasm and proliferating satellite cells located between the plasmalemma and the basal lamina. In regenerating myocytes, satellite cells migrate to the center of the damaged myocyte, form longitudinal rows and begin to produce sarcoplasm. In cross-section, regenerative myocytes have slightly basophilic sarcoplasm and centrally located nuclei corresponding to the rows of internal nuclei seen in longitudinal section (6).

There are numerous causes of myositis and myopathies. Attendees discussed a variety of general differentials which include nutritional causes such as vitamin E/selenium deficiency; exertional rhabdomyolysis; injection site injury and reaction to toxic drugs or plants. Toxins include ionophores such as monensin, salinomycin, narasin and lasalocid, which are commonly used as growth promoters in feedlot cattle and coccidiostats in poultry feed; gossypol from cottonseed meal and *Cassia* sp. plants (e.g. coffee senna). All have similar histological lesions including myocyte degeneration, necrosis, regeneration, variable degrees of histiocytic myositis and, in later stages, fibrosis. In horses, nutritional myopathies can be differentiated from ionophore toxicity by the presence of lesions in multiple stages of necrosis (multiphasic). Presumably, ionophore toxicity would be a one time event and result in uniform progression of myocyte necrosis with all lesions at the same stage (monophasic) (6).

The earliest published reports of muscle complications associated with fibrate administration date back to 1968 (7). Since then, there have been numerous reports of

similar findings with statins and fibrates resulting in the moniker, Cholesterol-lowering agent myopathy (CLAM).

Contributor: Abbott Laboratories, Department of Pathology, AP13A/R 469, 100 Abbott Park Road, Abbott Park, IL 60064

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CASE IV – C04-2863 (AFIP 2985457)

Signalment: 9-month-old, female C57BL/6 GM-CSF homozygous knockout (-/-) mouse (*Mus musculus*)
History: Mouse was used for breeding and underwent no experimental manipulation.

Gross Pathology: The mouse was received in good body condition. All lung lobes were mottled pale tan especially at the periphery, firm on palpation and sunk in formalin (Figure 1). The uterine horns were mildly dilated and thickened and contained small amounts of dark red material. In addition, there were multifocal to coalescing petechiae on the serosal surface of the ventral aspect of the left uterine horn covering a 0.5 x 0.5 cm area.

Histopathologic Description: Numerous alveoli throughout all lung lobes are filled with homogeneous and compact to granular, eosinophilic, acellular, PAS-positive material (Figures 2-4). Scattered alveoli also contain small numbers of large alveolar histiocytes with abundant, foamy cytoplasm. Alveolar septal walls are unaffected and acicular (cholesterol) clefts are not overtly apparent. Moderate to large numbers of lymphocytes and plasma cells expand the interstitium around blood vessels and bronchioles (Figure 5). Cystic endometrial hyperplasia was also present in the uterus (not submitted).

Contributor's Morphologic Diagnosis: Lung, alveolar proteinosis, multifocal to coalescing, chronic, moderate with perivascular and peribronchiolar lymphoplasmacytic infiltrates.

Contributor's Comment: Pulmonary alveolar proteinosis (PAP) is a rare condition characterized by the intra-alveolar accumulation of surfactant protein (1,2). There are three clinically distinct forms of the disease: congenital, secondary and acquired (1,2). Mutations in genes encoding surfactant proteins B or C, or the β chain of the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor result in fatal neonatal respiratory distress. Secondary PAP develops in association with various conditions in which there is functional impairment or reduced numbers of alveolar macrophages. Upwards of 90% of all cases are of unknown etiology and are classified as acquired. However, numerous reports have identified GM-CSF as playing a critical role in the pathogenesis of PAP since the initial description of the condition in 1958.

GM-CSF is a 23-kDa hematopoietic growth factor which modulates the function of mature myeloid cells, especially neutrophil and eosinophil granulocytes and monocytes/macrophages. In addition, GM-CSF is required for pulmonary surfactant homeostasis (2,3). This latter role was first recognized upon the generation of GM-CSF deficient mice which develop a characteristic pulmonary phenotype resembling PAP (3). In these mice, exons 1 and 2 and intron 1 of the GM-CSF gene are deleted; however, the mice are viable and have normal peripheral blood counts and bone marrow cellularity. Although normal at birth, all GM-CSF homozygous knockout (-/-) mice develop PAP by 3 weeks of age. The eosinophilic, PAS-positive, intra-alveolar material contains type C lamellar bodies, also present within phagolysosomes of alveolar macrophages, which are characteristic of surfactant. An additional feature of the pulmonary lesion in mice, which is not reported in human patients, is perivascular and peribronchiolar aggregates of lymphocytes (80% B and 20% CD4+ T) and plasma cells. The relationship between the perivascular and peribronchiolar lymphocytes and plasma cells is not known since similar aggregates are commonly observed in various tissues in mice, especially older mice, and are not necessarily indicative of pathology (4). Both GM-CSF deficient mice and human patients are at increased risk for developing opportunistic bacterial and fungal infections (1,3), although such infections were not a feature of this case.

Although mutations in GM-CSF are not found in human patients with acquired PAP, all patients do have GM-CSF-neutralizing autoantibodies in bronchoalveolar-lavage fluid and serum (1,2). These autoantibodies inhibit the activity of endogenous GM-CSF resulting in a state of functional GM-CSF deficiency similar to that in GM-CSF -/- mice.

Supplemental GM-CSF has been shown to be therapeutic in both mice and humans. Overexpression of GM-CSF targeted to the lung by the surfactant protein C gene in GM-CSF -/- mice resulted in complete resolution of alveolar proteinosis (1,5) as did daily aerosolization of GM-CSF in GM-CSF -/- mice (1,6). In addition, some human patients treated with subcutaneous GM-CSF have responded favorably (1,6).

Based on these observations, it is now clear that GM-CSF is critical for catabolism of surfactant by alveolar macrophages (1). Surfactant, which reduces surface tension and prevents alveolar collapse, is composed of surfactant proteins A, B, C and D that are synthesized, stored and secreted by type II pneumocytes. Approximately 70-80% of surfactant is taken up by type II pneumocytes and recycled or catabolized. Alveolar macrophages internalize and catabolize the remaining surfactant. In GM-CSF -/- mice and humans with PAP, biosynthesis and secretion of surfactant in GM-CSF is normal. Despite increased uptake by alveolar macrophages, catabolism is severely impaired. This results in intra-cellular and intra-alveolar accumulation of surfactant, thereby reducing the surface area available for gas exchange.

Spontaneous PAP has also been reported in CB.17 scid/scid mice (7), germ-free scid/scid-beige mice (8), a 3.5-year-old male Shih Tzu (9) and a 1-year-old Cocker Spaniel (9). PAP can be experimentally induced by crystallized silica (referred to as lipoproteinosis) or amphophilic drugs (referred to as phospholipidosis), and is seen in goats with caprine arthritis-encephalitis virus-induced interstitial pneumonia and lungworm infestation (10).

AFIP Diagnoses: 1. Lung: Alveolar proteinosis, diffuse, moderate, C57BL/6 GM-CSF (-/-) mouse, rodent.
2. Lung: Lymphoid infiltrates, peribronchiolar and perivascular, diffuse, moderate.

Conference Comment: The contributor provides a thorough review of the pathogenesis of pulmonary alveolar proteinosis (PAP) and the role GM-CSF plays in its development.

PAP not only occurs in mice and humans but has been reported in dogs. In one report, a 3_ yr old Shih Tzu diagnosed with PAP responded well to repeated bronchoalveolar lavages during which thick, turbid, white fluid was retrieved from the lungs and shown to contain globules of inspissated mucus, protein, and cholesterol clefts (9).

Attendees noted that the lungs in the provided gross photograph (Fig 1) had not collapsed, and discussed other differentials for mouse lungs which, on gross examination, fail to collapse. The list includes *Pneumocystis carinii* infection, lymphoma and acidophilic macrophage pneumonia. *P. carinii* is an atypical fungus which causes pulmonary infections predominantly in immunocompromised animals and humans. Lymphoma is very common in mice and can be broken down into several classifications with higher incidence rates among specific strains. Acidophilic macrophage pneumonia (AMP) is characterized by an accumulation of macrophages within alveoli with brightly eosinophilic intracytoplasmic crystalline material. Additional information on both *P. carinii* and AMP can be found by reviewing Wednesday Slide Conference number 3, case 1 from 2004-2005.

The conference closed with the moderator emphasizing the importance of conducting an extensive literature review when working with animal models.

Contributor: http://www.mskcc.org
http://www.med.cornell.edu/
http://www.rockefeller.edu

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