



WEDNESDAY SLIDE CONFERENCE 2007-2008

# Conference 7

31 October 2007

Moderator:

Dr. Wayne Anderson, DVM, PhD  
Principal Pathologist  
Wyeth Research  
Chazy, NY 12921

---

---

**CASE I – D6-29 (AFIP 3031647).**

**Signalment:** Female intact Crl:CFW(SW) mouse (*Mus musculus*), approximately 3.5-months-old

**History:** This mouse was in a group of mice exposed percutaneously via the tail to an average of 167 *Schistosoma mansoni* cercariae on 3/29/06 at the Biomedical Research Institute NIAID Schistosomiasis Resource Center (<http://www.schisto-resource.org>), which is a repository that provides schistosome parasites, snail vectors, and infected mammals for researchers. The mice were shipped to our animal facility 5 days following infection. Over a 3-day period 6.5 weeks post infection, several mice were found dead and were necropsied.

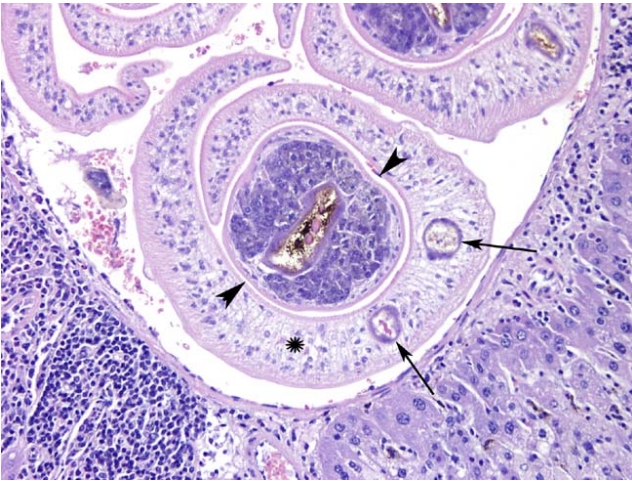
**Gross Pathology:** The mouse had moderate autolysis. The liver was brown and mottled with numerous yellow-tan irregularly shaped foci up to 3 mm diameter extending throughout the parenchyma.

**Histopathologic Description:** In sections of liver, dense accumulations of lymphocytes, eosinophils, epithelioid macrophages, and neutrophils with fewer multinucleated giant cells and plasma cells surround parasite ova or intravascular adult trematodes in periportal tissue or are randomly scattered in the parenchyma. The ova range up to approximately 110 µm by 50 µm, are nonoperculated,

have a thick yellow to brown shell occasionally with a sharp spiny process (lateral spine) protruding from one end of the eggs, and contain a miracidium. A dark yellow-brown granular pigment is present in occasional macrophages surrounding the eggs. Fibroblast proliferation is associated with the inflammation in some areas. Hepatic venules are expanded by adult trematode parasites filled with parenchyma and lacking a body cavity. The **trematodes (fig. 1-1)** are paired, with a smaller trematode (female) present within coiled larger trematodes (male). The caeca of the parasites contain yellow-brown granular pigment and red blood cells. The larger (male) parasites have surface undulations or protuberances, interpreted to be tegmental tuberculations. There are multiple areas of coagulative hepatocyte necrosis scattered throughout the liver. Most hepatocytes are moderately swollen by clear discrete vacuoles or lacy vacuolar change.

**Contributor's Morphologic Diagnosis:** Hepatitis, chronic, granulomatous, periportal to random, circumoval, with multifocal hepatic necrosis associated with intralesional schistosome eggs and intravascular adult schistosomes, liver

**Contributor's Comment:** Schistosomiasis is a disease affecting over 200 million people<sup>7</sup> and is caused by trematodes, most commonly *Schistosoma mansoni*, *Schisto-*



1-1 Liver, Crl:CFW(SW) mouse. Adult trematodes are paired, with the smaller female (Arrowheads) within a gynecophoric canal of the coiled, larger male. The parasites are composed of a tegument with underlying cortical muscle layer, and a spongy, solid body (star) within which are paired caecae (arrows) containing yellow-brown granular pigment and red blood cells. (H&E 200X)

*Soma haematobium*, and *Schistosoma japonicum*. Fresh-water snails (*Biomphalaria* for *S. mansoni*; *Bulinus* for *S. haematobium*, and *Oncomelania* for *S. japonicum*) serve as intermediate hosts.<sup>3</sup> Treatment programs help reduce morbidity, but the prolonged control efforts often cannot be sustained due to lack of resources in affected countries, and so vaccines can hopefully be developed to reduce the intensity of infection and disease severity. Schistosome research includes vaccine development, regulation of  $T_H1$  and  $T_H2$  responses, immunopathology of granulomas and fibrosis, and eosinophil function.<sup>5</sup>

**Life cycle<sup>3</sup>:** Only the cercarial stage released by fresh-water snails is infectious to humans. A schistosome miracidium hatched from an egg in the water penetrates a snail and multiplies into many cercariae, which escape back into the water. The cercariae penetrate the skin of the definitive host and then develop into schistosomules, which migrate into blood vessels and travel to the lungs. After a week, the schistosomules migrate to the liver and hepatic portal venules, where sexual maturity and pairing occurs. The adults travel to mesenteric venules, where *S. mansoni* eggs penetrate the vessel walls and pass through the wall of the intestine into the feces. Many embryonated eggs remain in body tissues. Eggs released in mesenteric venules can be carried in the intrahepatic portal system where they lodge in the hepatic sinusoids and provoke granulomas.

**Human Disease<sup>3</sup>:** In humans, symptoms of acute infection include fever, cough, asthma, hives, and diarrhea with marked eosinophilia. Hepatosplenomegaly and lymphadenopathy can occur. After the initial reaction to the eggs released by the parasites, immune downregulation can decrease these signs. In chronic infections, granulomas (eosinophils, plasma cells, lymphocytes, macrophages, and giant cells) occur around eggs, often with subsequent fibrosis. Symmers' pipestem fibrosis is seen grossly as tracts of portal fibrosis resembling white clay pipestems throughout the tissue, which contributes to portal hypertension and often occurs in patients that lack a downregulation of the immune response. Adult schistosomes in veins do not evoke a host response, but there can be significant reaction to dead worms following treatment or late stages of infection. Glomerulonephritis can also occur, probably due to immune complexes. Colonic polyps containing many schistosome eggs and adults can occur with *S. mansoni* and *S. haematobium* infections. Bilharziomas in the intestinal serosa and mesentery containing fibrous and inflammation around masses of eggs can develop. Cardiopulmonary changes can include pulmonary arteritis and cor pulmonale. Schistosome eggs can also enter the meninges and spinal cord causing meningitis and myelitis. Dermatitis and rashes can occur to the schistosomules. *S. haematobium* causes urogenital schistosomiasis.

**Mouse Model of Schistosomiasis<sup>7</sup>:** The mouse is a widely used model of experimental schistosomiasis. Mice are usually infected percutaneously through the tail or abdomen by exposure to cercaria in water or can be injected subcutaneously or intraperitoneally with cercaria.<sup>5</sup> Infected mice develop hepatic granulomas and immunoregulatory responses similar to humans. Somular antigens that cross-react with schistosomal egg antigens (SEA) induce T-lymphocyte activation and  $T_{M/E}$  cell expansion. Hepatic granulomas develop as a delayed-type hypersensitivity (DTH) response by SEA-reactive  $CD4^+$  ( $ab^+$ ) MHC-II-dependent T cells. Other cells present in the granulomas included  $CD8^+$  T cells, B cells, eosinophils, mast cells, NK cells, basophils, macrophages, neutrophils,  $gd^+$ , and fibrocytes. Mice develop granulomas in the liver, colon, and Peyer's patches, which begin to decrease in size about 8 weeks post infection due to downregulation of the immune response. Hepatic granuloma size was controlled by both  $T_H1$  and  $T_H2$  responses:  $T_H1$  cytokines (IL-2, IFN-g),  $T_H1$  cytokine receptor (IFN-g),  $T_H2$  cytokines (IL-4, IL-5, IL-10, IL-13, TNF-a), and a  $T_H2$  cytokine receptor (IL-4a). Fibrosis was associated with IL-4 and TGF-b, and was independent of the regulation of the hepatic granulomas.

Parasite description<sup>3,4</sup>: Schistosomes are unique from other trematodes infecting humans in that they live in blood vessels, they have separate sexes (most trematodes are hermaphroditic), the eggs are nonoperculated, and the metacercariae are not encysted. Anatomical features of mature schistosomes include an oral and ventral sucker at the anterior end, the lack of a body cavity, a brown granular schistosomal pigment often found in the parasite's cecum, tegmental tuberculations in male *S. mansoni* males, and a gynecophoral canal in males which holds the female. *S. mansoni* eggs are 114-175 µm by 45-68 µm and have a lateral spine.

**AFIP Diagnosis:** Liver: Hepatitis, granulomatous and eosinophilic, random and portal, moderate, with trematode eggs and intravascular trematodes etiology consistent with *Schistosoma mansoni*, CrI:CFW(SW) mouse (*Mus musculus*), rodent.

**Conference Comment:** The contributor gives an excellent review of schistosomiasis in animals and humans. *Schistosoma mansoni* is a member of the family Schistosomatidae, which are the blood flukes of mammals and birds. The three genera that make up this family include *Schistosoma*, *Heterobilharzia* and *Orientobilharzia*. Schistosomatidae of veterinary importance include *Heterobilharzia americana* in mammals of the southern USA, as well as *Orientobilharzia turkestanicum*, *O. dat-tai* and *O. bomfordi* in Asia. Other Schistosomatidae are separated into four groups depending on egg morphology and intermediate snail hosts.<sup>6</sup>

1. ***S. haematobium* group**

*S. bovis* – southern Europe, tropical Africa and Asia; portal and mesenteric veins (ruminants, horses, camels, pigs)

*S. mattheei* – central and southern Africa; stomach, urogenital, portal and mesenteric veins (ruminants)

*S. curassoni* – west Africa (ruminants)

*S. leiperi* – central Africa (artiodactyls)

2. ***S. mansoni* group** – central Africa

*S. rodhaini* (dogs, carnivores)

3. ***S. indicum* group** – India and southeast Asia

*S. spindale* – mesenteric veins (ruminants, horses, dogs)

*S. nasale* – nasal mucosal veins (cattle, goats, horses)

*S. indicum* – portal and mesenteric veins (herbivores)

*S. incognitum* – (swine, dogs)

4. ***S. japonicum* group** – Far East

*S. japonicum* – (human, domestic animals)

*S. mekongi* (dogs, humans)

Mixed infections may occur. *S. bovis* and *S. japonicum* are the most pathogenic in cattle and sheep.

Morphological characteristics of trematodes include a digestive tract and no body cavity.<sup>4</sup> Schistosome eggs contain a miracidium, a lateral spine, and have no operculum.<sup>3</sup>

This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant in veterinary parasitology. We are grateful to Dr. Gardiner for his comments and advice on this interesting case.

**Contributor:** Division of Laboratory Animal Medicine, Tufts University (Boston Campus), Boston, MA 02111 and Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA 02139.

**References:**

1. Anderson WI, Smiler KL, Leopard DM, Bell RM: Hepatic granulomas in a female *Cynomolgus* monkey (*Macaca fascicularis*). *Lab Animal* 24:17-19, 1995
2. Anderson WI, King JM, Uhl EM, Hornbuckle WE, Tennant BC: Pathology of experimental *Schistosoma mansoni* infection in the Eastern Woodchuck (*Marmota monax*). *Vet Pathol* 28:245-247, 1991
3. Cheever A, Neafie RC: Schistosomiasis. In: Pathology of Infectious Diseases, ed. Meyers WM, vol.1, pp. 23-47. Armed Forces Institute of Pathology and American Registry of Pathology, 2000
4. Gardiner CH, Poynton SL: Morphological characteristics of trematodes in tissue section. In: An Atlas of Metazoan Parasites in Animal Tissues. pp. 46-49. Armed Forces Institute of Pathology, Washington, D.C., 2006
5. Lewis F: Schistosomiasis. In: Current Protocols in Immunology, pp. 19.1.1-19.1.28. John Wiley & Sons, Inc., New York, NY, 1998
6. Maxie MG, Robinson WF: Cardiovascular system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 95-97. Elsevier Limited, St. Louis, MO, 2007
7. Stavitsky AB: Regulation of granulomatous inflammation in experimental models of schistosomiasis. *Infect Immun* 72:1-12, 2004



**CASE II – CASE 1 (AFIP 3066021).**

**Signalment:** Adult, male, Wistar Hannover rat (*Rattus norvegicus*)

**History:** An approximately 2-year-old male sentinel rat was found dead with no premonitory clinical signs

**Gross Pathology:** Gross findings included prominent, dark-red mesenteric vasculature. Additional findings included approximately 3 ml of hemorrhagic fluid in the abdomen, a deformed spleen that was constricted in the middle, an 2.0 cm diameter, thin walled cyst filled with clear fluid and extending from the pancreatic region, and a 1.0 cm diameter clear cyst protruding from the right kidney.

**Histopathologic Description:** Large and medium sized muscular mesenteric and pancreatic arteries are primarily affected. Some arteries have acute lesions of intimal and medial fibrinoid necrosis with thrombosis and luminal dilatation, destruction of the elastic laminae, mural hemorrhage, and segmental to global transmural infiltration of neutrophils, eosinophils, and mononuclear cells. Inflammatory infiltrates multifocally extend from the adventitia into the periarterial tissues. Other arteries have chronic lesions of irregular mural thickening due to fibrosis (sometimes causing narrowing of the lumen), mononuclear cell infiltrates, and organizing / recanalizing thrombi. Mural mineralization is also multifocally present within the medial layers of some arteries.

Other lesions on the slide include a dilated cystic structure adjacent to the pancreas interpreted to be a dilated lymph vessel as well as mesenteric lymph nodes with sinusoidal erythrocytosis and phagocyte hemosiderosis.

**Contributor's Morphologic Diagnosis:**

1. Mesenteric and pancreatic arteries; Polyarteritis, necrotizing, multifocal, with mixed cell inflammation, fibroplasia, mural hemorrhage, and occlusive thrombi, rat
2. Mesenteric arteries, media; Mineralization, moderate, multifocal
3. Lymph nodes (multiple); Erythrocytosis, sinusoidal, moderate, multifocal, with phagocyte hemosiderosis
4. Abdomen, peripancreatic; Dilated lymph vessel, focal

**Contributor's Comment:** The signalment, anatomical locations, gross appearance, and histologic characteristics of this case are consistent with those of polyarteritis nodosa (PAN). PAN is a progressive degenerative, inflammatory, and necrotizing disease that most commonly affects small to large arteries of the mesentery, pancreas, kidney, and testis.<sup>7</sup> The aorta, arterioles, and smaller caliber vessels are typically spared.<sup>7</sup> As observed in this case, the presence of acute, healing, and old lesions within a single animal is highly characteristic of PAN.<sup>7</sup> Clinically, polyarteritis nodosa tends to occur in aged rats (reports vary between 500 and 900 days). Although polyarteritis nodosa is most often an incidental finding in aged rats, it can be fatal if, as is likely in this case, se-

verely thrombosed mesenteric arteries rupture leading to fatal hemorrhaging into the abdominal cavity.<sup>2</sup>

PAN is typically considered to be an immune-mediated disease,<sup>7,12</sup> but the disease has also been associated in some studies with corticosteroid administration, estrogen treatment, exposure to chemical carcinogens, and hypersensitivity.<sup>2</sup> PAN has a high incidence in spontaneous hypertensive rat strains as well as in rats with late stage chronic nephropathy (which was present in this case).<sup>7,9</sup> The multifocal, moderate mineralization of the arterial media seen in this animals may also be secondary to chronic renal disease.<sup>7</sup>

Other related lesions observed microscopically in this animal included a focally extensive area of splenic coagulative necrosis with abundant intralesional thrombi, inflammation, hemorrhage, and hemosiderosis. This likely represents an infarct associated with the thrombi initiated by necrotizing polyarteritis.

**AFIP Diagnosis:**

1. Pancreas and mesentery: Arteritis and periarteritis, proliferative and necrotizing, chronic, multifocal, severe, with multifocal mineralization and thrombosis, Wistar Hannover rat (*Rattus norvegicus*), rodent.
2. Pancreas, exocrine: Atrophy, multifocal, mild.
3. Lymph node: Draining hemorrhage, chronic, with sinusoidal ectasia.

**Conference Comment:** The characteristic histologic lesion of polyarteritis nodosa (PAN) is segmental fibrinoid degeneration and thickening of the tunica media of affected arteries with an inflammatory infiltrate composed mainly of mononuclear cells with fewer neutrophils.<sup>9</sup> The size of the vessel lumen are markedly variable, with potential thrombosis with or without recanalization.<sup>9</sup> Both acute and chronic inflammatory processes may occur within the same individual.

In rats, lesions occur most commonly in medium-sized arteries of the mesentery, pancreas, pancreatic-duodenal arteries, and testis of male, Sprague-Dawley and spontaneous hypertensive rat (SHR) strains.<sup>9</sup> Microscopic lesions may occur in most organs except for the lungs.<sup>9</sup> In mice, most lesions occur within small and medium-sized arteries of the tongue, pancreas, heart, kidneys, mesentery, urinary bladder, uterus, testes, and gastrointestinal tract of MRL and NZB mice.<sup>9</sup>

Changes within the vessel walls can be highlighted with special stains. The modified Movat's pentachrome method stains elastic laminae black, collagen and reticular fibers yellow, ground substance and mucin blue, fibrin

intense red, and muscle fibers red.<sup>11</sup> With the Movat's pentachrome method, the quantity of intimal proliferation is readily apparent as are disruptions of the elastic laminae. Other stains such as Masson's trichrome and smooth muscle actin aid in differentiating increased amounts of intimal connective tissue from smooth muscle hyperplasia.<sup>11</sup>

In dogs, lesions similar to polyarteritis nodosa occur as a syndrome of unknown etiology termed juvenile polyarteritis syndrome or "beagle pain syndrome".<sup>13</sup> An immune-mediated etiology is suspected.

**Contributor:** Novartis Pharmaceuticals Corporation, One Health Plaza, East Hanover, NJ, 07936, USA.  
http://www.novartis.com

#### References:

1. Bishop SP: Animal models of vasculitis. *Toxicol Pathol* 17:109-117, 1989
2. Carlton WW, Engelhardt JA: Polyarteritis, rat. In: *Monographs on Pathology of Laboratory Animals*, eds. Jones TC, Mohr U, Hunt RD, vol. 9, pp. 71-76. Springer-Verlag, Berlin, 1991
3. Chamanza R, Parry NMA, Rogerson P, Nicol JR, Bradley AE: Spontaneous lesions of the cardiovascular system in purpose-bred laboratory nonhuman primates. *Toxicol Pathol* 34:357-363, 2006
4. Cohen JK, Cai LQ, Zhu YS, La Perle KM: Pancreaticoduodenal arterial rupture and hemoabdomen in ACI/SegHsd rats with polyarteritis nodosa. *Comp Med* 57:370-376, 2007
5. Kempner W, Peschel E, Black-Schaffer B: Effect of diet on experimental hypertension and on the development of polyarteritis nodosa in rats. *Circ Res* 3:73-78, 1955
6. Maxie MG, Robinson WF: Cardiovascular system. In: *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, ed. Maxie MG, 5th ed., vol. 3, pp. 69-73. Elsevier Limited, St. Louis, MO, 2007
7. Mohr U, Dungworth DL, Capen CC: Cardiovascular system. In: *Pathobiology of the Aging Rat*, vol. 1, pp. 306-309. ILSI press, Washington, D.C, 1996
8. Percy DH, Barthold SW: Mouse. In: *Pathology of Laboratory Rodents and Rabbits*, 3rd ed., p. 105. Blackwell Publishing, Ames, IA, 2007
9. Percy DH, Barthold SW: Rat. In: *Pathology of Laboratory Rodents and Rabbits*, 3rd ed., p. 164. Blackwell Publishing, Ames, IA, 2007
10. Porter, BF, Frost P, Hubbard GB: Polyarteritis nodosa in a Cynomolgus macaque (*Macaca fascicularis*). *Vet Pathol* 40:570-573, 2003
11. Prophet EB, Mills B, Arrington JB, Sobin LH: *Laboratory Methods in Histotechnology*, pp 128-130. American Registry of Pathology, Washington DC, 1994
12. Schoen FJ: Blood vessels. In: *Robbins and Cotran Pathologic Basis of Disease*, eds. Kumar V, Abbas, AK, Fausto N, 7th ed., pp. 539-540. Elsevier Saunders, Philadelphia, PA, 1999
13. Son WC: Idiopathic canine polyarteritis in control beagle dogs from toxicity studies. *J Vet Sci* 5:147-150, 2004
14. Van Vleet JF, Ferrans VJ: Cardiovascular system. In: *Pathologic Basis of Veterinary Disease*, eds. McGavin MD, Zachary JF, 4th ed., pp. 606-607. Elsevier, St. Louis, MO, 2007



#### CASE III – AN02-189, AN-02-326, AN02-395, AN04-734 (AFIP 3069049).

#### Signalment:

- AN-02-189-6: 3.5 Mo, male, B6, 129 hybrid, *Mus musculus*, mouse  
 AN-02-326-6: 5 Mo, male, B6, 129 hybrid, *Mus Musculus*, mouse  
 AN-02-395-8: 3 Mo, female, B6, 129 hybrid, *Mus musculus*, mouse  
 AN-04-734-8: 6 Mo, male, B6, 129 hybrid, *Mus musculus*, mouse

**History:** All four mice are homozygous mice for the p53 gene. The mice were on studies to access the influence of p53 on tumor development. All four mice were having labored breath and were submitted for necropsy.

**Gross Pathology:** A large pale gray mass filled the anterior thoracic cavity. One or more red nodules were present in various locations of the heart.

**Laboratory Results:** Lymphoma cells express CD3 and TdT. Vascular tumors had variable expression of CD31, CD34 and VEGFR-2.

**Histopathologic Description:** There are one or more nodules in the ventricular and/or septal wall of the heart and they consist of blood filled vascular channels of varying size. The vascular channels are lined by cells with spindle, round or oval nuclei with thin attenuated cytoplasm. The nuclei vary in size, have finely stippled to open chromatin and one or more small nucleoli. The

tumors have a high mitotic rate, and an occasional atypical mitosis. In focal areas at the periphery of the nodules the tumor cells infiltrate the adjacent myocardium or epicardium.

The tumors have multifocal CD31 expression, diffuse CD34 expression and diffuse VEGFR-2 expression in the cells lining the vascular channels. The cytological morphology and IHC profile are considered to be consistent with a hemangiosarcoma. In the lung, sections of some of the AN02-326 slides there are large tumor cells in a small artery and in the lymphomatous infiltrate surrounding the artery. The size of the nucleus of these cells suggests the cells may be metastatic hemangiosarcoma cells. Since it was not possible to confirm this with IHC, it is also possible the cells are lymphoma cells. In a few slides of AN-02-395 there are metastatic foci of tumor cells in the mediastinal fat. In one of the foci tumor is altering the integrity of a blood vessel.

A lymphoma is present in three of the cases associated with this submission. In case AN02-189 the lymphoma extensively involves the mediastinum, one of the mediastinal lymph nodes and the lung focally. In case AN02-326 the lymphoma involves both thymic lobes and does not extend outside of the thymic lobes. In case AN02-395 the lymphoma involves both thymic lobes, breaches the thymic capsule and extends into the adjacent mediastinal fat. The lymphoma associated with all three cases has a high mitotic rate, mild to moderate apoptosis and a starry sky appearance due to tingible body macrophages. The lymphoma cells have nuclei that are predominantly smaller than or equal to that of macrophage nuclei. The lymphoma cells have nuclei that are round, angular or irregular in shape and scanty cytoplasm. Predominantly, the nuclei have fine chromatin and inconspicuous or small nucleoli. However, a few cells have a large nucleus with medium size amphophilic nucleoli. The lymphoma cells of all three cases express CD3 and TdT with strong intensity. The cytological morphology and IHC profile of the lymphomas in all three cases are consistent with a T-cell lymphoblastic lymphoma.

**Contributor's Morphologic Diagnosis:**

AN-02-189; Heart, ventricular wall left: Hemangiosarcoma.

Mediastinum: T-lymphoblastic lymphoma.

AN-02-326; Heart; ventricular wall right left septum: Hemangiosarcoma multiple.

Thymus: T-lymphoblastic lymphoma, bilateral noninvasive nodule with invasion of the epicardium.

AN-02-395; Heart; ventricular, septum right: Hemangio-

sarcoma.

Mediastinum, fat: Hemangiosarcoma, multifocal, metastatic.

AN02-734; Heart; ventricular wall: Hemangiosarcoma, invasive.

Mediastinal, fat: Steatites.

**Contributor's Comment:** It is reported in the literature<sup>1,2,4,6</sup> that the majority of p53<sup>-/-</sup> mice die by six months of age due to their tumor load. Lymphomas, sarcomas and carcinomas occur in varying proportions depending on whether the mice are p53<sup>-/-</sup> or p53<sup>+/-</sup>.<sup>2,4</sup> Lymphomas are more common than sarcomas in p53<sup>-/-</sup> mice. In contrast, sarcomas are more common than lymphomas in p53<sup>+/-</sup> mice. The sarcomas consist of a variety of lineages with hemangiosarcomas and osteosarcomas being the most common.<sup>2,4</sup> Compared to wild type mice, hemangiosarcomas occur at a high frequency in both p53<sup>-/-</sup> and p53<sup>+/-</sup> mice. The incidence of hemangiosarcoma is 20-23% in p53<sup>-/-</sup> mice and 4-6% in p53<sup>+/-</sup> mice.<sup>1,2,4</sup> Except for one report, the incidences of hemangiosarcomas occurring in the heart of p53-deleted mice is not indicated and in that report a hemangiosarcoma was also present the perirenal fat.<sup>1</sup> The fact that a p53 deficient mouse may have a hemangiosarcoma in multiple tissues may account for the lack of tissue delineation of the hemangiosarcomas in these reports. In the contributor's experience hemangiosarcomas often occur in variety of organs including the heart in mice with a p53 deletion. The problem arises in determining whether the hemangiosarcomas are metastases of a monocentric tumor or whether the hemangiosarcomas in the various tissues are primary tumors of multicentric origin. Except for small metastatic foci in AN-02-326 and AN-02-395, the heart was the only organ identified at necropsy and histological examination of multiple tissues to have a hemangiosarcoma in the 4 cases associated with this submission. Therefore, it was felt the hemangiosarcoma in these cases represent examples of a primary hemangiosarcoma arising within the heart.

Lymphoma occurs in 60-70% of p53<sup>-/-</sup> mice and approximately 30% of p53<sup>+/-</sup> mice.<sup>1,2,4,6</sup> Approximately, 75% of the lymphomas in these mice are of T-lymphocytic lineage with the vast majority being of the lymphoblastic type. The lymphomas associated with 3 of the cases associated with this submission are a T-cell lymphoblast lymphoma typical of those that develop in p53<sup>-/-</sup> mice. The primary focus of the submission was to illustrate an example of primary hemangiosarcomas that occur in p53<sup>-/-</sup> mice. Strain background can affect the types and frequency of tumors in genetic manipulated mice. However, the incidence of lymphomas and heman-

Stage	Cyclin-CDK Complex	Inhibitors
G1	Cyclin D/ cdk 4	P16INK4a & p21
G1 → S	Cyclin E/ cdk 2	P27
S → G2 → M	Cyclin A/ cdk 2	
M	Cyclin B/ cdk 1	

giosarcomas are similar in p53<sup>-/-</sup> mice of the 129 background and p53<sup>-/-</sup> mice of the C57Bl/6,129 hybrid background.<sup>2,5</sup>

**AFIP Diagnosis:** 1. Heart, ventricle: Hemangiosarcoma, B6,129 Hybrid mouse (*Mus musculus*), rodent.  
2. Mediastinum; lymph node; thymus; lung: Lymphoma.

**Conference Comment:** The p53 gene encodes a transcriptional regulatory protein that is involved in regulation of the cell cycle and apoptosis following DNA damage.<sup>2</sup> In the normal cell cycle, progression from one stage to the next is controlled by certain cyclin and CDK complexes. The activity of the cyclin and CDK complexes are tightly controlled by CDK inhibitors of two main classes: Cip/Kip (p21 & p27), and INK4/ARF (p16INK4a & p14ARF). Transcriptional activation of the Cip/Kip inhibitor p21 is controlled by p53.<sup>7</sup>

Loss of functional p53 gene is linked to Li-Fraumeni syndrome in people, and results in an inherited predisposition to cancer development. Genetically engineered p53 knockout or deficient mice have greatly increased incidence of numerous neoplasms including osteosarcoma, soft-tissue sarcomas, and lymphomas.<sup>2</sup>

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

[www.stjude.org](http://www.stjude.org)

#### References:

- Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA, Butel JS, Bradley A: Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumors. *Nature* 356:215-221, 1992
- Donehower LA, Harvey M, Vogel H, McArthur MJ, Montgomery CA, Park SH, Thompson T, Ford RJ, Bradley A: Effects of genetic background on tumorigenesis in p53 deficient mice. *Mol Carcinog* 14:16-22, 1995

- Fry MM, McGavin MD: Bone marrow, blood cells, and lymphatic system. In: *Pathologic Basis of Veterinary Disease*, eds. McGavin MD, Zachary JF, 4th ed., pp. 798-802. Elsevier, St. Louis, MO, 2007
- Harvey M, McArthur MJ, Montgomery CA, Butel JS, Bradley A, Donehower LA: Spontaneous and carcinogen-induced tumorigenesis in p53 deficient mice. *Nat Genet* 5:225-229, 1993
- Harvey M, McArthur MJ, Montgomery CA, Bradley A, Donehower LA: Genetic background alters the spectrum of tumors that develop in p53-deficient mice. *FASEB J* 7:938-943, 1993
- Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, Bronson RT, Weinberg RA: Tumor spectrum analysis in p53-mutant mice. *Curr Biol* 4:1-7, 1994
- Kumar V, Abbas, AK, Fausto N: *Robbins and Cotran Pathologic Basis of Disease*, eds., 7th ed., pp. 289-292. Elsevier Saunders, Philadelphia, PA, 1999
- Mahler JF, Flagler ND, Malarkey DE, Mann PC, Haseman JK, Eastin W: Spontaneous and chemically induced proliferative lesions in Tg.AC transgenic and p53-heterozygous mice. *Toxicol Pathol* 26:501-511, 1998
- Maxie MG, Robinson WF: Cardiovascular system. In: *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, ed. Maxie MG, 5th ed., vol. 3, p. 209. Elsevier Limited, St. Louis, MO, 2007
- Percy DH, Barthold SW: *Pathology of Laboratory Rodents and Rabbits*, 3rd ed., pp. 48, 114-115, 170-170, 182-185, 205, Backwell Publishing, Ames, IA, 2007
- Petruska JM, Frank DW, Freeman GB, Evans EW, MacDonald JS: Toxicity and carcinogenicity studies of chlorpromazine hydrochloride and *p*-Cresidine in the p53 heterozygous mouse model. *Toxicol Pathol* 30:696-704, 2002
- Storer RD, French JE, Haseman J, Hajian G, LeGrand EK, Long GG, Mixson, LA, Ochoa R, Sagartz JE, Soper KA: p53<sup>+/-</sup> Hemizygous knockout mouse: overview of available data. *Toxicol Pathol* 29:30-50, 2001
- Usui T, Mutai M, Hisada S, Takoaka M, Soper KA, McCullough B, Alden C: CB6F1-rasH2 mouse: overview of available data. *Toxicol Pathol* 29:90-108, 2001
- Valli VEO: Hematopoietic system. In: *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, ed.

Maxie MG, 5th ed., vol. 3, pp. 209-210. Elsevier Limited, St. Louis, MO, 2007  
 15. Van Vleet JF, Ferrans VJ: Cardiovascular system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 593-597. Elsevier, St. Louis, MO, 2007



**CASE IV - V07 - 00474 (AFIP 3067193).**

**Signalment:** 3-year-old, female, New Zealand white rabbit, *Oryctolagus cuniculus*

**History:** The rabbit was not eating and drinking for three days; diet consisted of pellets. No other history provided.

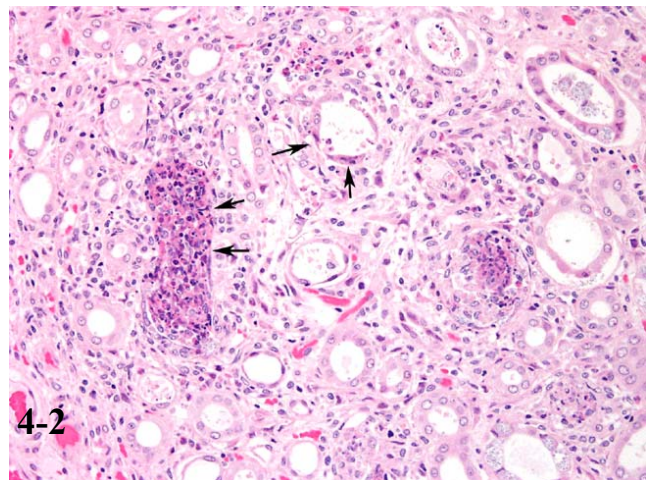
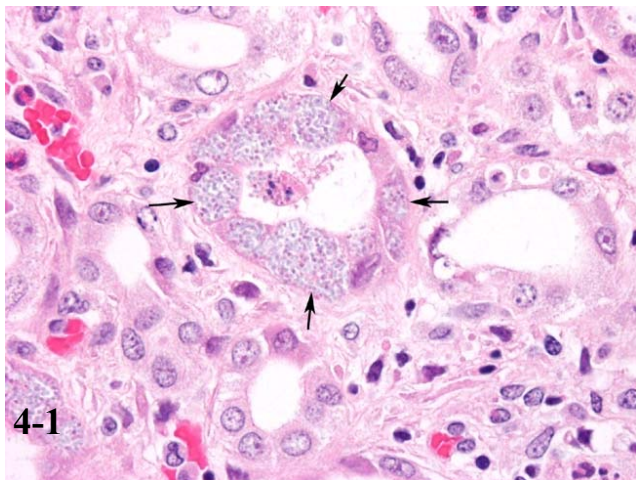
**Gross Pathology:** The rabbit was in poor body condition with mild postmortem autolysis. The lungs were moderately congested and edematous. No other gross lesions were present.

**Laboratory Results:** Aerobic culture from the lung identified rare coagulase negative *Staphylococcus* sp. Fecal

flotation was positive for *Eimeria* sp. oocysts.

**Histopathologic Description:** Numerous cortical and medullary tubules are dilated and are at least partially lined by swollen renal tubular epithelial cells that contain a cytoplasmic 10-15 micron diameter cyst (fig. 4-1) filled with myriad 3-5 micron diameter round to oval protozoal tachyzoites. Many of the affected renal tubular epithelial cells are **degenerate and necrotic** (fig. 4-2) with the tubular lumen filled with protozoa, cellular debris, and protein. The tubules with necrotic epithelium are lined by flattened and regenerative epithelial cells. Moderate numbers of the affected tubules are markedly dilated by numerous neutrophils and smaller numbers of macrophages, many of which contain cytoplasmic protozoa. The tubules filled with neutrophils are surrounded by macrophages, neutrophils, lymphocytes, and plasma cells. The renal pelvis contains a few perivascular foci of lymphocytes and plasma cells. The transitional epithelium of the renal pelvis is mildly hyperplastic.

**Contributor's Morphologic Diagnosis:** Kidney: Nephritis, tubulointerstitial, multifocal, moderate with tubular dilatation, tubular epithelial degeneration and necrosis with intralésional protozoa, New Zealand white rabbit, lagomorph, etiology consistent with *Encephalitozoon cuniculi*;



4-1 Kidney, New Zealand White rabbit. Tubular epithelial cells are greatly expanded by protozoal cysts (arrows) characterized by a 1 µm cyst wall bounding myriad 3-5 micron diameter round to oval tachyzoites. (H&E 600X)

4-2 Kidney, New Zealand White rabbit. Multifocally, there is tubular epithelium attenuation, degeneration and necrosis (arrows), and the lumina are filled with necrotic debris (cellular casts). (H&E 200X)

Kidney: Pyelitis, lymphoplasmacytic, perivascular mild, with mild transitional epithelial hyperplasia

**Contributor's Comment:** Encephalitozoonosis is caused by *E. cuniculi*. *E. cuniculi* and other microsporidial parasites have been regaining interest as opportunistic pathogens for individuals with a compromised immune systems.

*E. cuniculi* was first described as the etiologic agent of "infectious motor paralysis" in 1922 and was once thought to be a confounding disease affecting mainly laboratory rabbits.

*E. cuniculi* is a member of the phylum Microspora that includes single-celled, spore-forming, obligate intracellular parasites with a direct life cycle. *E. cuniculi* is the most extensively studied microsporidium that can infect a large variety of mammals.<sup>8</sup>

Microsporidia are traditionally considered protozoal organisms although novel genetic and molecular evidence suggest closer phylogenetic relationship to fungi. These findings include the presence of a particular mitochondrial heat shock protein that is more closely related to that of fungi as well as the composition of  $\alpha$ - and  $\beta$ -tubulin resemble more fungal tubulins. The organism contains chitin and trehalose that are also typical fungal components (reviewed in<sup>9</sup>).

*E. cuniculi* has three strains: strain I was found in rabbits and humans, strain II in rodents and blue foxes, and strain III in dogs and humans. Identification of microsporidia species in humans and animals suggest possible zoonotic transmission. In humans, mostly immunocompromised (such as HIV positive patients with AIDS) individuals are infected. Though strain III of *E. cuniculi* is found in humans and dogs, no direct evidence suggests that dogs can transmit the disease to humans.<sup>1</sup>

The organism contains a coiled polar filament in the mature spore stage. The sporoplasm following extrusion from the spore coat becomes capable of invading a susceptible host cell. Upon entry multiplication occurs in association with a cytoplasmic vacuole. Sporoblasts develop into mature spores and the ruptured host cell releases the organisms into the surroundings that can then infect other cells.<sup>7</sup> The organism does not contain mitochondria and peroxisomes. Intervening DNA sequences (introns) are rare in microsporidial DNA suggesting that the parasite is highly adapted to survive in their hosts.<sup>9</sup>

Infection in mammals most often occurs by ingestion or inhalation of contaminated urine or feces shed by in-

fecting hosts. Also, infection by transplacental transmission and traumatic inoculation has also been described.<sup>1</sup> In the large domestic rabbits the infection is usually sub-clinical and renal lesions are frequently found incidentally. Occasional nervous signs with mortality can occur in young, heavily infected New Zealand rabbits. Dwarf rabbits are especially susceptible. Infection in mice, guinea pigs, nonhuman primates, carnivores and other mammals have also been reviewed.<sup>9</sup> In neonatal dogs and blue foxes severe symptoms may develop.<sup>3</sup> A serological study in the UK among pet rabbits tested as either companion of infected rabbits or part of a health screen have shown 14 of 38 asymptomatic rabbits to be seropositive. 87 rabbits showing neurological, renal or ocular signs suggestive of encephalitozoonosis were also tested.<sup>2</sup>

For rabbits, the usual source of infection is spores shed in the urine from actively infected rabbits but they are also readily infected experimentally by oral or respiratory routes. The spores pass to the systemic circulation via infected mononuclear cells. Target organs initially are those with high blood flow such as lung, liver and kidney. At 1 month after oral inoculation moderate to marked lesions can be demonstrated primarily in lung, liver, kidney, and not in the CNS. At 3 months postinoculation, moderate to severe lesions are present in the kidney and minimal changes in heart, lung, liver, and also in the brain. Serum titers can be detected at 3-4 weeks and become high at 6-9 weeks. Spores are shed at 1 month in the urine up to 2 months. Shedding terminates at 3 months.<sup>7</sup>

The parasite is able to infect a large variety of cell types such as neurons, epithelial cells of ependyma and choroid plexus, renal tubular epithelium, endothelium and macrophages.<sup>3</sup> Lesions in the kidney occur as focal, irregular, depressed, 1-100  $\mu$ m diameter areas. In severe cases lesions may extend into the underlying cortex. Granulomatous lesions are evident in the interstitium of the lung, kidney and liver at 1 month postinoculation. In the lung, focal to diffuse interstitial pneumonitis with mononuclear cell infiltration may occur. Hepatic lesions are characterized by focal granulomatous inflammatory response with periportal lymphocytic infiltration. Focal lymphocytic infiltrates may also occur in the myocardium. Early lesions in the kidney display focal to segmental granulomatous interstitial nephritis with degenerated epithelial cells and mononuclear cell infiltration. Lesions minimally involve glomeruli. Spores may be present in epithelial cells, macrophages, inflammatory foci, or free within collecting tubules. At 1-2 months postinoculation organisms are already present in the kidneys. At a later stage, in renal lesions interstitial fibrosis, collapse of parenchyma, mononuclear cell infiltrations are typical. The

organism is eliminated from the kidneys by this time.

In the CNS, lesions do not occur until after the 1 month of exposure. Changes are focal nonsuppurative, granulomatous, meningoencephalomyelitis with astrogliosis and perivascular lymphocytic infiltration. Astroglial cells and granulomatous inflammatory foci contain organisms. Lesions can be detected in the absence of organisms. *E. cuniculi* infection has been also associated with cataractous changes. Organisms can be identified within the affected lens stroma. These cases are likely caused by intrauterine infections.<sup>7</sup> In the nervous system, the lesions are wide spread nonsuppurative meningoencephalomyelitis and the severity varies unpredictably in the different parts suggesting random localization of the organism and irregular distribution of inflammatory vascular changes. Small vessels are surrounded by focal gliosis and microscopic granulomas. Mononuclear cells form cuffs around larger vessels that show segmental fibrinoid change involving the adventitia and perivascular space and eventually appear similar to epithelial cells. Astrocytosis occurs in the surrounding parenchyma. The vascular lesions in the meninges, in the acute infection resemble polyarteritis nodosa and in the chronic disease become dominated by sclerotic changes with persisting perivascular cuffing and granulomatous reactions. Surviving puppies develop progressive renal disease.<sup>4</sup>

Staining properties of the organism and the nature of the inflammatory response can differentiate it from other infections such as *Toxoplasma gondii*. Gram positive stains mark organisms as 1.5-2.5  $\mu\text{m}$  in size. Carbon fuchsin stains them purple. In addition, serology tests are available such as modified India ink immunoreaction test as well as indirect immunofluorescent microscopy and a dot ELISA test. An intradermal skin test has been used also to detect infected rabbits.<sup>7</sup> Electron microscopy can reveal the organisms in parasitophorous vacuoles as well as the distinctive polar filaments.<sup>3</sup>

**AFIP Diagnosis:** Kidney: Nephritis, tubulointerstitial, necrotizing, chronic-active, multifocal, moderate, with myriad microsporidia, etiology consistent with *Encephalitozoon cuniculi*, New Zealand white rabbit (*Oryctolagus cuniculus*), lagomorph.

**Conference Comment:** The contributor gives an excellent review of encephalitozoonosis. The numerous organisms present in this case, is very unusual. Infections

are usually subclinical with very few organisms which are difficult to find.<sup>4</sup> Histologic changes are seen most commonly in the brain and kidney and are usually the result of a segmental vasculitis caused by parasitism of vascular endothelium.<sup>4</sup> In this case a mixture of chronic and acute inflammatory lesions are noted associated with the tubules.

**Contributor:** NMDA, Veterinary Diagnostic Services, P.O. Box 4700, Albuquerque, NM 87196-4700

#### References:

1. Didier PJ, Snowden K, Alvarez X, Didier ES: Microsporidiosis. In: Infectious Diseases of the Dog and Cat, ed. Green CE, 3rd ed., pp. 711 - 716. Saunders, St. Louis, MO, 2006
2. Harcourt-Brown FM, Holloway HK: *Encephalitozoon cuniculi* in pet rabbits. Vet Rec 152:427-431, 2003
3. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th Ed., pp. 575 - 578. Lippincott Williams & Wilkins, Baltimore, Maryland, 1997
4. Maxie MG, Youssef S: Nervous system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 433-435. Elsevier Limited, St. Louis, MO, 2007
5. Maxie MG, Newman SJ: Urinary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, p. 479. Elsevier Limited, St. Louis, MO, 2007
6. Newman SJ, Confer AW, Panciera RJ: Urinary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 662-664. Elsevier, St. Louis, MO, 2007
7. Percy DH, Barthold SW: Rabbit. In: Pathology of Laboratory Rodents and Rabbits, 3rd ed., pp. 290-294. Blackwell Publishing, Ames, IA, 2007
8. Szabo JR, Shaddock JA: Experimental encephalitozoonosis in neonatal dogs. Vet Pathol 24:99-108, 1987
9. Wasson K, Peper RL: Mammalian microsporidiosis. Vet Pathol 37: 13-28, 2000
10. Wilcock BP: Eye, eyelids, conjunctiva and orbit. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1396-1397. Elsevier, St. Louis, MO, 2007
11. Zeman DH, Baskin GB: Encephalitozoonosis in squirrel monkeys (*Saimiri sciureus*). Vet Pathol 22:24-31, 1985