The Armed Forces Institute of Pathology
Department of Veterinary Pathology
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CONFERENCE 21
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CASE I - SFFV-P-2 (AFIP 2841375)

Signalment: Adult, male, Swiss mouse (*Mus musculus*)

History: This mouse was sacrificed two weeks after being inoculated with Friend spleen focus forming virus (SFFV-P).

Gross Pathology: The spleen and liver were both greatly enlarged. The spleen weighed 1.2 grams.

Laboratory Results: The hematocrit was slightly elevated.

Contributor's Morphologic Diagnosis: Spleen: Erythroleukemia, NIH Swiss mouse.

Etiology: Friend spleen focus forming virus-P (SFFV-P).

Contributor's Comment: The majority of murine erythroleukemias are associated with experimental inoculation of either the Friend leukemia virus or the Rauscher virus (both retroviruses). Reports of spontaneously occurring erythroleukemia in mice are very rare. Erythroleukemia have been described in Tg.AC transgenic mice and occasional cases are experimentally induced with radiation or chemicals.
　　　　　The Friend virus complex consists of two components: the replication-competent murine leukemia "helper" virus and the replication-defective spleen focus-forming virus (SFFV). The SFFV component is responsible for the acute pathogenicity of this complex and has two well-known variants: SFFV-A (anemic form) and SFFV-P (polycythemic form).
　　　　　The disease process can be divided into two stages. The first stage consists of acute, erythroid hyperplasia resulting in marked splenomegaly. The second stage occurs only after viral integration and transformation of erythroid cells (erythroleukemia).
　　　　　SFFV encodes an Env glycoprotein (gp55) that can bind to and constitutively activate erythropoietin receptors (EpoR). This results in a state of continuous

erythropoietin-independent erythroid hyperplasia. Transformation, however, does not occur until after SFFV integrates into the genome (insertional mutagenesis). One common site for integration is at the Spi-1 locus, which results in elevated expression of the transcription factor PU.1. PU.1, which is normally expressed at very low levels in erythroid cells, inhibits differentiation of erythroid cells and facilitates the outgrowth of transformed cells. SFFV integration and transformation has also been associated with inactivation of the tumor suppressor protein p53. In addition to promoting genomic instability, loss of functional p53, especially in the presence of high levels of erythroid cell proliferation, further facilitates the development of transformed cells.

Determining where a particular histologic section lies in this multi-step process of transformation may be difficult. In the submitted section, erythroid hyperplasia is clearly elevated; however, there are also sheets (foci) of erythroblastic cells expanding the red pulp, compressing the adjacent white pulp and generally distorting the normal splenic architecture. These findings are suggestive of the early stages of erythroleukemia. Additional diagnostics such as a hemogram, bone marrow smears, impression smears (spleen, liver) and immunohistochemistry would all be helpful in confirming this diagnosis.

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AFIP Diagnoses: 1. Spleen, red pulp: Erythroleukemia, NIH Swiss mouse, rodent.
2. Spleen, red pulp: Extramedullary hematopoiesis, diffuse, moderate.
3. Pancreas: No significant lesions.

Conference Comment: Most conference participants agreed that this is a hematopoietic neoplasm, and they discussed the keys to diagnosis, which are the anatomic location of the neoplastic cells and the clinical history of retroviral inoculation. Generally, neoplastic cells of mouse granulocytic and monocytic leukemias are anatomically located in both red and white pulp. The neoplastic cells of erythroleukemia are present only in the red pulp and form sheets of erythroid precursors that replace myeloid progenitors and megakaryocytes with compression of periarteriolar lymphoid sheaths.

Cytology of a suspected hematopoietic neoplasm assists in characterization of cellular morphology to differentiate erythroid from myeloid origin. Additionally, a complete blood count characterizes the peripheral blood profile of the patient. In this case, by immunohistochemistry, neoplastic cells are positive for hemoglobin peroxidase, which confirms erythroid origin; and negative for CD68, lysozyme (myeloid markers) and CD3, CD79a (lymphoid markers).

The French American British (FAB) system classifies erythroleukemia in small animals as Acute Myeloid Leukemia (AML) M6A. Erythroleukemia is an acute leukemia with dual cell lineage, distinguished by the coproduction of erythroblasts and myeloblasts with greater than 50% erythroblasts and erythroid cells and less than 30% nonerythroid cells (myeloblasts). Erythroleukemia is rare and has been reported in few species, including mice, cats, and poultry.

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CASE II - 02-12316 (AFIP 2841394)

Signalment: 8-year-old castrated male Standardbred horse

History: This horse, currently in training, presented with anorexia and lethargy of 48 hours duration that rapidly progressed to circling and seizures in the six hours immediately prior to presentation. The horse was euthanized after preliminary diagnostic work-up due to the danger he presented to himself and to the hospital staff because of his severe neurologic problems.

Gross Pathology: Mucous membranes, sclera, body fat, and organs were diffusely yellow (jaundice). The liver was flattened with sharp margins, tough, and had a yellow-brown zonal pattern. Liver weight was 0.96% of body weight (normal 1.1%).

Laboratory Results:
Total bilirubin 486 umol/L (21-57)
Conjugated bilirubin 69 umol / L (2-3)

Free bilirubin 417 umol /L (18-55)
Alkaline phosphatase 389 U/L (119-329)
GGT 57 U/L (7-54)
AST 1060 U/L (259-595)
CK 1851 U/L (108-430)
Serum bile acids 136 umol / L (0-28)
Serum ammonia 970 umol / L (canine: 20-80)

Serum iron 122 umol / L (14-42)
Total iron binding capacity 122 umol /L (53-87)
Unbound iron 0
% saturation 100

Bacterial culture / liver: few *E.coli* and *Streptococcus sp*

Cell culture isolation: no virus isolated

Iron / liver: 4020 ug / g DW (toxic = 2100-3500 ug/g)
Copper / liver: 35 ug / g DW (toxic = >330ug /g)

Contributor's Morphologic Diagnosis:
Acute periacinar to midzonal hepatocellular necrosis with early regeneration - hepatic iron toxicosis

Contributor's Comment: Extensive hepatocellular necrosis and hemorrhage involved periacinar and midzonal regions. Few intact or markedly vacuolated hepatocytes remained in portal zones, and these were intermingled with multiple aggregates of proliferative oval cells and biliary epithelium, with occasional mitotic figures and megalocytes present among these populations. Portal connective tissue was diffusely infiltrated by densely packed lymphocytes and few plasma cells. In Perl's Prussian blue-stained sections from liver, multiple granular cytoplasmic aggregates staining positively for iron were present in the majority of degenerating and necrotic periacinar and midzonal hepatocytes.

Massive loss of hepatic parenchyma in this horse resulted in hepatic encephalopathy and the severe neurologic abnormalities identified clinically. Although the tentative diagnosis at necropsy was idiopathic 'serum hepatitis' (Theiler's disease) based on the collapsed appearance of the liver, the slightly unusual histologic appearance of liver, with better tissue preservation than expected for this entity and prominent proliferative and inflammatory responses, prompted further investigation of potential toxic causes of severe acute hepatic damage. Iron levels in liver were above those considered to be toxic in horses, consistent with the results of Perl's iron stain on this tissue. Total iron binding capacity and serum iron levels identified 100% saturation of TIBC. These combined findings were consistent with acute iron toxicity, likely resulting from administration of an injectable iron compound, as the cause of massive hepatic necrosis and resulting hepatic encephalopathy. Excessive iron is directly toxic to hepatocyte membranes through iron-catalyzed free radical production and resultant

membrane lipid peroxidation, as well as by direct iron-induced damage of hepatocellular DNA.

There is sparse documentation in the literature of hepatic iron toxicosis in adult horses. Administration of oral iron-containing compounds to neonatal foals has been associated with periportal to panlobular hepatocellular necrosis, biliary proliferation, and mild portal fibrosis. Attempts to induce hepatic injury by oral administration of iron in adult ponies failed to produce histologic liver lesions, suggesting that neonatal hepatocytes may be exquisitely sensitive to the free radical damage induced by excess iron absorbed through the portal circulation. Although the horse's owner declined mycotoxin analysis on feed, the absence of extensive megalocytosis and karyomegaly in residual hepatocytes and nonexistence of leukoencephalomalacia typical of equine fumonisin toxicosis did not support this mycotoxicosis as the cause of liver lesions.

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AFIP Diagnoses: 1. Liver: Hepatocellular degeneration, necrosis and loss, centrilobular to midzonal, diffuse, severe, with oval cell and biliary epithelial proliferation, megalocytosis, and intracytoplasmic golden brown pigment, Standardbred, equine.
2. Liver: Hepatitis, portal, lymphoplasmacytic, diffuse, mild.

Conference Comment: Conference participants reviewed the clinical pathology relevant to this case. The body's iron content is normally controlled by the rate of absorption, not by excretion. The quantity of stored iron and the rate of erythropoiesis regulate absorption. Iron is transported in blood bound to transferrin (measured as serum iron - SI) and by two copper-containing proteins (hephaestin, ceruloplasmin). The amount of iron that transferrin will bind is indirectly measured as total iron-binding capacity (TIBC). Iron typically occupies one-third of the transferrin binding sites, which is expressed as percent saturation. When excess iron is present, transferrin can bind more. Unbound iron-binding capacity (UIBC) is the numeric difference between TIBC and SI.

The marked hyperammonemia suggests decreased hepatic functional mass with decreased conversion of ammonia to urea via the hepatic urea cycle, which can result in hepatic encephalopathy. Marked hyperbilirubinemia also suggests decreased hepatic functional mass. Unconjugated bilirubin predominates in all cases of equine hyperbilirubinemia. This horse's mildly increased gamma-glutamyl transferase (GGT) suggests cholestasis.

The etiopathogenesis in this case is most likely iron toxicosis. However, participants could not exclude with certainty many other possibilities, including serum hepatitis (Theiler's disease), pyrrolizidine alkaloid containing plants and mycotoxicosis (aflatoxins). The AFIP's Department of Hepatic and Gastrointestinal Pathology consulted on this case and concurred that the exact etiology is unclear histologically, but suggestive of a toxic injury. In human medicine, iron toxicity primarily occurs in children who ingest large quantities. As in foals, the resulting hepatocellular necrosis is periportal.

Immunohistochemical assays were used to help evaluate the proliferating cells within the tissue. An assay specific for hepatocytes strongly marked residual

hepatocytes in periportal areas and produced lighter staining of large and/or multinucleated regenerative cells. A pan-cytokeratin assay positively stained the pre-existing biliary epithelium, but rarely and lightly marked proliferating ductal cells. As a side note, oval cells are liver stem cells that have the capability to differentiate into hepatocytes or biliary epithelium.

We are grateful to the Department of Hepatic and Gastrointestinal Pathology for their assistance in this case.

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CASE III - A02-817 (AFIP 2838925)

Signalment: 2.5-month-old female Pygmy goat, *Capra hircus*, caprine

History: This goat was presented to the teaching hospital for respiratory stridor and tachypnea (90 breaths/min). Cardiac ultrasound revealed a pericardial effusion with right atrial and ventricular enlargement. The goat was treated with diuretics and a blood

transfusion, and then sent home. Two weeks later, the goat was re-admitted with a non-regenerative anemia, leukopenia and thrombocytopenia. The owners elected euthanasia.

Gross Pathology: The goat was small, but fat stores were adequate. The pericardial sac contained 20 ml of clear fluid. There was generalized lymphadenomegaly and the lymph nodes were mottled red. Bone marrow was red and firm. A single 1 cm circumscribed red nodule was present in the hepatic parenchyma.

Laboratory Results: A complete blood count during the first presentation revealed severe anemia (HCT = 6%) and leukopenia (3500 cells/ul).

Contributor's Morphologic Diagnoses: 1. Diffuse myelofibrosis, bone marrow.
2. Marked extramedullary hematopoiesis, lymph nodes.

Contributor's Comment: There is extensive fibroplasia of the bone marrow with little to no fat within the marrow cavity. Small islands of erythropoiesis are widely separated by fibroplasia characterized by loosely arranged fibrous connective tissue and fusiform to stellate fibroblasts. Hematopoietic islands contain large blast-like cells. Megakaryocytes are prominent and only a few islands of myelopoiesis are present, but differentiation to mature neutrophils is present in some foci. In the lymph nodes, extensive extramedullary hematopoiesis fills subcapsular sinuses, medullary cords and sinusoids. Large megakaryocytes are most prominent. Also, erythropoiesis is present as well as myeloid differentiation with neutrophils and eosinophils. The nodule in the liver consists of similar hematopoietic tissue.

Myelofibrosis is described in 11 of 16 young pygmy goats from seven litters (G.R. Cain et. al., 1994). The morphologic features are similar to myelofibrosis in humans with dysmegakaryocytopoiesis and to dogs with myelofibrosis associated with congenital pyruvate kinase deficiency. The underlying cause for myelofibrosis in this goat and in the 11 goats previously described is unknown. Marked extramedullary hematopoiesis is in response to anemia, leukopenia, and thrombocytopenia caused by the myelofibrosis. The pathogenesis of myelofibrosis is incompletely understood, but is believed to be due to production of fibrogenic mediators from abnormal hematopoietic cells. Megakaryocytes have been implicated as the source for these mediators since abnormal megakaryocytes are often a prominent feature in myelofibrosis.

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AFIP Diagnoses: 1. Bone marrow: Myelofibrosis, diffuse, moderate, Pygmy goat, (*Capra hircus*), caprine.
2. Lymph node: Extramedullary hematopoiesis, diffuse, marked.

Conference Comment: Most veterinary cases of myelofibrosis are idiopathic or secondary to other conditions, such as irradiation or bone marrow necrosis. There has been an association of myelofibrosis/osteosclerosis with feline leukemia virus infected cats and in dogs and cats having myeloproliferative disease. An inconsistent but

characteristic finding in myelofibrosis is dacryocytosis (teardrop-shaped poikilocytes) in the peripheral blood. Dacryocytes have decreased deformability and imply the presence of developing myelofibrosis. The change in these poikilocytes may be due to alterations in the erythrocyte cytoskeleton. In cases of myelofibrosis, increased fibrous tissue in the bone marrow is demonstrated with reticulum or Masson's trichrome stains.

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CASE IV - PM02-035 (AFIP 2840433)

Signalment: 4-weeks-old female Fell Pony   (38/02)

History: Coughing at three weeks of age. Treated with proprietary cough medicine by owner. No improvement. Seen by Veterinary Surgeon one week later with mild nasal discharge. Investigated for suspected Fell Pony Immunodeficiency Syndrome. Treated with broad-spectrum antibiotic (Ceftiofur). Euthanized two days later after laboratory results available.

Gross Pathology: The foal had pale mucous membranes. There was a bilateral purulent nasal discharge. The mucous membranes of the upper respiratory tract were reddened. Bronchi were lined by glistening epithelium and contained plugs of mucopurulent material. Left and right apices showed well-defined areas of collapse. Other findings included glossal hyperkeratosis.

Microscopic Description: Section of lung with smooth pleura lined by mesothelium. Bronchi and bronchioles contain mucopurulent exudate. Bronchioles are lined by largely intact epithelium with moderate numbers of basophilic intranuclear inclusion bodies consistent with adenovirus infection. There is focal epithelial disruption and shedding. There is bronchiolitis with diffuse peribronchiolar infiltration by neutrophils, lymphocytes and macrophages. Epithelial proliferation is not apparent at this stage. Distal airways contain neutrophils and there is limited focal extension into alveoli. Atelectasis is also present. Syncytial cells are occasionally recognized.

Laboratory Results:

HAEMATOLOGY RESULTS

		26.6.02	28.6.02	Fell Pony** normal values n = 15		
Hb (g/dl)		4.8	5.9	9.1	-	13.9
PCV (1/1)		0.13	0.26	29	-	43
RBC (x 10^{12}/l)		2.91	3.66	6	-	14.1
MCV (fl)		45.0	45.0	35	-	53
MCH (pg)		16.5	16.1	10.6	-	13.6
MCHC (g/dl)		35.8	35.5	27	-	30
WBC (x 10^9/1)		5.6	6.2	8.3	-	14.5
Band Neutrophils	(x 10^9/1)	0.04	0.07	0	-	0.35
Segmented Neutrophils	"	4.29	4.60	2.4	-	8.9
Lymphocytes	"	1.00	1.33	4.27	-	11.07
Monocytes	"	0.27	0.16			
Eosinophils	"	<0.01	0.02			
Basophils	"	<0.01	<0.01			

Occasional Heinz bodies noted.

**Series examined in Department of Veterinary Pathology, University of Liverpool

CLINICAL CHEMISTRY

	26.6.02	28.6.02	Fell Pony** normal values n = 14		
Total protein (g/l)	54	56	48.5	-	72.1
Albumin	31	31	23.2	-	43.1
Globulin	23	25	3.7	-	12.5

LYMPHOCYTE PHENOTYPE

			Normal values
B cells as a % of lymphocytes (peripheral blood)	8	3	28 ± 10%

**Series examined in Department of Veterinary Pathology, University of Liverpool

BACTERIOLOGY

A light growth of *Streptococcus bovis* isolated from lung. The animal had received antibiotic therapy.

Contributor's Morphologic Diagnoses: 1. Bronchiolitis and peribronchiolitis, subacute, multifocal, suppurative, with bronchiolar intraepithelial intranuclear inclusion bodies consistent with adenovirus infection.
2. Atelectasis partial.

Contributor's Comment: Fell Ponies are a native breed in the United Kingdom. They were used extensively in Cumbria as draft animals and are now generally found in North West England. They are used for riding and showing and there is a flourishing Fell Pony Society. Over the last 15 years, a syndrome of increased mortality and morbidity in Fell Pony foals has been recognized. Clinical signs include anaemia, failure to thrive and susceptibility to a range of infections. It is believed that this is as a result of an inherited B cell immunodeficiency. Studies on lymphocytes in peripheral blood show a decline in circulating B lymphocytes. In normal Fell Pony foals, the percentage of B lymphocytes is 18-38% of total lymphocytes. Foals with immunodeficiency consistently have values below 10% (Thomas - Personal communication). The cause of the anaemia is not understood. Other post mortem findings have included glossal hyperkeratosis, granulomatous enteritis, cryptosporidiosis and respiratory infections. Lymphoid tissue is often inconspicuous. Susceptibility to equine adenovirus infection is well recognized in congenital combined immunodeficiency in Arab foals (SCID). It is perhaps not surprising that adenovirus infection also occurs in these Fell Pony foals. The main lesion is a bronchiolitis, which varies from necrotising to proliferative. Airway obstruction by detached epithelium and exudates is responsible for the widespread atelectasis. The relatively low proportion of currently infected cells in this case may account for the overall preservation of airway epithelium. Epithelial proliferation is observed in later stages of the disease. Secondary bacterial infection may also occur. This animal had received antibiotic therapy and only a light growth of *Streptococcus bovis* was isolated, which was not considered significant. This animal was anaemic and lymphopaenic with a reduction in circulating B cells. An autosomal recessive mode of inheritance is under consideration.

AFIP Diagnosis: Lung: Bronchitis and bronchiolitis, suppurative, diffuse, moderate, with multifocal necrosis and epithelial basophilic intranuclear inclusion bodies, Fell pony, equine.

Conference Comment: The contributor has provided a concise summary of the recently recognized syndrome of anemia, ganglionopathy and immunodeficiency in Fell ponies. Severe combined immunodeficiency (SCID) occurs in Arabian foals, dogs and mice. In Arabian foals, there is an autosomal recessive, inherited failure to produce functional B and T lymphocytes due to a mutation in DNA-dependent protein kinase. This enzyme is needed for the receptor gene rearrangements in B and T cells necessary to make mature, functional lymphocytes. SCID foals often succumb to opportunistic pathogens, most commonly adenovirus, *Cryptosporidium parvum*, *Pneumocystis carinii*, and *Rhodococcus equi*. X-linked SCID occurs in Bassett Hounds and Welsh Corgis. Although affected dogs may have normal T cell numbers, the T cells do not respond to mitogenic stimuli due to a defect in the gamma chain of the IL-2 receptor.

Some conference participants preferred the diagnosis of bronchopneumonia. However, like the contributor, we favor the above diagnosis due to the minimal extension of the suppurative inflammation beyond the airways into the surrounding tissue. Gram stains did not demonstrate a secondary bacterial infection.

Reticulocytosis is one parameter used to classify anemia as regenerative versus nonregenerative. Other signs of regeneration include polychromasia, macrocytosis, hypochromasia, erythroid hyperplasia within the bone marrow, and in ruminants, basophilic stippling. Horses are an exception since reticulocytes are not released into their peripheral blood, and therefore reticulocytes are not present in regenerative anemia in horses. This lack of reticulocytosis causes all equine anemias to appear nonregenerative; consequently, repeated complete blood counts and bone marrow evaluation are useful to evaluate regeneration in this species. New methylene blue (NMB) is a fast, nonpermanent preparation used on peripheral blood smears to stain reticulocytes in species other than the horse.

Heinz bodies are protruding round structures of the erythrocyte membrane, that are composed of denatured, precipitated hemoglobin. Heinz bodies stain dark blue with NMB and are indicative of erythrocyte oxidative injury. Heinz body hemolytic anemia occurs in horses, cattle and dogs ingesting onions; in horses consuming wilted red maple (*Acer rubrum*) leaves or receiving phenothiazine; in cattle fed *Brassica* sp., and in dogs, sheep and humans with an inherited deficiency of the glucose-6-phosphate-dehydrogenase enzyme.

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