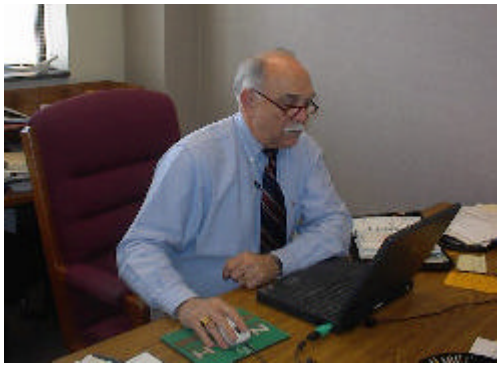
Winter 2001



FROM THE DIRECTOR

H. Leon Thacker, DVM, PhD

Working in the Diagnostic Laboratory is like veterinary practice in many ways, not the least of which is the fact that no two days at work are the same. No danger of getting bored. Dr. Kanitz has recently isolated encephalomyocarditis virus from a pygmy hippopotamus and from the African elephant; the significance of these isolates is still being investigated. We have made diagnosis of enterovirus polioencephalomyelitis causing very high mortality in fattening swine; recurrence of the infection on one premises has presented the problem of identifying effective preventative or control measures. Mycoplasma gallisepticum infection in a group of adult turkeys has presented as an atypical occurrence, tissues and body fluids were positive by PCR diagnosis, gross lesions consistent with the infection in turkeys were present, and some birds showed sero-conversion; however, attempts in our laboratory and two others have been unsuccessful in isolating the agent so that further evaluation by fingerprinting of the particular isolate involved has not been possible.

We will soon be interviewing candidates for a new molecular diagnostician position in the ADDL. We look forward to the individual coming into this position to further develop and evaluate new testing procedures and assist in making those procedures routinely available to diagnostic situations.

Hope this missive finds you enjoying the new year in health and spirit. January 1 could have been better for the Boilermakers, but we were proud of them and they had a great season.

FINAL DIAGNOSIS

Sheep-associated Malignant Catarrhal Fever (by histopathology and PCR)

In each issue, we will feature a case submitted to ADDL that we hope will be of interest to you.

Signalment:

9-month-old Holstein heifer

Clinical history:

Short history of pyrexia, lethargy and nasal discharge. History also indicated this animal was in close association with sheep on the farm.

Gross necropsy findings:

Coronary bands of both front limbs were hyperemic and mildly swollen. There were multiple mucosal erosions/ulcerations of the oral cavity, esophagus, gastrointestinal tract. Nasal turbinates were hyperemic and covered with fibrinonecrotic material. Mesenteric lymph nodes were enlarged and, on cross section, were dark red with areas of necrosis. The meninges were congested. The urinary bladder was hyperemic. The mucosa was covered in some areas by fibrinonecrotic material.

Histopathology findings:

Histopathology revealed lymphocytic to necrotizing/ulcerative lesions associated with vasculitis in oropharyngeal, esophageal and abomasal sections. Histopathology of the cerebrum and cerebellum revealed a moderate lymphocytic meningitis. The meninges were infiltrated by large lymphoblastic-type lymphocytes, and fewer macrophages that extended along Virchow-Robins space and concentrated around cerebral vessels. In some areas, the infiltrates extended into the adjacent neuropil. The urinary bladder was denuded of surface epithelium and overlain by a layer of fibrinohemorrhagic material. The propria submucosa was infiltrated by moderate numbers of lymphocytes, fewer plasma cells and macrophages. The tunica media and adventitia of several small to medium caliber arteries were infiltrated by large lymphoblastic-type lymphocytes. In some vessels, the tunica media was segmentally necrotic and expanded by fibrin deposits. Endothelial cells were swollen.

Morphologic diagnosis:

Multiple tissues; Multifocal ulcerations, erosions, marked, with lymphocytic to necrotizing vasculitis

Brain; Lymphocytic meningitis and Vasculitis

Etiology:

Sheep-associated Malignant Catarrhal Fever virus

Discussion:

Malignant catarrhal fever (MCF) is the clinical manifestation of infection of certain ruminant species with one of a group of pathogenic gammaherpes viruses known as MCF viruses. The disease is sporadic to epidemic and is distributed worldwide. Most domestic cattle and numerous exotic species, such as banteng and gaur are susceptible to clinical disease. Bison, moose and some species of deer are highly susceptible.

The disease syndromes associated with these viruses range from acute, severe inflammatory disease with a short clinical course to a more chronic syndrome. The acute disease is characterized by high fever, lymph node swelling, and widespread inflammation of mucosal surfaces. Lymphoproliferation and vasculitis are the main histologic lesions.

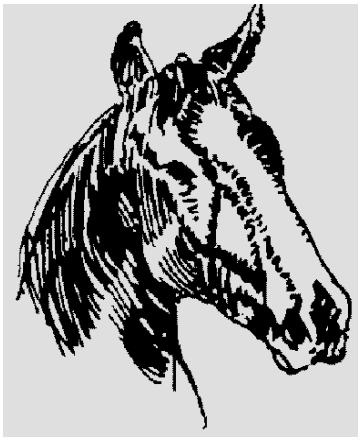
There are two known pathogenic viruses that are etiologically associated with MCF-1) the sheep-associated and 2) the wildebeest-associated. These two viruses are closely related antigenically and genetically. The virus that is endemic in and well-adapted to wildebeest alcelaphine herpesvirus 1 (AHV-1), can be readily isolated. Wildebeest are the principal reservoir hosts. The virus that is endemic in sheep, though never isolated, has been designated ovine herpesvirus 2(OHV-2).

The gross lesions of MCF are similar to those associated with bovine viral diarrhea, infectious bovine rhinotracheitis and bluetongue. In the case described here, indirect immunofluorescence assays did not

detect antiviral antibodies or antigens in lung, spleen and kidney tissue samples for these viruses. Sheep-associated malignant catarrhal fever was highly suspected based on gross and histologic lesions of lymphocytic to fibrinonecrotizing vasculitis in various tissues, and was confirmed by OHV-2 specific PCR. Lymphocytic to necrotizing vasculitis in the brain of cattle, together with fibrinonecrotizing vasculitis in several tissues are characteristic of MCF. The recent development of molecular diagnostic assays has provided effective diagnostic tools for MCF viruses. A serological assay, a competitive-inhibition enzyme-linked immunosorbent assay (CI-ELISA) and PCR for the OHV-2 and AHV-1 strains of MCF viruses have dramatically improved the accuracy of diagnosis of MCF in clinically infected animals.

The pathogenesis of MCF is not well understood, but the early involvement of cytotoxic lymphocytes has been proposed. Sheep are latent carriers of OHV-2 and close association with cattle must be avoided to avoid transmission.

-by Victoria Owiredu-Laast, DVM,
ADDL Graduate student



Equine Polysaccharide Storage Myopathy

Equine polysaccharide storage myopathy (EPSM) is a sub-type of exertional rhabdomyolysis characterized by a defect in glycogen storage in skeletal muscle. This disease is seen in many different breeds including Quarter horses, draft horses and crosses of these breeds. There is evidence that the disease is heritable in Quarter horses; this question has not been answered for other breeds. Skeletal muscles in affected horses have higher amounts of stored glycogen than in normal horses. Affected horses also have higher levels of a complex polysaccharide which is resistant to amylase digestion, aiding identification of the disease by histopathology as described below.

Clinical signs of EPSM are similar to those of all forms of rhabdomyolysis: stiff gait, pain, muscle cramping, and reluctance to move after exercise. However, in some subclinically affected horses, EPSM is not discovered until unusual events (such as anesthesia and subsequent postanesthetic recumbency) occur. A diagnosis of rhabdomyolysis can be made from clinical signs and serum chemistry values. Muscle enzymes such as creatine kinase (CK), lactate dehydrogenase (LDH) and aspartate transminase (AST) will be high after episodes of rhabdomyolysis. Furthermore, diagnosis of rhabdomyolysis does not specify the underlying metabolic problem. In humans and other species there are numerous

metabolic disturbances in glycogenolysis or glycolysis that cause rhabdomyolysis. To date, however, the specific etiology of EPSM has not been elucidated. Studies have shown that EPSM is not a defect in glycogenolysis or glycolysis. Instead, it is believed to be due to increased glucose uptake, possibly due to up-regulation of insulin receptors because affected horses have an exaggerated response to insulin.

EPSM must be differentiated from equine motor neuron disease (EMND), colic, equine protozoal myeloencephalitis (EPM), musculoskeletal injury, hyperkalemic periodic paralysis (HYPP) and even Lyme disease. EMND is an idiopathic degenerative disease of somatic nerves originating in the ventral horn of the spinal cord. These horses will show weakness and trembling due to neurogenic atrophy of type 1, versus type 2 in EPSM, muscle fibers which make up the postural muscles. EPSM can mimic colic because affected horses show signs of discomfort; however, a thorough examination of the gastrointestinal tract will rule out colic. Horses with EPM will often have asymmetrical atrophy. The stiff gait of horses with EPSM may mimic the proprioceptive deficits associated with EPM, but a thorough neurological exam will reveal that horses with EPSM do not cross their pelvic limbs on tight turns. EPSM can look like musculoskeletal injury, as a crouched gait can be a sign of back injury. Stiff gate with decreased flexion is common in both disorders. A thorough physical examination, radiographs, and nuclear scintigraphy should determine if the horse actually has a musculoskeletal disorder. As there is a familial basis in Quarter horses for both EPSM and HYPP, the two must be differentiated. HYPP DNA testing can be done by submitting a blood sample to Veterinary Genetics Laboratory (phone 916.752.7416 for instructions.) Finally, Lyme disease can manifest as shifting leg lameness, stiffness and discomfort. Polymerase chain reaction of synovial fluid of affected horses will reveal infection with *Borrelia burgdorferi* if the horse is infected with Lyme disease. If the above diseases and syndromes cannot be differentiated, a muscle biopsy can be done.

EPSM and EMND are best differentiated by muscle biopsy. The epaxial muscles, specifically the *sacrocaudalis dorsalis medialis*, are made of type 1 fibers and are good samples for EMND diagnosis. For diagnosis of EPSM, a biopsy of the *semitendinosus* muscle is a good choice as this is a type 2 muscle, therefore locomotory, and is affected in EPSM. To obtain the best biopsy sample, the horse should be sedated and administered local anesthesia with either a caudal epidural or line block over the incision site, without actually injecting into the muscle to be sampled. Make a 5 cm longitudinal skin incision parallel to the muscle fibers. Repeat through the fascia. Obtain a strip of muscle approximately 5 cm in length by 1 cm in diameter. Be sure to undermine the muscle first so it does not retract when one end is cut. Place the tissue sample in 10% formalin and submit for histopathologic examination. Punch biopsies do not provide enough tissue for evaluation of EPSM or EMND.

The best tissue stain to identify EMND is Masson's trichrome. EPSM is identified best by staining with both PAS and PAS with amylase digestion. In both EMND and EPSM, there will be excessive variation in muscle fiber size, internal nuclei, and hypertrophy. If the horse has EMND, the muscle cells will have fibrosis and fat infiltration, scattered necrotic and regenerative areas, and intramuscular nerves will be atrophied. In the case of EPSM, the muscle cells will have vacuoles, small rounded fibers, normal intramuscular nerves and glycogen/polysaccharide granules which will not be digested by amylase.

Treatment for EPSM, once diagnosis is made, is primarily dietary therapy. In the past, standard therapy for horses with rhabdomyolysis has been to remove excess carbohydrate, often in the form of grain, from the diet. In the case of EPSM, this is not enough as the horse still makes too much abnormal polysaccharide. Replacement of carbohydrates with a diet high in fat (up to 25%) and protein has worked well to control

signs of EPSM by forcing the horse to metabolize fat and protein for energy. Within six months, many horses treated with this diet resumed training to previous levels, without signs of EPSM and without elevated CK, LDH or AST. Furthermore, horses benefited most from as little rest as possible and recovered sooner than horses treated only with diet modification.

Horses with EPSM often have abnormal thyroid hormone levels despite normally functioning thyroid glands ("euthyroid sick syndrome"); therefore thyroid supplements do not help. Interestingly, horses on the high fat diet resolved their thyroid and vitamin E/ selenium imbalances. This is likely due to better muscle function with fat as an energy source. Once diagnosed, EPSM is treatable; the animal has a reasonable prognosis for return to work. However, because EPSM may be inherited, the veterinarian should advise clients against breeding affected horses.

- by Catherine Alinovi, Class of 2001
- edited by Karen Tucker-Gillum, DVM, ADDL Graduate Student

Nitrate Toxicity in Ruminants

Nitrates are relatively non-toxic; toxicity results when they are reduced to nitrites in foodstuffs or by ruminal flora in ruminants. The main hazard to ruminants is ingestion of plants that have accumulated excessive amounts of nitrates or nitrites. Drought conditions and the usage of manure as fertilizer heighten nitrate accumulation in cereal grasses (wheat, rye, oats), corn, sorghum, sugar beets, and many weeds, including pigweed (*Amaranthus retroflexus)* and variegated thistle (*Silybun marianum),* found in pasture. The highest concentration of nitrates is in the stems, followed by roots and leaves. Nitrate toxicity may also result from ingestion of polluted water rich in nitrates and ingestion of nitrate-based fertilizers (ammonium or potassium nitrate).

Nitrates can have an irritant effect on the gastrointestinal tract and cause vasodilation. In addition, they can also be irritants to the kidneys and urinary tract. However, in ruminants in which reduction of nitrate to nitrite occurs, the more toxic nitrites cause the iron component of blood to become oxidized and thus unable to transport oxygen. This may cause the blood to be discolored brown, often described as "chocolate-colered." If the level of methemoglobineria reaches 20-40%, the mucous membranes will start to appear cyanotic and the animal may become dyspneic; tachycardia is also often noted. In extreme cases, this may lead to coma and death. Clinically, nitrate intoxication may also cause weakness, ataxia and convulsions. Chronic sublethal nitrate poisoning has also been reported. Clinical manifestations include reproductive problems such as abortion, infertility and lower birth rate, hypothyroidism, and depletion of dietary vitamin A.

Gross lesions that may be used to diagnose nitrate intoxication include brown discoloration of the blood, which is present in approximately 64% of cases. The discoloration fades within five hours after death so it may not be detected at postmortem examination. Pale, blanched areas are sometimes noted on the myocardium along with epicardial hemorrhages. The lungs may show intense congestion and reddish-brown coloration.

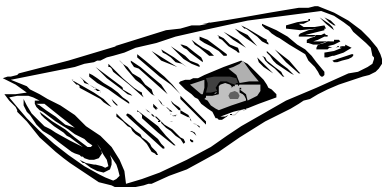
Laboratory confirmation of nitrate poisoning can be achieved by checking suspected plants, water, serum, ocular fluid, and heparinized whole blood. Nitrate levels in forage should not exceed 1% dry matter weight. A preliminary field test using 1% diphenylamine blue in concentrated sulfuric acid may be used. If the plant material changes to a dark color quickly, the nitrate levels are excessively high. All field tests are presumptive, however, and should be confirmed with laboratory tests. Samples of

grass or forage (about 1 kg) should be collected from several locations of the pasture or from several different bales of hay. At least 500 ml of water should be collected and submitted in a sterile bottle. Diagnosis of nitrate intoxication can also be based on finding excessive amounts of methemoglobin in the whole blood. Heparinized blood must be collected within two hours of death and promptly assayed in order for this test to be valid; otherwise, blood must be rapidly frozen to prevent the spontaneous reduction of methemoglobin back to hemoglobin.

Serum and ocular fluid are the most commonly used samples to assess nitrate levels (nitrites are typically not detected due to their short *in vivo* half-life). Samples should be sent to the laboratory immediately. Nitrate in serum is stable for one week if refrigeration and one month if frozen. The nitrate levels in ocular fluid remain stable for over 24 hours after death and diagnostically significant for up to 60 hours after death. Ocular fluid is thus often the only valid sample available. Nitrate crosses the placental barrier in cattle and diffuses into the fetus. Amniotic fluid and stomach contents from fetuses thus also represent valid diagnostic samples.

Treatment in cattle consists of administering an 8.8 mg/kg dose of a 1% isotonic saline solution of methylene blue IV; sheep may need up to 20 mg/kg. Repetition of this regimen may be necessary after an interval of 6-8 hours. It must be noted, however, that methylene blue is not approved by the FDA for use in food producing animals. Symptomatic treatment involving cardiorespiratory stimulants, gastric antacids, and oral antibiotics to reduce nitrite production by gastrointestinal flora may be indicated. Ensiling high-nitrate forages with other feeds and adding trace mineral supplements are useful means to prevent nitrate intoxication.

-by Theresa Boulineau, Class of 2001
-edited by Melissa Popielarczyk,
VPB Graduate Student



ADDL STAFF NEWS

Dr. Matti Kuipel, ADDL Graduate student, has won the Ernst-Reuter Award for excellence in PhD theses. Each year, the Ernst Reuter Society selects the best thesis of all PhD graduates of the universities in Berlin, Germany. Dr. Kiupel is the first veterinarian to win this prestigious award. His thesis was titled "Prognostic Factors for Treated Canine Malignant Lymphoma". The official ceremony will be on December 4, 2000 and the award will be presented through the Secretary of State for Education and Science.

The ADDL has successfully completed the Pseudorabies G1 differential proficiency check test administered by the National Veterinary Services Laboratory, Ames, Iowa. Congratulations to Tammy Crowell, Serology Technician.

Purdue football fans Leon Thacker, Bob Smith, Dan Harrington, Mary Fran Nelson, Tammy Crowell and Cheryl Parker attended the Rose Bowl festivities in Pasadena, CA. Bob and Mary Fran also helped decorate floats for the Rose Bowl parade.

ON THE ROAD

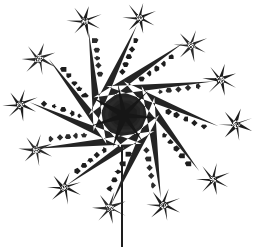
Dr. Randy White traveled to northern Indiana to consult on fish health issues with managers of Bodine State Fish Hatchery and Curtis Creek Trout Rearing Station.

Drs. Evan Janovitz, Bill Van Alstine, Brad Njaa, Victoria Owiredu-Laast, Janice Lacey and Matti Kiupel attended the American College of Veterinary Pathologists annual meeting in Amelia Island, Florida, December 2000.

Drs. Brad Njaa and Duane Murphy attended the Foreign Animal Disease Diagnostician training course for Veterinary Laboratory Diagnosticians and Pathologists at the Foreign Animal Disease Diagnostic Laboratory on Plum Island, New York, November 2000.

Dr. Greg Stevenson was a featured speaker at the Iowa Swine Disease Conference, Ames, Iowa, November, 2000

Drs. Leon Thacker and Greg Stevenson attended the Indiana Swine Group meeting in Indianapolis, December, 2000.



Purdue ADDL and Heeke ADDL will be closed on the following dates.

Memorial Day	May 28, 2001
Independence Day	July 4, 2001

Please plan accordingly

****

Please remember Purdue ADDL and Heeke ADDL hours are 8-5 Monday through Friday. Samples from U.S. mail, UPS or courier services cannot be accepted on Saturday, Sunday or holidays.

The dates that Purdue ADDL and Heeke ADDL are closed will appear 3-6 months in advance in the Diagnostic Forum.

A pathologist can always be reached by calling 765.494.7440 and following the instructions

****

Visit our web site at www.addl.purdue.edu

Toxicology – Sample Submission

Many poisons/poisonings can be diagnosed from proper tissue and/or feed samples. If a toxicosis of unknown origin is suspected in a live animal, samples which can be of value in analysis are whole blood, urine, vomitus (or gastric contents from lavage) and feed. In general, clot separator tubes should not be used for collection of blood for toxicological analyses. The material in these tubes can make analysis difficult or impossible. Urine and stomach contents should be frozen. Feed should be thoroughly mixed and a representative sample submitted. If an unknown poisoning is suspected at necropsy, samples to be collected include liver, kidney, fat, brain, stomach contents, urine and blood (if available). With the exception of blood, ¼ to ½ pound (100 to 200 grams) of tissue (or all that is available for small animals) should be collected, wrapped in aluminum foil, placed in individually labeled plastic bags, sealed and frozen. Brains of large animals should be cut longitudinally and ½ of the brain wrapped, labeled, sealed, frozen and submitted. Representative samples of tissues should also be fixed in formalin for histological examination. Following are guidelines which can be used for future reference. Please feel free to phone ADDL at 765-494-7440 with any questions regarding samples or fees.

-by Stephen Hooser, DVM, PhD, Head
Toxicology Laboratory
Robert Everson, PhD, Chemist
Christina Wilson, Assistant Chemist
Regina Bedel, Lab Technician

KEEP FOR FUTURE REFERENCE

Test	**Sample to submit**
Mineral/Elemental Analyses	
Arsenic	liver, ingesta
Copper	feed, serum, liver
Iron	serum, liver
Lead	Whole blood (Not in clot separator tubes), liver, kidney, paint chips, bone, urine
Magnesium	serum, ocular fluid
Potassium	feed
Selenium	serum, liver
Sodium	feed, serum/CSF fluid, brain
Zinc	liver, serum (No rubber stoppers, use plastic)
ICP Mineral Analyses	serum, soil, tissue, urine, vitreous humor, water

Bone Analysis

Calcium/Phosphorus	feed, bone (whole femur)
Bone Ash/Density	bone (whole femur)

Organics

Alkaloid screen (strychnine, nicotine, caffeine, theobromine)	ingesta, liver, urine
Anticoagulant rodenticides	blood (in EDTA tube), liver
Cholinesterase inhibition	heparinized blood, brain
Pesticide screen (organophosphate, carbamate, organochlorine Insecticides, herbicides)	ingesta, liver, milk, fat, feed, water, serum

Mycotoxins

Aflatoxin, deoxynivalenol, Ochratoxin, T-2, DAS, Zearalenone, Fumonisin B1,B2	feed

Other feed-related toxicants/tests

Ammonia/urea	water, rumen contents, feed (freeze quickly)
Cyanide	ingesta, plant (freeze quickly)
Nitrate	feed, plants, water, ocular fluid
Nitrite	water, ocular fluid
Monensin	feed
Vitamin A	serum, liver, feed
Vitamin D	serum, kidney feed
Vitamin E	serum (must not be hemolyzed), liver, feed

MINIMUM AMOUNTS OF SAMPLES TO SUBMIT

Whole blood	4 ml
Serum	2 ml
	3 ml for both Vitamin E and Selenium
Liver	½ pound (200 g)
Kidney	1 whole, large animal/ both, small animal
Brain	½ cut longitudinally (large animal)
	Whole, small animal
Fat	¼ pound (100 g)
Urine	All available
Ingesta (vomitus)	1 pound (450 g) or all available
Ocular fluid	All available
Feed	1 pound (450 g)

If these amounts of tissues cannot be obtained from the animal, then send all that is available after removing a section for formalin fixation and histological examination.

Plant and seed identification is also available.