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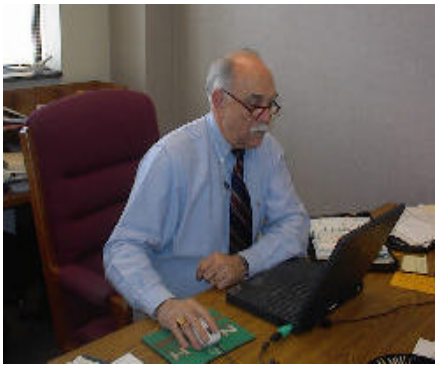
Winter 2004



FROM THE DIRECTOR

H. Leon Thacker, DVM, PhD

Good day from the Purdue ADDL. Hope this finds you warm and enjoying Indiana winter. This too shall pass.

The finding of Bovine Spongiform Encephalopathy (BSE) positive cow in Washington state just before Christmas this year certainly increased the ringing of the telephones around here and elsewhere on the Purdue campus as well as at the Board of Animal Health. It is encouraging to see that the first finding of BSE in the U.S. has been met with maintenance of confidence of the meat-consuming public. Confidence in the safety of our meat supply is deserved and I believe that the safeguards that have been initiated in our feeding and slaughter practices in this country have been well thought, adhered to, and effective in breaking the chain of events necessary for an outbreak of BSE in our cattle or of variant Creutzfeldt-Jacob disease in our meat consuming public. An example of the effectiveness of eliminating a prion-derived disease by eliminating the cycle of cause is seen in the elimination of the human disease Kuru which occurred in a native tribe of New Guinea until the practice of consuming the prion carrying brains were eliminated from the diets of the people. The ban of feeding bovine-derived protein or specified risk material to cattle will prove to be effective in eliminating the cycle required to propagate this disease also.

In other news from the activities of the ADDL, the check of the corn samples taken around the state of the '03 crop revealed some low levels of deoxynivalenol and of fumonisin so that the possibility of problems from these mycotoxins may be recognized. We are continuing to run the samples of deer brains in search of Chronic Wasting Disease in Indiana deer harvested in both '02 and '03. So far no positive samples have been found. Requests for performing tests on skin samples from cattle for persistent infection of BVD have been high. The fluorescent antibody test we are running has been proven to be a fast and effective method for this testing. Faculty and staff of the ADDL have been involved with training exercised required by the new Quality System which has been initiated in the Lab. Our lab is due for an accreditation visit during the coming year for renewal of our full accreditation status; we're in preparation for that visit though the date has not yet been set.

Please keep us apprised of your diagnostic needs. It is our desire to provide you with animal disease diagnostics in the most accurate and timely manner currently available to veterinary application.

FINAL DIAGNOSIS

White Snakeroot Intoxication in a Calf

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

History: A 6-month-old male, Aberdeen Angus, partially weaned calf was presented to the Purdue University Veterinary Teaching Hospital for generalized muscle weakness characterized by collapse and muscle fasiculations following exercise. Per clinical history, the calf was anorexic, tachycardic and pyrexic, with moderate ketonuria. ECG, hematology, serum biochemistry, and rumen contents were reportedly within normal limits. It initially responded to supportive therapy, but deteriorated and died the following day. The calf was submitted to ADDL for necropsy. The owners indicated that white snakeroot was present in the pasture grazed by the herd, and that the dam had also developed muscle tremors and subsequently recovered.

Gross findings: Significant gross lesions were not observed.

Histopathologic findings: Samples of the quadriceps femoris and shoulder muscles contained focally extensive areas of degeneration characterized by myocyte swelling with sarcoplasmic hyalinization, fragmentation, and loss of cross striations. Rarely, myocytes were segmentally mineralized. The interstitium was expanded by edema, numerous macrophages and fewer neutrophils. Variable numbers of macrophages infiltrated the remnants of degenerate myocytes. Regenerative myofibers with basophilic sarcoplasm and large, centrally located nuclei (myotubes) were scattered throughout the damaged muscle tissue.

Toxicology: Rumen contents from the calf were submitted for microscopic analysis. Toxic plants or seeds were not identified in the rumen contents.

Discussion: White snakeroot (*Eupatorium rugosum*) is a member of the daisy family that can cause severe toxicities in livestock and humans. The perennial herb grows 1-3 feet tall from a shallow mat of fibrous roots, has oval, opposite, serrated leaves with pointed tips, and clusters of small white tubular flowers that bloom in late summer. White snakeroot is common throughout Indiana and the eastern United States. The plant is found in low, moist, shady areas and in clearings and thickets bordering woodlands. Consumption of 0.5-2.0% of body weight in green plant is associated with signs of intoxication in livestock. The toxic effect is cumulative. All parts of the leaves and stems are toxic. Tremetol content is highest in the green plant and remains toxic even when dried in hay. Frost does not inactivate the toxin. Following ingestion, plant components presumably become toxic after microsomal activation by cytochrome P-450 enzymes in the liver. The apparent toxic principle in white snakeroot may be tremetol (or its ketone, tremetone), a fat-soluble, high molecular weight alcohol. Crude tremetol has been experimentally separated into a toxic fraction and a nontoxic sterol fraction. Tremetol is fat soluble and excreted in the milk of lactating animals. Lactating animals are generally slower to show clinical signs of toxicity, although their nursing young will be affected.

In cattle, white snakeroot intoxication has been called “trembles” because of

the characteristic muscle tremors. Clinical signs in cattle and other ruminants include depression, lethargy, listlessness, acetone breath (ketosis), constipation, and weakness that often progresses to recumbency, coma, and death in 2-10 days. Muscle tremors are most severe in the muzzle and legs and tend to occur after exercise.

In horses, white snakeroot intoxication is associated with congestive heart failure. Clinical signs include sweating, stumbling, depression, jugular pulses, tachycardia, cardiac arrythmias, and difficulty swallowing. Electrocardiogram changes include increased heart rate, ST elevation, variable QRS complexes, and ventricular premature beats. Muscle tremors are inconsistently observed in horses with white snakeroot poisoning.

White snakeroot intoxication was the cause of "milk sickness" in 18th and 19th century America. Milk sickness in humans begins as weakness, loss of appetite, abdominal pain, and violent vomiting followed by constipation, severe thirst, vomiting, tremors, acetone breath, prostration, delirium, coma, and death. Abraham Lincoln's mother reportedly died from milk sickness in 1818 after drinking milk from a cow that had been grazed on white snakeroot. Although tremetol is not inactivated by pasteurization, human disease is uncommon today due to current practices of animal husbandry and the pooling of milk from many producers.

Mortality in livestock is high. Treatment consists of supportive care, including extra bedding in stalls to prevent development of pressure sores in recumbent animals. Exercise and excitement should be avoided. Lactating animals should be frequently milked out and the milk discarded. Cattle, horses, and other livestock should be restricted from access to pastures containing white snakeroot.

There are no specific routine diagnostic tests for tremetol (or tremetone). Diagnosis is based on clinical signs, presence of white snakeroot in the hay or pasture, elevated muscle enzymes (CK, ALP and AST), histologic evidence of cardiac and/or skeletal muscle degeneration and rule-out of other causes. Rumen or stomach contents may be submitted for microscopic identification of white snakeroot leaves. Gross lesions associated with white snakeroot and other toxic myopathies are often unremarkable, and may be difficult to distinguish microscopically from acute nutritional or exertional myopathies.

Differential diagnoses include botulism, organophosphate intoxication, nutritional or exertional myopathies, other toxic myopathies, rabies and esophageal obstruction in horses. Other plants that cause tremorgenic syndromes in livestock include dallies grass, rayless goldenrod (*Isocoma wrightii*), Jimmy fern (*Notholaena sinuate*) and western mountain laurel (*Sophora secundiflora*). Most of these plants are found in the southern and southwestern United States.

-by Dr. Kim Maratea, ADDL Graduate Student

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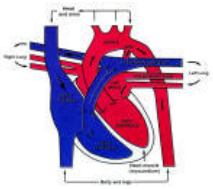
Patent Ductus Arteriosis



Patent ductus arteriosis (PDA) is the most common congenital heart disease in dogs. Commonly affected breeds include Miniature poodles, Collies, Pomeranians, Cocker spaniels, Chihuahua, Maltese, German shepherds, Irish setters, Keeshounds, and Shetland Sheep dogs. The disease occurs more frequently in females than in males (2:1).

The ductus arteriosis is the fetal connection between the descending aorta and the main pulmonary artery, allowing the shunting of fetal oxygenated placental blood from the pulmonary artery to the systemic circulation bypassing the atelectic non-functional fetal lung.

Following parturition and the onset of breathing, the rapid increase in arterial oxygen tension causes constriction of ductal smooth muscle and functional closure of the ductus. Anatomic obliteration occurs by necrosis and fibrosis during the first few weeks of life. Patency of the ductus arteriosus beyond 7-10 days of age is considered abnormal in dogs.

Failure of ductal closure appears to be the result of histologic abnormalities within the ductal wall. The normal ductal wall contains a loose branching pattern of circumferential smooth muscle throughout its length. In prenatal pups bred to have a high probability of PDA, varying portions of the ductal wall resemble the wall of the aorta, lacking constricting smooth muscle.

The anatomic appearance of the patent ductus is varied. There is a progression from a ductus diverticulum that is a blind, funnel-shaped outpouching of the aorta at the site of ductus, to a ductus that approaches the size of the aorta. The length and diameter of the ductus also vary. In some cases, there is virtually no ductus but, instead, an opening between the aorta and pulmonary artery.

Left to right Patent Ductus Arteriosus:

Left to right is the typical form of PDA in which the aortic pressure is higher than pulmonary artery pressure throughout the cardiac cycle, and blood shunts continuously from the aorta to the pulmonary artery. This results in a continuous cardiac murmur, increased pulmonary flow, volume overloading, and diastolic dilatation of the left atrium and left ventricle. The left ventricle also undergoes eccentric hypertrophy and increased left ventricular end-diastolic pressure. If the defect is wide enough to allow a large shunt and pulmonary vascular resistance remains low, the end result may be left ventricular failure with pulmonary edema. Left ventricular stroke volume is increased (Frank-Starling principle) and is reflected in an increased arterial systolic pressure. In addition, rapid run-off blood from the aorta via the ductus causes a decreased aortic and arterial diastolic pressure. The wide arterial pulse pressure is felt as a bounding, hyperkinetic arterial pulse. Increased volume flow in the aortic arch and pulmonary artery causes dilatation of the aorta and main pulmonary artery. The right ventricle never handles the shunted blood and remains normal unless there are increases in pulmonary vascular resistance and pulmonary arterial pressure. Experiments in dogs involving aortopulmonary ducts indicate that a communication of 3 mm or less in diameter may lead to the slow development of left ventricular hypertrophy, but it is otherwise well tolerated. A shunt of 5 mm diameter may lead to pulmonary hypertension, with degenerative changes in pulmonary vessels and congestive heart failure. When the pulmonary artery resistance

increases, right- to-left, or reverse, shunting PDA develops; this condition will be described later in this article.

Clinical signs rarely develop within the first week of life, and many diagnoses are made at the time of initial examination at 6-8 weeks or later. In all but the mildest cases with very small shunts, arterial pulse is hyperkinetic (bounding). Mucous membranes are pink in the absence of heart failure. A continuous trill may be palpated at the heart base and a continuous murmur is audible at the same point.

Noninvasive studies are usually diagnostic. Electrocardiography usually indicates left ventricular enlargement by a normal frontal plane QRS axis and increased R-waves in leads II, III, Vf, V2 and V4. Left atrial enlargement may also be indicated by widening of P waves. Radiography demonstrates cardiomegaly, pulmonary hyper-vascularity, left atrial and ventricular enlargement. The most specific finding is the appearance of an aortic bulge near the origin of the ductus. The diagnosis could be proved by echocardiography in almost all cases. Ductus diverticulum, a hidden form of PDA, can be diagnosed only by angiography or necropsy.

Approximately 64% of the dogs diagnosed with left-to-right shunting PDA will die from complications within a year of diagnosis without surgical correction. Some dogs with modest shunts will survive to maturity, and a few may live 10 years or more.

Right-to-left Patent Ductus Arteriosus

Right-to-left, or reverse, shunting PDA occurs when there is an increase in pulmonary vasculature. Dogs with reverse PDA exhibit diminished pulmonary flow, a comparatively small left ventricle, and marked hypertrophy of the right ventricle. The exact mechanism by which the pulmonary vasculature resistance increases is not completely understood, but anatomic description of the pulmonary microvasculature is similar for both humans and animals. Histologic changes within small pulmonary arteries include hypertrophy of the media, thickening of the intima, reduction of lumen dimensions, and plexiform lesions of the vessel wall. Most of these changes are considered irreversible.

Many owners do not recognize obvious clinical signs in their pet during the first 6-12 months of life. Clinical examination is very different from the more common left-to-right PDA. Physical examination reveals either no murmur or only a soft, early systolic murmur at the left heart base. Differential cyanosis (cyanosis of the caudal mucous membranes with pink cranial membranes) may be observed, but recognition may require examination after exercise. Differential cyanosis is caused by the location of the PDA, which shunts right to left from the pulmonary artery into the descending aorta. Perfusion of the kidneys with hypoxemic blood leads to secondary polycythemia and hyperviscosity, with the PCV gradually increasing to 65% or greater. Polycythemia may occur during the first year but often does not become severe until 18-24 months of age, therefore, many times owners do not recognize clinical signs until the condition has progressed.

The electrocardiogram shows changes of right hypertrophy. Thoracic radiographs and echocardiography demonstrate right ventricular hypertrophy and a dilated main pulmonary artery.

Animals with reversed PDA often live 3-5 years; a few survive beyond 7 years if the PCV is kept below 65%.

Summary: Left to right shunting Patent Ductus Arteriosus is the most common congenital heart disease in dogs and is caused by failure of ductal closure after birth. Almost all affected

dogs will die from complications before maturity if the condition is not treated. After surgical treatment, the prognosis is excellent.

Right to left Patent Ductus Arteriosus occurs when there is an increase in pulmonary vasculature pressure and is an uncommon condition.

-by Horia Popa, ECFVG Student
-edited by Dr. Alok Sharma, ADDL Graduate Student

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Purdue ADDL and Heeke ADDL will be closed on the following University holidays

January 19, 2004.....Martin Luther King
May 31, 2004.....Memorial Day

Drs. Leon Thacker, Greg Stevenson, Ching Ching Wu, Robert Everson, Steve Hooser, Ramesh Vemulapalli, Peg Miller, Jose Ramos-Vara, Ingeborg Langohr, and **Steve Vollmer** and **Linda Hendrickson** attended the annual meeting of the American Association of Veterinary Laboratory Diagnosticians in San Diego, October, 2003.

Drs. **Ching Ching Wu, Tom Bryan** and **Mohamed Abdelwahab** attended the North Central Avian Disease Conference in Cleveland, Ohio, September, 2003.

Dr. Zheko Kounev attended the Turkey Market Development Council meeting in Jasper, IN, September 2003.

Drs. Peg Miller and **Bill VanAlstine** attended the American College of Veterinary Pathologists annual meeting in Calgary, Canada, November, 2003

Dr. Leon Thacker attended the Indiana Veterinary Medical Association annual meeting in Indianapolis, January 2004.

Equine Acute Laminitis
A Current View

Laminitis has long been considered as inflammation and edema of the sensitive laminae of the hoof following transient ischemia that is often associated with a coagulopathy and causing breakdown and degeneration of the union between the horny and sensitive laminae. Recent reports, however, suggest that these changes may more likely result from failure of the distal phalanx-hoof wall bond rather than being the initial cause.

During normal hoof growth, attachments between epidermal lamellae and basement membrane are constantly being broken down and then reformed. It is proposed that the cell-cell and cell-basement membrane attachments are released under the influence of active matrix metalloproteinases (MMPs). MMPs have been isolated from normal lamellar tissues and are increased in lamellar tissues of horses affected by laminitis. Recent studies show that bacterial proteases activate MMPs. These results suggest that bacteria can produce potent MMP activators that probably facilitate host invasion.

The enzymatic theory of laminitis based on lamellae MMP activation challenges the alternative view that laminitis develops because of vascular changes to the circulation of the foot. Traditionally, it was suggested that vasoconstriction and compartment syndrome decreased the flow of blood in the lamellar microcirculation to induce ischemic necrosis of epidermal lamellae. A recent study showed that epidermal cell necrosis, intravascular coagulation, and edema were not recognized in sections processed from tissue in the early stages of laminitis. The paper also reported that the vessels in the primary dermal lamella are for the most part fully open without evidence of microvascular thrombi. Further, no abnormalities in the systemic coagulation and fibrinolytic cascades are found in horses with carbohydrate-induced acute laminitis. The gross anatomical appearance of freshly dissected laminitis tissue is one of dryness. Collectively, these results suggest that in the acute phase of laminitis, the enzymatic effect might be the earliest and most influential effect on laminar structures.

The most common causes of laminitis include excessive ingestion of carbohydrates (grain overload), grazing of lush pastures (especially in ponies) and excessive exercise in adult horses. It also may occur secondary to post-parturient metritis, endotoxemia, colic, enteritis, or administration of excess corticosteroids.

Clinical signs of laminitis are often only noted after the disease has progressed and, by that time, the inciting cause may be difficult to ascertain. Once clinical signs of laminitis are present, the clinical course of the disease is typically correlated to the degree of laminar damage. Horses with mild laminar damage have less severe signs and usually respond quickly to therapy. Horses with extensive laminar changes have more severe signs and either respond less quickly or not at all to medical therapy. In general, the acute laminitis affects both front feet, but all four feet could be involved. On palpation, heat may be present over the hoof wall and coronary band. An increased bounding digital pulse is evident. The degree of pain may be reflected by tachycardia, muscle tremors, and sweating. In severe cases, examination is difficult since the horse will often not allow the feet to be picked up and examined.

Obel categorized the severity of lameness by the following criteria: Grade 1, at rest the horse alternately

and incessantly lifts the feet, often at intervals of a few seconds; lameness is not noted at a trot. At Grade 2, the horse moves willingly at a walk, but the gait is stilted; a foot can be lifted off the ground without difficulty. At Grade 3, the horse moves very reluctantly and vigorously resists attempts to lift the front foot off the ground. At Grade 4, the horse refuses to move unless forced.

The diagnosis of laminitis is based on clinical signs, physical examination and radiography. Examination with a hoof tester will reveal pain over the sole, particularly at the toe, and tapping on the hoof wall may cause pain. Radiographs should be taken at the first sign of acute laminitis to develop a baseline for continuous radiographic comparison. Early radiographic signs in laminitis include mild bony reaction along the dorsal aspect of the distal phalanx in addition to widening the distance between the distal phalanx and the dorsal hoof wall. This distance should be less than 18 mm in normal horses or less than 30% of the palmer length of the distal phalanx measured from the tip of the bone to its articulation with the navicular bone. Palmar or plantar rotation of the distal phalanx away from the dorsal hoof wall confirms the diagnosis of laminitis. The mean palmar and plantar rotation of the distal phalanx in normal horses is thought to be 0.5 $\pm$ 1.3 degrees and less than 4 degrees. Digital venogram and vascular perfusion casts have been used to identify perfusion deficits which, if present, usually indicate a poor prognosis.

The goals of treatment are 1) to prevent the further development of laminitis, 2) to reduce the pain or hypertension cycle, 3) to reduce or prevent permanent laminar damager, 4) to improve dermal laminar capillary demodynamics, and 5) to prevent movement of the distal phalanx. Acute laminitis should be considered a medical emergency, and treatment should be initiated as soon as possible, preferably before clinical signs develop. Since circulating endotoxin and infectious processes are found in cases of laminitis, treatment for endotoxemia and sepsis should be attempted. When a horse is suspected of grain overload, one gallon of mineral oil by stomach tube acts as a laxative and tends to prevent absorption of toxic material from the gastrointestinal tract. Recommended treatments include intravenous fluids, parenteral antimicrobials, flunixin meglumine, and hyperimmune serum or plasma. Additional laminitis-preventative measures include the administration of anti-inflammatory drugs, vasodilator, heparin, oral aspirin, and placement of the horse in the stall. Some cases need trimming of the hoof. As for packing of the hoof, a recent study suggests that hot packs used early in the course of the disease may be more beneficial. Non-steroid anti-inflammatory drugs are the cornerstone of most therapeutic regimens.

Predicting the prognosis and survival of horses with acute laminitis can be difficult. One study shows the majority of horses with less than 5.5 degree rotation returned to former athletic function, but those with more than 11.5 degree rotation were no longer able to perform properly, although another paper reported the degree of rotation or distal displacement of the distal phalanx observed on radiographies did not correlate with the outcome of the horses. Based on multiple studies, lameness severity in horses with laminitis likely correlates with the severity or quantity of permanent laminar damage that has, or is likely to, occur.

-by Yasuyuki Usami, ECFVG student
-edited by Dr. Theresa Boulineau, ADDL Graduate Student

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ADDL NEWS

Paula Brost has been named supervisor of the histology laboratory. Paula has been in the ADDL histology lab for the past 28 years.

ADDL Staff Members **Cheryl Parker** (Serology lab technician), **Phyllis Lockard** (electron microscopy technician) and **Denise Riley** (Heeke ADDL clerical staff) were recently honored for their years of service to Purdue. Cheryl and Phyllis have been at Purdue for 25 years, Denise for 15. Congratulations and thanks for your contributions to ADDL.

Hairy Vetch Toxicosis

Hairy vetch (*Vicia villosa*) is often used as a forage legume or cover crop throughout many temperate areas of the world, including the United States. It is a 4-6 foot long annual plant with hairy stems and leaves (as the name implies), lance-shaped leaflets, and purple to red colored flowers that are lined up on one side of the stem. Though commonly found in pastures, hairy vetch can cause a systemic granulomatous disease, occasionally in cattle and rarely in horses. The disease prevalence is greatest when the major component of the forage is hairy vetch or when the plant is reaching maturity in mid to late

spring. The plant is less likely to cause a problem in hay or when ensiled.

In cattle, the ingestion of *Vicia* spp. has been associated with three apparently different clinical manifestations. One clinical form is characterized by acute neurological signs compatible with the cyanogenic glycosides contained in the seeds. In another form, the animals may present with subcutaneous swelling, ulcers of the oral mucous membranes, purulent nasal discharge, cough, alopecia, weakness, and loss of appetite. The third form of V. *villosa* (and, to a lesser extent, other *Vicia* species) poisoning, which is the best studied and documented of the three forms, consists of pruritic dermatitis with alopecia (not restricted to non-pigmented areas as seen with photo-sensitivity), diarrhea, weight loss, drop in milk yield, and sporadic abortions and red-tinged urine. The body temperature is usually normal. Lymphocytosis and hyperproteinemia are typical clinical pathologic alterations in affected animals. In horses, reported clinical signs include generalized dermatitis, with alopecia, crusting and scaling, blepharitis, conjunctivitis, corneal ulceration, lymphadenomegaly, depen-dent edema, diarrhea, and wasting, accompanied by lymphocytosis and hyperproteinemia.

On postmortem examination of affected animals, there are multifocal to coalescing, gray-yellow, soft to moderately firm nodules disrupting the normal architecture of several organs, including liver, kidneys, spleen, heart, lymph nodes, and adrenal gland. Microscopically, the lesions consist of perivascular granulomatous inflammatory infiltrate composed of epitheloid macrophages, lymphocytes, plasma cells, occasional multinucleated giant cells, and, often, eosinophils.

The pathogenic mechanism associated with hairy vetch toxicosis has not yet been determined. It has been proposed that the vetch lectins induce a type IV, or cell mediated, hypersensitivity reaction that would account for the inflammatory reaction seen in this condition. Another hypothesis is that the vetch lectins might directly activate T lymphocytes, thereby initiating the multisystemic granulomatous disease. The facts that the plant is frequently consumed by cattle without any apparent problem, that several unsuccessful attempts have been made to experimentally induce the disease in younger animals, and that it was reproduced only once in a cow that had recovered from the disease one year earlier are claimed as further evidence to the hypersensitivity theory. A genetic predisposition is also suggested because vetch-associated systemic granulomatous disease occurs mainly in Holstein and Angus cattle.

In cattle, vetch-associated disease is prevalent and more severe in cattle $\geq$ 3 years old. Affected younger animals usually have mild disease, but can still be fatally affected. Sex predilection is not apparent. Outbreaks are most common during the season of maximal vetch growth, although sporadic cases are observed throughout the year. Cattle that develop the clinical disease usually have been grazing pasture that contained hairy vetch for at least 2 to 6 weeks, and sometimes clinical signs can become apparent only after the cattle have been removed from the hairy vetch pastures. The interval between the appearance of clinical signs and death ranges from 3 days to 5 weeks, but is usually between 10 and 20 days. Morbidity rates vary from 1-68% (but in most outbreaks is approximately 10%); lethality rates are usually high (50-100%; therefore, recovery of animals that develop severe disease is unlikely.

In horses, systemic granulomatous disease is most likely multifactorial. Many cases of this disease occur in this species without known exposure to vetch pasture. Also in cattle, several clinically and pathologically similar (if not

identical) diseases have been reported in animals that have not had access to vetch. These have been referred to as "vetch-like diseases", and have been associated with the consumption of rations containing diureido-isobutance (DUIB) in the Netherlands, the ingestion of silage preserved with Sylade (a commercial silage additive consisting of a combination of formalin and sulphuric acid) in Wales, and the consumption of citrus pulp in the United States, England, and Brazil.

-by Bethany Lovaas, Class of 2004
-edited by Dr. Ingeborg Langohr, ADDL Graduate student

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Painting the surgical margins of a tumor biopsy

A common problem faced by pathologists is interpreting surgical margins of tumor biopsies. Although it is often easy to distinguish surgical margins from those produced during the trimming process in the histology laboratory, it is sometimes difficult and frustrating for both pathologists and clinicians. Diagnosticians know that the three most common questions posed by the clinician regarding a neoplastic process are "What kind of tumor is it?", "Is it benign or malignant?" and "Are the margins free of neoplastic cells?"

In human medicine, surgical margins of a biopsy are commonly painted with a dye that adheres to tissue and is visible under the microscope. This procedure is simple and does not interfere with histologic evaluation. Its advantage is that it clearly distinguishes surgical margins from trimming margins. This is essential to assess complete/incomplete excision of a tumor.

Biopsy margin painting can be done on unfixed or fixed tissues; however, painting unfixed tissues is easier and preferred. There are several commercially available products for this purpose (call the ADDL for additional information if needed). The use of different colors (black, blue, green, red, yellow, etc) for different aspects of mass orientation is superior to using sutures of different colors. Painting biopsy margins is also inexpensive; a 20 ml bottle of dye will last several years. ADDL staff can paint fixed specimens, but an additional fee with accrue.

How to do it
You don't have to be a Picasso!

1) Blot the biopsy margins of the mass.
2) Select the dye color.
3) With a wooden applicator stick or cotton swab, "paint" the biopsy margin.

Do not pour dye on the surface; just apply as if painting.

4) Let the dye dry for 5-10 minutes and immerse the sample in regular fixative solution. Some of the dye will dissolve with the fixative; that's OK. If the sample is thicker than 4-6 mm, section it to improve penetration of fixative as you usually do.

In summary, you will get more from your biopsy report if you mark biopsy margins with dye.

Images of the technique are posted on the ADDL website (www.addl.purdue.edu). If you have questions, please call the histology section of the ADDL.

Percent of Micro-organisms that are Resistant to Selected Antibiotics from Jan.- June and Jul.- Dec. 2003

Antibiotic	Canine										Equine												Feline						
	E. Coli		Enterococcus sp.		Pse. aeruginosa		Staph. aureus		Staph. intermedius		E. Coli		Salmonella sp.		Staph. aureus		Staph. epidermidis		Strep. equi		Strep. zooepidemicus		E. Coli		Enterococcus sp.		Pse. aeruginosa		Staph. aureus
	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	July-Dec.	July-Dec.	July-Dec.	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June
Amikacin	1	0	18	47	0	0	0	0	0	0	0	0	0	100	0	0	0	7	83	100	88	92	0	0	100	75	0	0	0
Amoxycillin/Clauvulinic acid	12	24	27	42	100	100	29	55	16	8	6	5	0	100	33	43	18	43	0	0	0	0	12	11	0	25	100	100	0
Ampicillin	46	61	27	42	100	100	57	91	48	57	44	44	14	100	67	100	45	64	0	0	0	0	47	36	0	25	100	100	50
Cefazolin	11	28	82	79	100	100	0	36	0	0	13	7	0	100	17	14	0	29	0	0	0	0	6	7	100	100	100	100	0
Cefotaxime	1	11	27	56	25	8	0	36	0	0	0	0	0	100	17	17	0	33	0	0	0	0	0	4	0	25	0	30	0
Cefoxitin	10	23	73	89	100	100	0	36	0	0	6	7	0	100	17	14	0	43	0	0	0	0	12	14	0	75	100	100	0
Ceftiofur	8	23	91	84	100	100	0	36	0	0	6	7	0	100	17	14	0	29	0	0	0	0	0	4	100	75	75	92	0
Cephalothin	18	39	73	79	100	100	0	36	0	0	19	9	0	100	17	14	0	36	0	0	0	0	6	11	0	50	100	100	0
Chloramphenicol	24	24	0	0	92	93	0	0	0	3	13	21	14	100	0	0	0	14	0	0	0	8	0	11	0	0	75	100	0
Ciprofloxacin	14	25	18	44	0	0	29	36	0	0	0	0	0	100	0	0	0	17	0	0	0	0	0	4	0	25	0	0	0
Clindamycin	99	100	73	83	100	100	14	27	13	0	100	100	100	0	0	0	0	29	100	0	100	0	100	100	100	50	100	100	0
Enrofloxacin	13	24	27	58	42	50	43	36	0	8	0	2	0	100	0	0	9	14	100	0	60	0	0	4	0	25	25	42	0
Erythromycin	98	100	18	37	100	100	29	18	16	3	100	100	100	0	17	14	18	21	100	50	100	23	100	100	0	25	100	100	50
Gentamicin 500 microgm/ml	0	0	0	6	0	0	0	0	0	22	0	0	0	100	0	0	18	0	0	0	0	0	0	4	0	25	0	0	0
Gentamicin	14	17	9	42	25	0	14	0	10	0	38	28	14	100	67	0	0	14	100	0	88	69	0	4	100	25	0	0	0
Sulphadimethoxine/Ormetoprim	29	41	0	6	100	100	14	9	6	14	16	100	14	100	33	17	0	17	0	0	4	0	6	12	0	0	75	100	0
Oxacillin + 2% NaCl	100	99	91	83	100	100	0	36	0	0	100	100	100	0	17	14	0	29	0	0	0	0	100	100	0	100	100	92	0
Penicillin	100	100	27	47	100	100	57	91	35	49	100	100	100	0	67	71	27	57	0	0	12	0	100	100	0	25	100	100	50
Rifampin	97	94	27	5	100	100	0	0	0	37	100	95	100	0	0	0	9	14	0	0	0	0	100	82	0	25	100	100	0
Tetracycline	43	46	45	58	42	33	14	0	26	27	56	49	14	100	33	14	27	21	0	0	40	38	18	7	0	50	25	58	0
Ticarcillin	45	55	27	47	25	20	57	91	35	49	38	44	14	71	67	71	36	64	0	0	0	0	47	36	0	25	0	17	50
Tribrissen	29	41	0	6	33	58	14	18	23	34	56	39	14	100	50	33	18	17	0	0	0	0	0	12	0	0	50	40	0
Vancomycin	100	100	0	0	100	100	0	0	0	0	100	100	100	0	0	0	0	0	0	0	0	0	100	100	0	0	100	100	0
number of isolates	68	83	11	19	12	16	7	11	31	37	16	43	7	7	6	7	11	14	6	2	25	13	17	28	1	4	4	12	2

Percent of Micro-organisms that are Resistant to Selected Antibiotics from Jan.- June and Jul.- Dec. 2003

Antibiotic	Beef								Dairy										Swine							
	E. coli		Past. Haemolitica		Past. Multocida		Salmonella sp.		E. coli		Past. Haemolitica		Past. Multocida		Staph. aureus		Salmonella sp.		APP		E. coli		Salmonella sp.		Strep. suis	
	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June	July-Dec.
Ampicillin	25	28	33	14	11	8	40	50	52	59	0	50	22	0	35	32	30	54	17	18	58	59	47	63	1.83	3.8
Apramycin	6	3	17	71	56	100	20	0	28	22	17	0	56	70	na	na	9	0	7	5	20	17	8	8	45.8	28.8
Ceftiofur	11	3	0	0	11	8	40	50	25	20	0	0	0	0	0	5	30	50	0	0	13	13	18	30	4.29	9.4
Chlortetracycline	59	52	0	14	11	8	40	50	86	78	0	50	0	0	na	na	39	53	14	5	94	92	78	83	96.6	84.8
Clindamycin	100	100	100	100	100	100	100	100	96	96	100	50	100	90	na	na	100	100	14	27	99	100	100	100	85.1	85
Enrofloxacin	5	14	0	14	11	8	0	0	11	15	0	0	0	0	na	na	0	0	2	0	0	0	0	0	7.04	0.86
Erythromycin	98	100	0	14	22	38	100	100	96	98	0	0	11	8	10	5	100	98	2	5	99	100	100	100	82.4	84.4
Florphenicol	100	100	0	14	11	25	100	100	95	98	0	0	11	10	na	na	100	100	0	0	100	99	98	100	78.1	57.8
Gentamicin	13	17	0	0	0	25	20	25	49	40	0	0	0	0	na	na	17	11	0	0	23	21	6	5	3.71	6.98
Neomycin	24	21	83	43	22	75	20	50	75	67	33	50	56	20	na	na	22	25	2	5	45	40	25	28	60.7	49.3
Oxytetracycline	67	62	67	29	44	63	40	50	86	82	33	50	56	20	na	na	43	55	43	36	95	96	78	85	95.4	89.2
Penicillin	100	100	50	57	22	25	100	100	96	98	100	100	33	17	35	32	100	100	88	68	100	100	100	100	5.2	8.46
Sulphadimethoxine	52	48	83	43	78	75	40	50	63	57	33	50	67	42	85	100	39	61	12	18	74	69	75	65	55	60.4
Spectinomycin	32	41	100	71	67	75	100	100	76	64	83	50	89	20	na	na	91	93	9	32	55	62	92	95	22.1	35.8
Sulphachloropyridazine	52	48	0	43	67	75	40	50	84	80	33	0	67	80	na	na	35	55	22	18	73	69	65	63	58.8	59.3
Sulphathiazole	52	48	83	57	89	75	40	50	84	78	50	50	78	60	na	na	39	55	19	14	76	68	65	63	60.6	61.3
Tiamulin	100	100	50	71	78	88	100	100	96	96	50	50	67	90	na	na	100	100	9	9	100	100	100	100	18.4	24.4
Tilmicosin	100	93	0	14	22	63	100	100	96	95	17	0	22	10	na	na	100	100	2	9	100	98	100	100	81.8	85.5
Triple Sulfa	16	24	0	14	11	0	20	25	68	56	33	0	0	0	na	na	13	4	0	0	17	14	16	18	0.62	2.94
Tylosin	100	100	100	100	100	75	100	100	96	96	100	100	100	90	na	na	100	100	na	na	100	100	100	100	na	na
number of isolates	63	29	6	7	9	8	5	4	80	55	6	2	9	10	20	22	23	55	58	22	128	121	51	40	109	105