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Winter 2006

FROM THE DIRECTOR

H. Leon Thacker, DVM, PhD

As this is written, winter has been mild and quite pleasant compared with some of those of past years. OK with me if it continues. Activity in the ADDL continues to be high; the faculty and staff of the Laboratory continue to provide dedicated, beyond the call of duty, hustle to diagnostic requests and submissions. We have recently completed testing by the immunohistochemistry method samples from 1256 hunter-killed deer from Indiana; all samples were found to be 'no resistant prions detected, i.e. no prions diagnostic of Chronic Wasting Disease of deer were found. We continue to support the national surveillance program for detecting Scrapie of sheep and goats. We are running 600-1000 samples by IHC per week. The federally assisted state program for Johne's disease surveillance in Indiana continues to generate samples for fecal culture or serum ELISA testing for Johne's disease.

Our laboratory was recently selected as one of the members of the National Animal Health Laboratory Network laboratories to participate in an interlaboratory comparison study for a newly developed Lawrence Livermore National Laboratory multiplex system capable of testing for a number of diseases from the same animal tissue/fluid sample by multiplex polymerase chain reaction methodology. High technology equipment has been installed and calibrated in the ADDL and we have two technicians in addition to the head of our molecular diagnostics area, Dr. Ramesh Vemulapalli, who have been trained on the equipment operation.

I was recently presented with a letter of resignation from Dr. Zheko Kounev who has been a member of our faculty for the past three years as avian diagnostician and food safety specialist. Dr. Kounev was presented with an opportunity with an Illinois nutrition company who will assign Dr. Kounev to activity in Bulgaria for major timeframes as part of his employment responsibilities. As Zheko and his wife are natives of Bulgaria and they yet own properties there, it was an opportunity that felt they could not decline. We wish Zheko and his family the best of times in the future. ADDL will begin a search for a replacement for Dr. Kounev's avian activities ASAP.

We continue to receive queries regarding Avian Influenza presence and testing. To date, we have tested birds of several species, the most numerous being chickens and turkeys; we have found no evidence of AI presence in Indiana. Tests available in ADDL for AI include PCR, virus isolation, antigen capture ELISA and agar gel immunodiffusion.

In August of 2005, we sent out a survey of ADDL laboratory users to get ideas of means whereby we can improve services. We were very well pleased with the satisfaction reflected by the returned survey results. Some areas of perceived needed improvement were identified; we are working on them.

We hope to see many of the veterinarians who receive this newsletter at the annual meeting of the Indiana Veterinary Medical Association in Indy the end of this month.

FINAL DIAGNOSIS: This column in the Winter 2006 issue is being replaced by an article written by Carla Vega de la Cruz, Purdue Merck-Merial Summer Research Scholar, Tuskegee University

Bone Marrow Fat Analysis as a Measure of Starvation in Animals

Summary: Making a definitive diagnosis of starvation in animals is difficult because there are few quantitative measures of starvation available at postmortem examination. The Toxicology and Analytical Chemistry section of the Purdue ADDL is currently developing a method which will be used to relate severely decreased bone marrow fat to clinical starvation. This will be done by developing an analytical method for bone marrow fat analysis, establishing a database of values for the normal percentage of bone marrow fat in domestic animals, and relating severely decreased bone marrow fat to clinical starvation. At this time, we welcome inquiries regarding the submission of femurs for analysis.

Rationale and Significance: Malnutrition is a state in which a diet does not provide the optimal amount of nutrients. The long-term effect of inadequate intake of food is starvation. Inadequate food intake can be exacerbated by a physiological condition or disease state as well as by extreme environmental factors such as those that occur in winter. Malnutrition and starvation are a natural cause of death in wildlife. Management practices resulting in malnutrition and starvation can also occur in domestic livestock. When this occurs, there can be legal ramifications related to mismanagement and mistreatment. However, diagnostically, there are few quantitative measures of starvation available at post-mortem examination. This is why making the definitive diagnosis of starvation is many times difficult, especially if the cause and time of death are unknown (Ballard, 1995). Therefore, a need exists for a validated, quantitative analytical method which can be used to support a post-mortem diagnosis of starvation.

Literature Review: Malnutrition is defined as the inadequate intake and/or malabsorption of any required nutrients (Stedman's, 1995). This can occur in an animal which is eating, but is not able to ingest, digest, absorb, and/or utilize a sufficient quantity of nutrients (Radostits, 2000). In addition to simple lack of food/nutritional intake, malnutrition can be related to injuries, bad teeth, parasitism, neoplasia, toxins, or infectious disease (Hungerford, 1990). Starvation is characterized by a lengthy and continuous deprivation of food (Stedman's, 1995). They both can be caused by diseases, injuries, management conditions, and/or the environmental conditions in which the animals live. In the northern hemisphere, winter can bring on additional stress to outdoor livestock due to a lack of food-related negative energy balance brought about by poor quality/inadequate forages, cold weather, and increased energy demands (Radostits, 2000).

In wild ruminants such as deer and moose, analysis of bone marrow fat content by various methods has been used for several decades for diagnosis of starvation because, following harsh winters, bones are frequently the only sample which can be found for evaluation (Cheatum, 1949, Bischoff, 1954, Greer, 1968, Neiland, 1970, Verme and Holland, 1973, Franzmann and Arneson, 1976). In those studies, a fat solvent extraction method was generally found to provide the most consistent results when compared to other methods even though some of the other air-drying or compression methods are more rapid and easier to perform in the field (Greer, 1968, Meiland, 1970, Verme and Holland, 1973). In wildlife, the femur has been used as a standard when evaluating bone marrow fat content (Ballard, 1995). The femur is used because it is readily obtained, has a large marrow content, an abundant blood supply, and is one of the last fat sources to be utilized. The bone marrow of a normal healthy animal is solid, white and waxy due to the high fat content (Cheatum, 1949). In a state of malnutrition, the bone marrow is red, solid, and slightly fatty to the touch (Cheatum, 1949). In an advanced state of starvation, the bone marrow is red to yellow, gelatinous, and glistening and wet to the touch due to the high water content (Cheatum, 1949). In addition to the applicability of the solvent extraction method, findings of those wildlife studies pertinent to domestic livestock include: 1) with a high degree of accuracy, a gelatinous bone marrow ,regardless of its color, is indicative of a poor animal resulting directly or indirectly from malnutrition as in one study, 95% of gelatinous marrows were found in poor deer and 97% were low in marrow fat (low defined in that study as less than 19%, Bischoff, 1954), 2) no definite conclusion can be made from a solid marrow concerning deer condition as in some cases they can appear solid down to approximately 40%-50% fat (Bischoff, 1954), 3) tibia marrow does not correspond to femur marrow (Bischoff, 1954), 4) in another study,

femur bone marrow fat content (by solvent extraction) in winter-killed elk was less than 0.25% (n=12) although the fat content in other live elk at the end of winter could be as low as 1% (Greer, 1968), and 5) in an additional study, femur bone marrow fat from winter-killed moose was as low as 6.1% fat in calves and 5.5% in adults by a dry-weight method which includes non-fat residue (Franzman and Arneson, 1976). However, while these studies have been performed on wildlife, there are no published reports of the use of bone marrow fat for diagnosis of starvation in domestic livestock.

The body utilizes different sources (carbohydrates, protein and fat) for energy. Generally, the first source utilized is carbohydrate in the form of glycogen. However, glycogen stores are relatively rapidly exhausted and the next source for energy is predominantly fat. Bone marrow fat is one of the last body stores of fat to be used. Late in the course of starvation, when glycogen and fat stores have been depleted, the only source available for energy is protein, the catabolism of which results in the development of ketosis (ketone bodies in blood and urine). If this negative energy balance is not corrected the animal will die. In general, clinical signs and gross pathological findings related to malnutrition/starvation include animals that are weak and underweight, have a loss of skin turgor, have dull hair coats, sunken eyes, tucked-up abdomens, prominence of the bones of shoulders, ribs, vertebra and pelvis, atrophy of muscles, and a decrease or absence of subcutaneous, perirenal, pericardial and bone marrow fat which can be described as serous atrophy of fat.

Objective: Our goal is to develop and validate an analytical method for the quantification of bone marrow fat from the femur and utilize that method to establish a database of normal values in different animals for use in suspected cases of starvation. The ADDL Toxicology and Analytical Chemistry section has begun to develop and validate the analytical method. We will then develop a database of normal values that can be used to determine cases of starvation in animals.

- edited by the Toxicology and Analytical Chemistry Section
Dr. Steve Hooser, Section Head
Dr. Robert Everson, Analytical Chemist
Christina Wilson, Assistant Chemist
Kim Meyerholtz, Laboratory Technician

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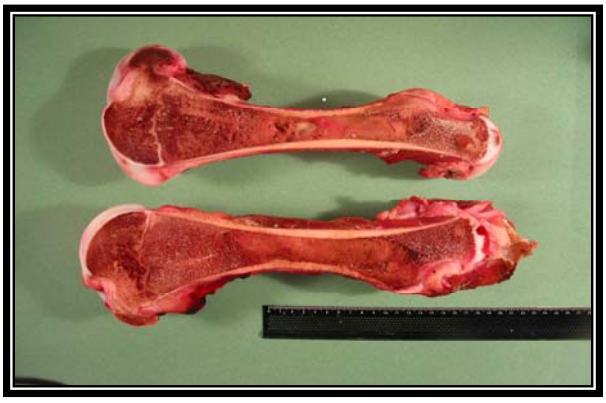

Normal bone marrow

Leptospiral Reproductive Losses in Cattle

Traditionally, leptospiral abortions were thought of as late-term losses and the source of infection was a contaminated environment due to wildlife, dog, or swine reservoirs. These late-term abortions are caused by several serovars including *Leptospira interrogans* serovar hardjo (type: hardjo-prajitno), *Leptospira interrogans* serovar pomona, *Leptospira interrogans* serovar canicola, *Leptospira interrogans* serovar icterohemorrhagiae, and *Leptospira kirschneri* serovar grippotyphosa. The standard 5-way leptospiral vaccines provide protection from all of these pathogens; however, an additional leptospiral threat exists, *Leptospira borgpetersenii* serovar hardjo (type: hardjo-bovis).

For hardjo-bovis, cattle are the maintenance host. The reproductive tracts and kidneys are colonized and cattle serve as the source of infection in a herd. The traditional late-term abortions can occur, but the real effect is overall reduced reproductive performance. Herds see reduced conception and pregnancy rates, increased early embryonic losses, and stillbirths and weak calves.

Eliminating carriers is key to minimizing losses associated with leptospirosis once a diagnosis has been established within a herd. To diagnose a herd problem with hardjo-bovis, serum and urine samples are required from a representative sample of the herd. Any open cows should be included in that sampling. To facilitate flushing the leptospiral organisms from the kidneys, cows are given furosemide and the urine sample is collected from dilute urine that is voided after the initial concentrated urine is voided. The urine is chilled in red-top tubes. Although serology is typically more useful with the *L. interrogans* and *L. kirschneri* serovars, serum samples should also be collected from the same animals that provided the urine samples. If aborted fetuses are available, useful samples include kidney, liver, lung, urine, and thoracic or abdominal fluids. Contacting your diagnostic laboratory for guidance on what samples to submit and how to package them for shipment is always recommended.

Once the diagnosis of hardjo-bovis is established, carriers are eliminated by treatment with oxytetracycline. A diligent herd vaccination program that includes not only the traditional 5-way vaccines, but the new Spirovac vaccine for *Leptospira borgpetersenii* serovar hardjo (type: hardjo-bovis) is necessary. Spirovac is safe and effective in calves as early as 4 weeks of age. Initial vaccination includes 2 doses at 4-6 weeks apart. Once the entire herd is initially vaccinated, annual vaccination is required to maintain protection. Additionally, any additions to the herd should be isolated and follow the same treatment and vaccination schedule used to initially clear the herd.

-by Dr. Julie Davis, Class of 2005
-edited by Dr. Leon Thacker, ADDL Director

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ADDL Schedule

Purdue ADDL and Heeke ADDL will be closed on the following University holidays in 2006.

January 16……Martin Luther King Day
May 29……Memorial Day
July 4……Independence Day
September 4……Labor Day
November 23-24……..Thanksgiving
December 22-26…….. Christmas

Equine Mandibular Juvenile Ossifying Fibromas

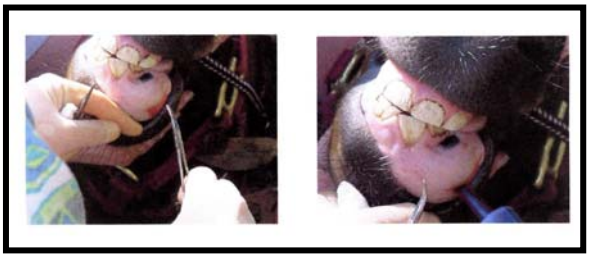
Although equine tumors are fairly uncommon, a significant portion of those that do arise occur in the head and neck region. Specifically, tumors of the oral cavity may originate in the mandible, gums, tongue, etc., often extending into the surrounding tissues. One of these tumors is the ossifying fibroma that tends to develop from the intramembranous bone of the mandible. This tumor has a high occurrence in young (2-14 months of age) horses, no breed or sex predilection has been shown, and genetic predisposition has yet to be determined. Many believe this tumor can be diagnosed through history, signalment, physical examination and radiographic findings, as it presents with highly characteristic features. Nevertheless, in order to definitively diagnose an ossifying fibroma and distinguish it from similar proliferative lesions in the mandible (osteoma, osteosarcoma, fibrous dysplasia, fibrous osteodystrophy), histo-pathology should be key in the diagnostic plan.

This neoplasm is locally aggressive, with extensive bony proliferation and trabecular destruction, yet there are no reports of it possessing metastatic qualities. Medical and/or surgical options may be employed to successfully treat the neoplasm, avoiding further occurrence. However, as with other invasive neoplastic processes, inadequate resection of margins or incomplete treatment often leads to rapid, extensive and increased regrowth. Prognosis is often dependent on the extent of mandibular involvement (prehension difficulty) and aesthetic appearance of the horse (owner's visual value).

Clinical Presentation and History: Clinical signs associated with the juvenile ossifying fibroma depend on location and size of the tumor. They may include difficulty with prehension, lymphadenopathy, and intermittent oral mucosal bleeding. Early diagnosis is not often made because the anatomy of the oral cavity allows for a considerable amount of involvement and progression of the ossifying fibroma before obvious clinical signs are noted. When clinical signs are apparent, advanced local infiltration is often present. The most common presentation is as a sub-gingival, bony proliferation on the rostral mandible in a young horse. The mass is uniformly firm and does not elicit signs of pain when manipulated. The

mucosa covering the mass is usually ulcerated. Upon palpation, the teeth in the affected area may be loose.

It is controversial whether the juvenile mandibular ossifying fibroma is caused by trauma, or whether an undetected, immature fibroma causes the bone to be brittle and more susceptible to minor trauma. Many of the reported cases cite an injury as the cause of the bony proliferation. Trauma affecting the mandible through a fall, kick or self-inflicted injury (e.g., running into objects), will often result in gingival ulcerations and tears that will not heal despite weeks to months of treatment, progressing to the growth of a prominent hard structure at the site of injury. The ossifying fibroma arises from mutations in normal bony remodeling that would otherwise reconstruct the mandible. The mass will proliferate until the lips are no longer apposed (allowing visualization of the mass), prehension difficulty is noted, and weight loss occurs as a result of not eating. Many feel that the trauma sustained by the mandible should not be substantial enough to elicit such an injury with obvious prolonged healing time. Therefore, another facet to the trauma theory is that the ossifying fibroma already existed in the bone, had weakened its trabecular structure, and made the mandible more susceptible to minor injury. Extensive and rapid growth is then stimulated by the trauma.



Diagnosis: A tentative diagnosis can be made based on the history, as well as on the gross, clinical and radiographic aspects of the lesion.

Histological examination of the mass is used to confirm the presumptive diagnosis. This can be done on a core biopsy or an en bloc excisional biopsy. The juvenile mandibular ossifying fibroma is characterized by well differentiated, moderately vascularized, abundant, dense fibroblastic stroma, with isomorphic fibroblasts transforming into osteoblasts that rim bony spicules. The histologic alterations tend to be very uniform in appearance throughout the mass.

To distinguish the juvenile mandibular ossifying fibroma from other closely resembling non-neoplastic and neoplastic lesions, histologic morphology plays an important role. Unlike ossifying fibroma, bony spicules in fibrous

dysplasia are rarely lined by osteoblasts, and only mature lesions contain deposits of lamellar bone. Another differential diagnosis can be osteoma. These are bony growths that are initially formed of cancellous bone with intertrabecular fatty or hematopoietic marrow; they may become increasingly compact with time. Because of the morphological similarity between ossifying fibroma and some cases of osteoma, it is thought that ossifying fibromas may mature into osteomas. Finally, in osteosarcomas, neoplastic cells have a high mitotic index and are pleomorphic, features which are lacking in ossifying fibromas.

Treatment: Treatment includes surgical (mandibulectomy, hemi-mandibulectomy) and medical (radiation therapy) management. Combinations of these therapies may also be employed.

Surgical management requires the extensive removal of the entire mass and involved structures (teeth), with achievement of adequate clean margins. It has been widely reported that local excision of a juvenile mandibular ossifying fibroma often results in rapid and proliferative recurrence unless the surgical excision includes wide surgical margins. The choice of which surgical procedure to use is based on diagnostic imaging (radiographs, computerized tomography), which determines the extent of bony involvement. If diagnosed or suspected early in the growth process, a rostral mandibulectomy or rostral hemi-mandibulectomy may suffice as proper treatment. If there is significant bony involvement, more drastic surgical procedures (complete mandibulectomy or hemi-mandibulectomy) are recommended. When the ossifying fibroma has grown from the rostral mandible, involved the entire mandibular symphysis, and extended to both hemi-mandibles, internal fixation (metal implants) must be used to create a pseudosymphysis upon removal of the neoplasm. This allows for proper apposition of dentition, as well as stabilization of the grinding forces of the jaw during mastication. If complete removal of the juvenile ossifying fibroma is achieved, there is a very low probability of recurrence, even years post surgery. If regrowth is to occur, most studies have shown that this takes place within the first six months post surgery.

Radiation therapy, the other therapeutic option in cases of mandibular ossifying fibromas, uses ionizing radiation to treat the neoplasm and to limit the neoplastic growth. While not surpassing the normal tissue tolerance of the healthy tissue surrounding the ossifying fibroma, radiation therapy delivers a sufficient lethal dose of radiation to the tumor tissues. Radiation therapy can be delivered through brachytherapy or, more commonly, through an external beam. External beam therapy includes gamma or X-rays from megavoltage equipment with Cobalt-60, linear accelerators or orthovoltage machines. Success has been obtained by treating the ossifying fibroma with a bilateral parallel opposed pair technique. The radiation margins should include the tumor and a border of normal, healthy tissue. After several successive treatments, the mass initially appears to be the same size, but less radiodense using diagnostic imaging. Over time, the ossifying fibroma progressively decreases in size to the point of no visible external existence.

As with radiation treatment, serial follow-up radiographs are extremely important in the surgical post-operative monitoring of the patient. Radiation therapy can be combined with surgery; surgery can be used to either debulk the mass for radiation therapy or used in en bloc excision to expose transitional margins primed for radiation therapy.

In summary, mandibular juvenile ossifying fibroma is a locally invasive, proliferative, fibro-osseous tumor that is most commonly found in the mandible of young horses. Though aggressive in nature, the neoplasm is benign, as no incidents of metastasis have been reported. Grossly it is very distinct, yet in order to definitively diagnose this mass, histopathology must be employed. If diagnosed prior to significant mandibular involvement, treatment options yield a fair to good prognosis. Both surgical and radiation therapies have resulted in extremely low recurrence rates when adequately employed, with the horse returning to normal prehension, activity and visual aesthetics post treatment.

-by Araba Oglesby, Class of 2006
-edited by Dr. Ingeborg Langohr

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Blood tube boxes

Please remember…

The blood tube boxes that we provide to veterinarians are to be used only for blood samples sent to Purdue ADDL.

Granulomatous meningoencephalomyelitis (GME) in dogs

GME is an acute, progressive inflammatory disease of the central nervous system (CNS) of dogs. GME is a common differential for dogs that are affected by focal or diffuse neurological diseases. An inflammatory disease like GME can cause severe and often irreversible damage to the CNS. Consequently, a better understanding of the disease is essential.

Etiology: GME has been reported around the world and can affect most breeds and ages of dogs; however, middle aged, small breed dogs such as terriers and poodles are more susceptible (Thomas, 1998). GME accounts for up to 25% of all canine CNS disorders reported in the United States (Cuddon, 1984). No specific etiological agent has been described for this disease.

Clinical signs: The clinical signs of the disease are variable depending on the location of the lesion in the CNS. Three syndromes of GME have been recognized based on the location of the lesion:

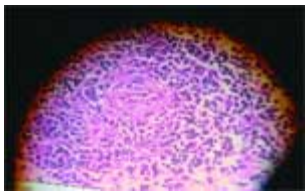
a) *Focal GME* – this is a chronic progressive condition (3-6 months) and the clinical signs occur secondary to nodular granuloma formation and mimic the effects of space occupying tumor/masses.

b) *Multifocal or disseminated GME* – This is an acute, progressive condition (2-6 weeks). The most common sites affected are lower brain stem, cervical spinal cord and meninges. Up to 25% of the dogs are dead within a week (Wong and Sutton, 1002).

c) *Ocular form*- this can be acute, progressive or static and can affect eyes unilaterally or bilaterally.

Depending on the location of the lesions, the clinical signs can vary, but neurological deficits and pain from meningeal involvement are common.

Pathology: At necropsy, gross lesions are evident if the angiocentric inflammation is severe and can be seen as areas of swelling and yellow to gray discoloration. Histopathologic lesions are characterized by perivascular cuffs of monocytes, macrophages, lymphocytes and plasma cells. These perivascular cuffs merge at adjacent blood vessels to form cellular whorls that can evolve into nodular granulomas (Ryan et al (Ryan et al, 2001). Immunohistochemical characterization of the inflammatory cells in the granulomatous lesions of GME showed that the lesions consist of MHC class II and CD3+ T-cells indicating a T-cell mediated delayed hypersensitivity reaction (Kipar, 1998).



Diagnosis: GME diagnosis is supported by the exclusion of neoplastic, infectious and other inflammatory conditions (e.g., canine necrotizing meningoencephalitis, NME). CT and MRI can sometimes be of use in detection of the CNS lesions but it is difficult to differentiate the lesions from neoplasia. Cell characteristics such as cytologic atypia and mitotic figures might be useful to differentiate this condition from neoplasia or neoplastic reticulosis. Granulomatous inflammation due to viruses (e.g., rabies or canine distemper), protozoa (e.g., *Toxoplasma* and *Neosporum*), and fungi (e.g., *Cryptococcus*) can be ruled out by demonstration of specific antigens in CSF or serum antibody titers for the various etiologic agents. NME can be differentiated based on breed predilection (small-size breeds, especially Pugs) and lack of obvious granulomas.

Treatment: B) Corticosteroids are the mainstay of treatment for GME. Response to therapy is variable and discontinuation results in recurrence of clinical signs and progression of the disease. B) Leflunomide a de novo pyrimidine synthesis inhibitor can also be used because of the immune component (T-cell mediated) in the disease. However, these drugs are expensive and controlled clinical trial results are not available. C) Radiation therapy can prolong the mean survival (MST) of dogs.

Prognosis: The prognosis is generally poor for GME. MST for all dogs with GME is 14 days (range 1-1215 days – Munana, 1998). Dogs with focal signs in forebrain have an MST of >359 days while dogs with focal signs elsewhere have an MST of 59 days. Dogs with multifocal signs have an MST of 8 days. Dogs that receive radiation therapy for focal signs can survive >404 days. Corticosteroid therapy may induce a transient remission of clinical signs and can prolong the MST.

-by Jeetendra Eswaraka, EVFVG Student
-edited by Dr. Vimala Vemireddi, ADDL Graduate Student

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On the Road

Drs. Leon Thacker, Greg Stevenson, Bob Everson, Ching Ching Wu, Duane Murphy, Steve Hooser, Jose Ramos-Vara, Ramesh Vemulapalli, Roman Pogranichniy, and Linda Hendrickson and Steve Vollmer attended the annual meeting of the American Association of Veterinary Laboratory Diagnosticians in Hershey, PA, November, 2005.

Drs. Roman Pogranichniy and Ching Ching Wu attended the Conference for Research Workers in Animal Diseases/International PRRS Symposium in St. Louis, December, 2005.

Drs. Margaret Miller, Ingrid Pardo and Gopa Gopalakrishnan attended the annual American College of Veterinary Pathologists meeting in Boston, December, 2005

ADDL NEWS

Our congratulations to **Dr. Gopa Gopalakrishnan and Dr. Alok Sharma**, ADDL Graduate Students, both of whom were presented awards at the recent American College of Veterinary Pathologists meeting in Boston, December, 2005. Dr. Gopalakrishnan was awarded one of three ACVP Young Investigator Awards for his poster presentation in the Diagnostic Pathology category entitled “Esophagitis in Camelids: Report of 3 Cases.

Dr. Sharma was awarded the C.L. Davis DVM Foundation Student Scholarship Award in Veterinary Pathology, an award given to a student who displays superior knowledge of pathology as well as leadership, dedication and accomplishment.

Reporting Results

In an effort to provide results to our users more quickly, ADDL has upgraded our processes to enable "AutoFaxing". This allows case reports to be generated, posted to the website, and faxed shortly after the results are entered into the computer system, even if it is after hours or on week-ends.

If you would prefer to have your case results emailed instead of (or in addition to) being faxed, send us a request at addl@purdue.edu. Case reports will be mailed as Adobe PDF files.

From the Virology Section

Testing for Persistently Infected BVD animals by antigen capturing ELISA

Bovine viral diarrhea virus (BVDV) belongs to the family *Flaviviridae*, the genus pestiviruses. There are several members of the genus and two types of BVD viruses. All of them are highly infectious and economically important to the livestock industry. If a fetus becomes infected *in utero* with BVD virus in the early stages of pregnancy, the newborn calf will become persistently infected (PI) without immune response to this virus for the rest of its life. This animal will shed the BVD virus in the herd and infect other animals. If a PI animal is not identified in the herd, it will be a source of infection until it is removed. Elimination of BVD from the herd requires removing PI animals from the herd.

There are several assays available to identify PI animals: Polymerase chain reaction (PCR), virus isolation (VI), immunohistochemistry (IHC) and antigen capturing (Agc) ELISA. The AgcELISA for BVD virus is a rapid diagnostic tool that can identify PI animals in the herd if appropriate samples are submitted. Serum and ear notch samples are suitable for PI animal testing by AgcELISA for BVD viral antigen. To provide optimum service on this assay, the following guidelines should be followed when sending ear notch samples to Purdue ADDL for AgcELISA BVD PI testing in animals of all ages.

1. Ear notches should be taken with a sharp adult-sized pig ear notching tool.
   - Baby pig ear notches, punches and other cutting and punching tools are **NOT** recommended; the sample they provide is too small for an accurate test.
   - Dull notches can damage animals and samples are not sufficient.

2. Ear notches need to be fresh and the samples need to be approximately **1 cm x 1 cm** in size.
   - Avoid testing scabby or frostbitten ears.
   - **DO NOT** put samples in formalin.
   - Samples should be submitted fresh and chilled. For short term storage (1-2 days), samples can be refrigerated at 4º C; for long term storage, samples can be stored at -20º or colder.

3. Package individually in snap cap tubes (size 12x75 mm). These can be obtained from the following vendors: Fisher Scientific at www.fishersci.com (catalog #14-959-2A) and VWR at www.vsrsp.com (catalog #60818-419).
   - **DO NOT** use Whirl packs to submit samples
   - Number tubes 1,2,3,etc..with animal identification to match information on accession form. Numbers should be clearly marked and legible.
   - It is not recommended to submit pooled samples for testing or to request pooling of samples.
   - If submitting more than 200 ear notches, please call the ADDL at 765-494-7440 prior to submission to allow for the quickest possible processing samples and reporting.

Percent of Micro-organisms that are Resistence to Selected Antibiotics from Jul.- Dec. 2004 and Jan.-June 2005.

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	
	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.	Jan.-June	July-Dec.	Jan.-June	July-Dec.	Jan.-June
Amikacin	0	1	19	56	4	0	0	0	0	0	0	11	0	0	0	0	0	0	67	50	83	15	0	0	30	0	0	0	14	0
Amoxycillin/Clauvulinic acid	23	19	15	17	96	95	18	11	32	0	6	11	57	17	20	0	38	23	0	0	0	0	0	22	20	0	100	100	86	50
Ampicillin	40	43	15	17	96	100	55	68	58	53	35	25	64	33	40	50	38	50	0	0	0	0	24	35	20	0	100	100	100	0
Cefazolin	22	23	63	89	96	100	0	11	11	0	6	14	57	17	20	0	23	18	0	0	0	0	3	22	50	0	100	100	41	0
Cefotaxime	50	21	0	100	0	100	0	11	0	0	0	14	0	17	nt	0	nt	18	nt	0	0	0	nt	22	nt	0	nt	100	nt	0
Cefpodoxime	nt	20	nt	100	nt	100	nt	0	nt	0	nt	15	nt	33	nt	0	nt	29	nt	0	nt	0	nt	22	nt	0	nt	100	nt	0
Ceftiofur	18	16	74	89	88	100	0	11	13	0	6	11	57	17	20	0	23	18	0	0	0	0	0	22	90	0	100	100	41	0
Cephalothin	24	27	52	89	96	100	0	11	13	0	13	17	57	17	20	0	15	18	0	0	0	0	3	17	50	0	100	100	41	0
Chloramphenicol	11	18	4	6	91	90	0	0	0	0	16	17	64	33	0	0	0	9	0	0	0	0	0	17	0	0	100	100	0	0
Clindamycin	99	100	81	83	96	100	0	11	13	4	100	100	100	100	20	0	8	14	0	0	0	0	100	100	80	0	100	100	57	0
Enrofloxacin	20	19	48	39	54	43	27	11	21	4	13	0	0	0	0	0	0	9	67	0	38	6	0	13	50	0	0	0	57	50
Erythromycin	99	99	33	28	100	100	0	26	18	9	100	100	100	100	20	0	38	23	0	0	8	0	100	96	20	0	100	100	71	0
Gentamicin	10	18	11	11	8	5	0	0	5	4	23	17	43	33	40	25	8	9	33	50	71	0	3	17	40	0	0	0	14	0
Imipenem	1	1	15	17	0	5	0	11	14	0	0	0	0	0	20	0	15	18	0	0	0	0	0	0	20	0	0	0	71	0
Marbofloxacin	nt	1	nt	0	nt	0	nt	0	nt	0	nt	0	nt	0	nt	0	nt	0	nt	0	nt	0	nt	0	nt	nt	nt	0	nt	nt
Orbifloxacin	24	19	31	28	39	38	0	11	19	4	13	0	0	0	0	0	0	9	0	0	0	0	0	13	10	0	0	0	57	0
Oxacillin + 2% NaCl	99	100	27	100	87	100	0	11	13	55	100	100	100	100	20	0	15	18	0	0	0	100	100	96	60	0	100	100	71	0
Penicillin	99	100	26	22	96	100	55	68	59	0	100	100	100	100	40	50	31	45	0	0	0	0	100	100	20	0	100	100	100	0
Rifampin	94	87	26	33	100	100	0	0	0	0	100	97	100	100	0	0	0	9	0	0	0	0	82	70	10	0	100	100	14	0
Tetracycline	26	29	41	72	75	81	27	100	21	15	39	33	71	33	40	13	8	14	0	0	54	18	21	17	80	0	43	50	14	0
Ticarcillin	34	41	22	17	25	5	55	21	62	55	35	22	64	17	40	50	38	50	0	0	0	0	24	26	20	0	14	0	100	0
Ticarcillin/Clavulanic Acid	nt	15	nt	22	nt	5	nt	68	nt	0	nt	3	nt	17	nt	0	nt	23	nt	0	nt	0	nt	9	nt	0	nt	0	nt	0
Trimethoprim/Sulphamethoxazole	nt	27	nt	11	nt	90	nt	0	nt	0	nt	36	nt	17	nt	13	nt	9	nt	0	nt	3	nt	9	nt	0	nt	100	nt	0
number of isolates	128	150	27	18	23	21	11	19	39	47	31	36	14	6	5	8	13	22	3	2	24	33	34	23	10	0	7	2	7	2

nt - not tested

Percent of Micro-organisms that are Resistence to Selected Antibiotics from Jul.- Dec. 2004 and Jan.-June 2005.

Antibiotic	Beef								Dairy										Swine							
	E. coli		Man. haemolitica		Past. multocida		Salmonella sp.		E. coli		Man. haemolitica		Past. multocida		Staph. aureus		Salmonella sp.		Haemophilus sp.		E. coli		Salmonella sp.		Strep. suis	
	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.	Jan.-June
Ampicillin	35	29	0	0	0	0	67	70	52	61	13	63	0	8	89	47	66	52	13	20	69	71	48	68	3	3
Apramycin	8	0	33	100	100	100	0	0	19	23	50	0	71	71	nt	nt	0	0	4	9	20	20	0	13	43	25
Ceftiofur	19	3	0	0	0	0	0	0	27	28	0	0	0	0	0	0	63	52	0	4	25	28	10	28	12	11
Chlortetracycline	65	63	0	0	0	0	0	63	84	85	13	38	0	0	nt	nt	69	56	0	2	98	95	81	85	92	92
Clindamycin	96	100	100	100	100	100	100	100	95	99	100	100	100	100	nt	nt	100	100	13	20	100	99	100	100	82	88
Enrofloxacin	15	3	0	0	1	0	0	0	19	15	25	25	0	0	nt	nt	0	0	0	2	0	1	0	0	0	6
Erythromycin	96	99	0	0	50	0	100	100	96	99	13	50	25	17	0	0	100	96	8	6	100	99	95	98	81	86
Florphenicol	96	100	0	0	33	0	100	88	95	99	0	25	14	10	nt	nt	100	96	0	0	100	99	95	100	69	66
Gentamicin	23	9	0	0	17	0	0	0	43	44	0	25	0	0	nt	nt	13	4	0	0	20	21	10	15	5	8
Neomycin	35	23	33	0	83	50	67	63	69	74	25	63	57	80	nt	nt	53	44	13	29	53	49	14	30	64	38
Oxytetracycline	69	69	33	0	83	0	67	63	86	86	25	75	57	50	nt	nt	69	56	63	24	99	98	81	90	94	96
Penicillin	96	100	33	0	67	0	100	100	96	99	75	75	25	25	89	47	100	100	50	55	100	100	95	100	16	17
Sulphadimethoxine	50	60	33	0	83	25	67	80	62	74	25	63	50	58	100	87	69	68	17	22	77	84	86	90	62	72
Spectinomycin	54	33	100	50	50	0	100	100	73	72	100	75	86	40	nt	nt	100	76	67	39	53	66	95	98	29	20
Sulphachloropyridazine	50	61	33	0	83	100	67	88	80	88	0	63	86	80	nt	nt	69	76	13	29	77	84	81	88	62	76
Sulphathiazole	50	60	33	50	83	50	67	75	80	86	25	88	71	70	nt	nt	72	68	50	45	77	82	81	85	60	80
Tiamulin	96	100	67	50	83	50	100	100	95	99	75	63	100	80	nt	nt	100	100	8	16	100	99	100	100	22	26
Tilmicosin	96	94	0	0	50	0	100	100	94	99	0	38	29	20	nt	nt	100	100	9	2	100	99	95	100	81	87
Triple Sulfa	31	19	0	0	33	0	0	10	56	62	13	50	14	20	nt	nt	34	28	0	0	32	25	0	15	0	6
Tylosin	96	100	100	100	83	75	100	100	95	99	100	100	100	70	nt	nt	100	100	nt	nt	100	100	100	100	nt	nt
number of isolates	26	70	3	2	6	4	3	10	81	81	8	8	7	10	9	15	32	25	24	49	101	170	21	40	86	104

nt - not tested