Spring 2000

FROM THE ASSISTANT DIRECTOR

W.G. Van Alstine, DVM, PhD

Effective July 1, 2000, Dr. Stephen Hooser will be assuming much of the leadership role as Assistant Director of the Animal Disease Diagnostic Laboratory. This change in leadership will allow me the time to resume my duties as a pathologist and to pursue a new direction in research at the ADDL and within the School of Veterinary Medicine.

I consider it an honor and a privelege to have served the past ;18 months as Assistant Director. The faculty and staff of the ADDL are a dynamic group and are dedicated to excellence in veterinary diagnostics – service, teaching, and research. They are a tremendous asset to Indiana veterinarians, the Indiana animal-owning public and our Indiana animal industries. I thank Drs. Thacker, Rebar, Woodson and Lechtenburg for the opportunity to serve as Assistant Director; I have enjoyed the challenge of ADDL administration.

Dr. Hooser is a toxicologist and a Diplomate of the ABVT. He received his DVM degree from the University of Illinois in 1982 and his PhD degree from from the same school in 1989. He is currently an Associate Professor in the Department of Veterinary Pathobiology and the Chief of Toxicology Services in the ADDL. He has several active research projects in toxicology and has been active in the area of toxicology in several national and international organizations such as the American Association of Veterinary Laboratory Diagnosticians.

I'm sure Steve will do an excellent job in helping Dr. Thacker with the leadership of the ADDL. I hope you will joint me in welcoming Dr. Hooser to this position and lend him all your supports.

As always, we are just a phone call, fax, or email away. Let us hear from you!

FINAL DIAGNOSIS

Feline Heartworm

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

Signalment: 6 year old, neutered male Domestic Shorthair cat

Clinical history: Cat was adopted as a stray one year ago and presented to rDVM with acute, progressive dyspnea. The cat died naturally.

Gross necropsy findings: Dirofilariasis, right ventricle dilation, lung edema, hepatopathy, nephropathy, kidney congestion

Histopathologic findings: Histopathology of the lung revealed a chronic, diffuse eosinophilic endarteritis with myointimal proliferation and fibrosis. The walls of all elastic blood vessels within this section were thickened by hyperplasia and hypertrophy of the tunica media. The smooth muscle cells were swollen and some were vacuolated with pyknotic nuclei. The internal elastic lamina was disrupted and the tunica intima was infiltrated by moderate numbers of eosinophils and plasma cells. The endothelial cells were diffusely plump and in some areas, were stratified. In some affected vessels, the tunica intima was expanded by homogeneous eosinophilic material (collagen) that, in occasional vessels, partially occluded the lumen with villous projections. The surrounding alveoli contained numerous eosinophils and alveolar macrophages. The alveolar septae were diffusely congested with slight hemorrhage into the alveoli, although few hemosiderin-laden macrophages and erythrophagocytes were observed. Smooth muscle bundles in the alveolar septae were prominent and rounded (hypertrophy). The peribronchial glands were increased in number and the stroma was congested and infiltrated by low numbers of eosinophils.

Morphologic Diagnoses: (1) Chronic, diffuse, proliferative and degenerative, eosinophilic endarteritis (2) Eosinophilic peribronchiolitis

Pathogenesis: Adult Dirofilaria immitis in ventricle → pulmonary arteries → eosinophilic endarteritis (Type I hyper-sensitivity) → myointimal proliferation → Type III/IV hypersensitivities → endothelial damage/platelet activation → thrombosis → recanalization → fibrosis

Discussion: Cats are generally less easily infected with Dirofilaria immitis than are dogs; however, because of small body size, cats with low worm burdens may have severe disease. Male cats are more frequently and more heavily infected. Cats have a greater tendency to spontaneously eliminate the parasite or die from infection. The life span of the parasite is shorter in cats; infections tend to be self-limiting after two years.

Aberrant migration of fourth stage larvae occurs more frequently in cats; therefore, ectopic heartworms may be found in body cavities and in the central nervous system. The most severe clinical signs in cats are associated with the arrival of larvae in the pulmonary arteries and with thromboembolism following death of one or more mature worms. The caval syndrome in dogs, caused partly by large numbers of heartworms interfering with the tricuspid valve, is rarely seen in cats due to light infection. Arterial intimal proliferation, as occurs in dogs, also develops in the small peripheral and major lobar pulmonary arteries of cats. Right ventricular hypertrophy or right heart failure due to pulmonary hypertension, however, is rare in cats. Cats will also develop interstitial pneumonia; however, unlike dogs, may also have extensive alveolar type II cell hyperplasia. These lesions play an important role in the acute respiratory distress seen in cats at presentation. Some cats may go into remission and eventually recover; however, sudden death, usually associated with degenerating worms in pulmonary arteries, can occur.

Similar lesions may be seen with infection by Angiostrongylus vasorum.

-by Kaori Sakamoto, DVM, ADDL Graduate Student

Diagnosing Bovine Viral Diarrhea in Persistently Infected Animals

Bovine Viral Diarrhea (or BVD) is a disease complex with many manifestations including subclinical infections, immunosuppression, repeat breeding problems, abortion and mummification, congenital defects, persistent infection (PI), and acute and chronic mucosal disease. The PI animal results from a viral infection of the fetus during the first four months of gestation. PI calves may be born prematurely, grow poorly, exhibit lethargy, have difficulty nursing, have reduced resistance to disease, or die at a young age. Most PI calves will die within the first 18 to 24 months of life; however, PI calves may appear healthy and grow at a normal rate).

PI cattle in herds or newly introduced to herds are the main source of BVD virus in cattle herds. Accurate identification of these infected cattle is critical for eliminating infected animals from herds. There are countries currently in the European Union that are attempting to eliminate or have already successfully eliminated BVD virus.

Clinically apparent PI animals and PI calves with mucosal disease are not a diagnostic challenge, but accurate identification of clinically silent PI animals requires an accurate herd history and specialized diagnostic tests. The current "gold standard" for demonstrating BVD virus is virus isolation. A high level of viremia is typical in most PI animals so virus is often easily isolated from serum or buffy coats from whole blood. Virus can also be isolated from nearly every tissue in the body, but greatest results typically come from lymphoid organs (spleen, Peyer's patches, mesenteric lymph nodes, and thymus). This test can be time consuming and very expensive if many animals are to be tested. In addition, circulating maternal antibodies to BVD virus can interfere with the virus isolation in very young calves.

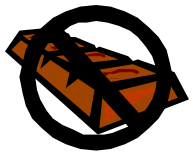
There are other molecular techniques available around the country and across the globe that can aid in the diagnosis of BVD. One of these techniques is immunohistochemical detection of BVD viral antigen using frozen or fixed tissues. Since most veterinarians submit fixed tissue, most immunohistochemistry has been developed for formalin-fixed tissues. An antibody is applied to the surface of fixed tissue that is tagged with a signal. This antibody is specific for BVD antigen and, if it finds its target, a chemical reaction takes place which amplifies the signal. In PI animals, this technique can detect the virus is nearly any tissue examined.

A second popular technique is Polymerase chain reaction (PCR). This test is designed to be specific for a portion of the viral genetic material. When the viral RNA is detected, the signal is amplified again so that the signal can be visualized. This method is very sensitive and can detect extremely small amounts of BVD virus. PCR can be used on pooled samples, such as bulk milk tank samples, thus screening large numbers of animals in lots of several animals and, if a positive signal is detected, the individual animals in that lot can be retested to determine which animal or animals are positive.

In summary, there are many different tests available that can detect PI animals in a herd. Blood, milk or tissues can be submitted for virus isolation, or can be sent to various diagnostic labs that routinely perform other molecular diagnostic techniques such as immunohistochemistry or PCR. Virus isolation remains the gold standard for definitive diagnosis of BVD virus, but can become cost prohibitive if applied to large herds and can yield false results when testing very young calves that have circulation colostral antibodies.

If you have questions regarding diagnosis of BVD please do not hesitate to call and we will discuss this with you further.

-by Lorrie Culver, Class of 2000
-edited by Brad Njaa, DVM, MVSc



Chocolate Toxicity

Chocolate is readily available, particularly at certain holiday times, and represents a potential lethal toxin for dogs. Many species are susceptible, but the dog is most commonly affected. Excessive ingestion of chocolate was recently reported as one of the top 20 most common intoxicants in the dog. Cocoa bean hulls or waste used as bedding for large animals, most commonly horses, have been a source of toxicosis as well.

Etiology: Theobromine is found in chocolate, cocoa beans, baker's chocolate, cola and tea and is believed to be the toxic component of chocolate. Baking chocolate is the most concentrated form of theobromine containing approximately 390 mg/oz versus 44 mg/oz found in milk chocolate. It is readily absorbed orally and widely distributed throughout the body. Theobromine is metabolized by the liver with primarily urinary excretion. In dogs, the LD_{50} for theobromine is approximately 250-500 mg/kg, however deaths have occurred following ingestion of 115 mg/kg. The half-life of theobromine is very long in dogs (17.5 hours) compared to other species. This may help to account for the susceptibility of canines to theobromine toxicity.

Pathogenesis: Theobromine is a methylxanthine that stimulates the central nervous system, cardiac and skeletal muscle, promotes diuresis and induces smooth muscle relaxation. Mechanisms of action, at the molecular level, include increased intracellular calcium, cAMP accumulation, and release of catecholamines. In aggregate, these cellular events lead to increased skeletal and cardiac muscle activity, and irritability of the sensory cortex resulting in exaggerated responses to normal stimuli. In severe cases, cardiac arrhythmias can lead to death.

Clinical findings: Acutely, dogs may show signs which include restlessness, hyperactivity, vomiting, diarrhea, cardiac arrhythmias, tachycardia, polypnea, ataxia, muscle tremors, hyperthermia, seizures and, if severe enough, coma. If seizures develop, they are typically tonic to tetanic and occur late in the clinical course.

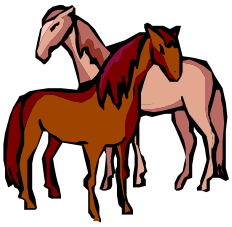
Diagnosis: Generally, historical evidence of chocolate ingestion, clinical signs compatible with chocolate toxicosis and the presence of chocolate in the GI tract are methods of diagnosing theobromine intoxication. Additionally, theobromine levels can be determined by high-performance liquid chromatography (HPLC) in stomach contents, serum or urine.

Gross lesions are nonspecific and may include hyperemia of the gastric and duodenal mucosa, and diffuse organ congestion.

Treatment: There is no specific antidote for theobromine; however, a combination of detoxification, supportive and symptomatic treatment can be successful. Detoxification includes emetics within the first 3-4 hours of ingestion, gastric lavage, activated charcoal administration, and oral cathartics. Remember, theobromine has an unusually long half-life in dogs so detoxification should be continued for at least 72 hours. Supportive therapy includes intravenous fluids to prevent dehydration, urinary catheterization to prevent reabsorption of toxins, appropriate therapy if the animal is in shock and minimizing levels of excitement and stress. Administration of muscle relaxants and control of seizure activity are important symptomatic therapies.

Ultimately, the best solution is prevention. Informing owners of the dangers of feeding chocolate to their pets is crucial to minimizing intoxication by theobromine-containing foods. Limiting pet access to chocolate, particularly during holiday seasons, is one of the most important first steps toward prevention.

-by Jennifer Iannaccone, Class 1999
-edited by Brad Njaa, DVM, MVSc and Stephen Hooser, DVM, PhD



Equine Infectious Anemia

Equine Infectious Anemia (EIA) is a Lentivirus from the family Retroviridae. It has been known to infect equine since the 1900's, yet there is still no effective vaccination or treatment. Infection is for life and many infected horses show no clinical signs. EIA is related to the HIV in humans and this discovery has made new research into the disease available.

Transmission is via hematophagous insects such as deerflies and horseflies. Iatrogenic transmission via hypodermic needles and tattoo equipment can also occur. Due to the pain involved with the feeding of these insects, they do not normally finish their blood meal on one host. Transmission of the disease takes place when they are interrupted. Since horses are housed very closely together, this allows for easy transmission. The large mouth parts of these insects can hold up to 10 nL of blood. The virus only lives for 30 minutes to 4 hours in the mouth. The insects prefer to finish their blood meal rapidly, even though their flight range can be 4 miles. If animals are separated by more than 200 yards, the fly will most likely try to finish the meal on the original animal. Fly control is one of the major defenses against this disease.

The disease can present with acute or chronic signs; the acute clinical signs include high fever, anemia, and thrombocytopenia. Chronic disease presents with recurrent fever, weight loss, severe anemia, and ventral edema. The strain and dose of virus in the horse's system, along with immune response, are the determinant factors for disease. The more virulent the strain, the higher the fever, which is usually 7-30 days post-infection. The highest concentrations of virus at this time are found in the serum, liver, spleen, lymph nodes, bone marrow, lung and kidney. The replication of the virus occurs within tissue macrophages, rather than circulating monocytes. The majority of horses with EIA are chronically infected and overcome the initial infection. They appear clinically normal for days to weeks, sometimes up to a year. At this time, they may or may not develop recurring fever, thrombocytopenia, and depression. Viremia occurs during the febrile period (viral particles are 1,000 – 10,000 times higher than when afebrile). During the non-febrile period, the virus is cell-associated and not free in the plasma. After approximately twelve months, most horses become inapparent carriers. These horses go unnoticed and can serve as a reservoir for infection to others. As carrier horses become immunocompromised, the virus re-appears in the blood stream.

Reproductive ramifications include decreased fertility, abortion (if mare was infected on or before 203 days of gestation and occurs 21-64 days post-infection), transplacental transfer, colostral or milk transmission and , rarely, venereal transmission.

Clinical diagnosis of EIA is via clinical signs, history and diagnostic testing. Tests include agar gel immunodiffusion (AGID) or Coggins and competitive enzyme-linked immunosorbent assay (C-ELISA) on serum. Different states recognize one or both of these tests. These tests both detect antibody to p26 core protein of EIA. The Coggins test was developed in the early 1970s and is highly sensitive and specific. It has a 95% accuracy rate and is the most used test for EIA. It is performed on a Petri dish in a layer of agar. The G-ELISA has not been used as frequently as the Coggins, but is believed to have similar sensitivity and specificity. Western immunoblot assay (this tests for virus specific antibodies) can be used for horses with questionable Coggins and C-ELISA results. PCR can also be used on peripheral blood mononuclear cells and/or buffy coat cells. This is another method of confirming the diagnosis.

Gross necropsy lesions during febrile disease include generalized lymph node

enlargement, an enlarged liver with a prominent lobular pattern, an enlarged meaty spleen, mucosal and visceral hemorrhages, ventral subcutaneous edema and vascular thrombosis. Histopathology of these tissues usually reveals accumulations of lymphocytes and macrophages in sinusoids and portal areas of the liver, in medullary sinus of lymph nodes, adrenal glands, spleen, meninges, and lung. This lymphoproliferation may be due to an attempt to control the infection by the T-lymphocytes. There is also marked extramedullary hematopoiesis. Other liver lesions include fatty degeneration and hepatocellular necrosis. Kupffer cells are swollen with hemosiderin accumulation. In infected horses with no clinical signs, gross lesions are generally unremarkable, although some may have glomerulitis, retinal depigmentation, and choroiditis.

Although horses mount a strong humoral and cell-mediated immune response, they are unable to completely clear the virus and are infected for life. Control is dependent on isolation, hygiene (needle usage), identification of positive animals, and fly control. Interstate travel requires a negative EIA test. A negative test is also required for most horse shows, as well as the sale of horses at public auction. Action should be taken by owners, veterinarians, and regulatory officials to keep EIA under control.

-by Angie Johnson, Class of 1999
-by Melanie Greeley, DVM

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

May 29, 2000 Memorial Day
July 4, 2000 Independence Day
September 4, 2000 Labor Day

Please plan accordingly

Bovine Trichomoniasis

Trichomoniasis is a venereal disease of cattle, characterized primarily by early pregnancy loss and, occasionally, by abortion and pyometra. The causative agent, *Tritrichomonas foetus*, is a flagellated protozoan parasite transmitted from infected, asymptomatic bulls to heifers or cows at the time of coitus. Trichomoniasis has a worldwide distribution and is a major cause of infertility in naturally bred cattle in many countries. The incidence in the United States is obscure because of the non-reportable status of the disease.

Tritrichomonas foetus is confined to all regions of the reproductive tract where trophozoites multiply by binary fission to form two daughter trophozoites. In cows, the trophozoites attach to the surfaces of epithelial cells lining the reproductive tract. Examples of colonization in heifers and cows include the vagina, uterus, and oviduct. *T. foetus* can be found in secretions from these sites, including the mild mucopurulent discharge associated with vaginitis and endometritis. Bulls carry the protozoa only on the penis and preputial membranes, localizing in the secretions (smegma) of the epithelial lining of the penis, prepuce, and distal portion of the urethra. There are no lesions of diagnostic significance in bulls and the parasite does not affect either semen quality or sexual behavior. A scant purulent preputial discharge may be noted within the first two weeks of infection, but generally, the infected bull serves as an asymptomatic carrier of the parasite. Older bulls tend to become permanent carriers of *T. foetus*, perhaps as a result of the development of epithelial crypts in the preputial cavity of older bulls. The parasite transmission rate

from male to female at breeding may be as great as 42%. *T. foetus* is rarely transmitted by artificial insemination of cattle if appropriate procedures for bull testing and hygiene are practiced.

The pathogenesis of pregnancy loss is not yet well understood. A likely cause of abortion is the direct cytotoxic insult of maternal endometrium and/or fibroblasts and the fetal chorionic trophectoderm. Another potential virulence factor is the battery of extracellular cysteine proteinases that are elaborated by *T. foetus*. At physiologic pH, these enzymes are very active against a wide variety of proteins including immunoglobulin, fibronectin, and lactoferrin. Although *T. foetus* can bind immunoglobulin molecules in a nonspecific manner, whether this binding offers the parasite any protection from specific immune attack or whether it precedes proteinase degradation of immunoglobulin is not known.

Overt clinical signs are rare as the apparent infertility due to embryonic death is the most common result. Pyometra and abortion often are the first signs of trichomoniasis noticed in a herd, but they occur in relatively few animals. When abortion occurs, it is usually within the first third to one-half of gestation.

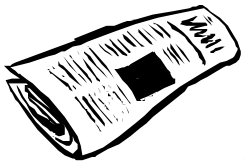
Grossly, the degree of autolysis in fetuses and placentas can vary from mild to marked. Placentas are edematous, but otherwise unremarkable. Fetuses may have no discernible lesions; however, enlarged livers and non-inflated, enlarged, firm lungs may be present on some fetuses. Emphysematous bullae involving the splenic and hepatic capsules and the parietal peritoneum have been reported.

Because infection is inapparent in bulls and mild vaginitis is found only occasionally in cows, a definitive diagnosis requires the identification of parasites in infected animals. *T. foetus* is best located in preputial or vaginal secretions and, to a lesser extent, amniotic, allantoic, or abomasal fluids from the infrequently aborted fetuses. The flagellated parasites can be identified by direct microscopic examination of these fluids. More commonly, samples are inoculated into one of the several media, most notably Diamond's or Claussen's media, and allowed to grow in vitro until sufficient numbers of parasites are present to allow detection by light microscopy. In bulls, the organisms are in the prepuce, frequently in small numbers. Microscopic examination of preputial smegma for trichomonads is the most common method to confirm a herd diagnosis. The characteristic aimless, jerky motion of the flagellate is diagnostic. Culturing increases sensitivity markedly over direct exam. Even with culturing, there is a 10-20% probability than an infected bull will be missed by a single culture; hence, multiple cultures are recommended. In the field, the In-Pouch media system (Biomed Diagnostics, Santa Clara, CA) are convenient to use and have a long shelf-life.

Reaching a diagnosis is only the beginning of the problem. No legal treatment exists for bovine trichomoniasis. Given the lifelong nature of most bull infections and lack of legal treatments, a veterinarian must recommend slaughter of infected bulls. Artificial insemination has reduced the incidence of trichomoniasis over the past three decades and has proven to be the best control measure available. Other control measures are possible if artificial insemination is not feasible. The herd should be divided into exposed and unexposed groups. The exposed group should be treated for recognizable uterine disease and allowed three months of sexual rest. One may recommend eliminating all bulls greater than three years of age and using only younger bulls for mating. This is based on the relative lack of susceptibility of young bulls to trichomonad infection. Immunoprophylaxis for bovine trichomoniasis has been a priority due to the prevalence of the disease and its economic impact. A killed *T. foetus* vaccine is available which can be used in both cows and bulls. Two infections are required, 2-4 weeks apart, prior to breeding season. Extensive field testing reveals that when used properly, protection can be obtained in

over 90% of the animals vaccinated. Annual vaccinations are required. Natural immunity in the cow will develop after 1-3 heat cycles, but is of short duration (6-12 months). The available vaccine (Trich Guard-Fort Dodge) is partially efficacious in the cow, but has no known efficacy in the bull; hence, disease control involves both identification and culling of infected bulls along with vaccination (prebreeding) of cows to decrease the incidence of infertility until the disease is eradicated from the herd. It is also important to remember that younger bulls are less susceptible to persistent infection than are older bulls.

-by Trish LaSala, Class of 2000
-edited by Arlen Mills, DVM, Purdue University Large Animal Hospital



ADDL STAFF NEWS

Dr. Marlon Rebelatto, ADDL Graduate Student, was recently awarded a Ph. D. from Veterinary Pathobiology. His thesis, entitled Nasal-Associated Lymphoid Tissue and Intranasal Immunizations in Cattle, focused on lymphocyte populations, adhesion molecules expression, and cytokine expression of tonsils in cattle and the development of subunit intranasal vaccines for cattle. Dr. Rebelatto is also a finalist for the Phi Zeta award in Basic Science Research.

Dr. Melanie Greeley, ADDL Graduate Student, completed her Masters program and plans to pursue a PhD in Environmental Pathology at UC Davis.

On THE Road

Dr. Chuck Kanitz, Head of Virology/Serology, attended the inaugural meeting of the National Institute for Animals in Agriculture, Corpus Christi, Texas April 2000

Alan Bunning, Computer Systems Programmer attended a VisuaLab Users meeting, Dallas, Texas , April 2000

Mary Woodruff, Virology Lab Superviser, attended The Association of Veterinary Microbiologists meeting, Manhattan, Kansas April 2000

Dr. Ching Ching Wu, Bacteriology and Avian department head, participated in the USDA external review for the infectious bursal disease project, Tel Aviv, Israel, March 2000

Drs. Ching Ching Wu and Christine Hanika attended the Indiana State Poultry Association meeting in Indianapolis , March 2000

Dr. Charles Kanitz and Min Wang, Virology graduate Student, will attend the American Society of Microbiologists meeting in Los Angeles, California in May, 2000.

Dr. Greg Stevenson traveled to the Peoples Republic of China to consult with the swine industry on diseases of swine in April 2000.

Ajf;iarhjg;mvbi;daflgjk;aldnmfbgjk;f;lkg