Summer 2002

FROM THE DIRECTOR

H. Leon Thacker, DVM, PhD

It is great to live in a part of the world with changing climates. It is good to see summer come bringing with it the growing crops, county fairs, family get-togethers and the other enjoyments along with the heat. But it will also be good to see the cooler weather, cool nights and the changed social activities of fall. Activities at the ADDL continue to keep us busy, changes to the testing requirements for county fairs have slowed the amount of testing for these exhibitions, but the increases of sophistication of diagnostic testing and increased requests for them fill the void.

The first of July saw an increase in the fees for service charged by the ADDL. The ADDL operated for many years with services provided free of charge, it was mandated in 1979 that a fee schedule should be established for out of state submissions. It was ten years later that fees for Indiana submissions were initiated and the first fee schedule was established. The fees charged by the Lab have not been substantially changed since 1991 and the present fees are judged to be well in line with the prevailing charges in surrounding states and other laboratories. We are always adding new tests and testing procedures to the offering of the Laboratory to utilize new technology in health and disease testing and to improve expediency and accuracy of replaced procedures. We hope that the services we provide are meeting the needs of users of the Laboratory. If there are things we are doing that should be changed or procedures that we are pursuing that could be better provided, we hope that you will let us know your thoughts.

Have a good summer; we're here to assist you with your diagnostic needs.

FINAL DIAGNOSIS

Candida fungemia
Enterococcus septicemia
Bone marrow aplasia

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

History: A 6-year-old, male German shepherd dog was submitted to the Animal Disease Diagnostic laboratory for necropsy. Reportedly, the dog had lethargy, diarrhea, vomiting, weight loss, polyuria and polydypsia, and persistent leukopenia. Reported pertinent clinicopathological data included a CBC consistent with pancytopenia characterized by neutropenia, monocytopenia, lymphopenia, thrombocytopenia and anemia, and a bone marrow aspirate revealing marked myeloid hypoplasia and mild erythroid and megakaryocytic hypoplasia. Per clinical history, the dog was icteric, had elevated liver enzymes (ALT, ALKP and GGT) and a prolonged activated partial thromboplastin time (PPT).

Gross Findings: The carcass was emaciated, icteric and had multiple petechiae, ecchymotic and/or effusive hemorrhages within the subcutis, diaphragm, intercostal muscles, lungs, liver, mesenteric lymph nodes, kidney, urinary bladder, gastric and intestinal mucosa, and serosa. The gingival mucosa had multiple ulcers measuring 1.0 x 0.5 cm in greatest dimension. Pleural and abdominal cavities both contained serosanguinous effusions admixed with fibrin strands. Fibrinous strands covered serosal surfaces of the diaphragm, lungs, liver, stomach and intestine and caused adherence between the diaphragm and liver and between intestinal loops. The liver was diffusely yellow-green, diffusely enlarged and friable with multiple perivascular necrotic foci which measured 0.3 cm in greatest dimension and were rimmed by hemorrhage. Mesenteric lymph nodes were diffusely enlarged, dark-red and bulged on cut surface. Renal cortices were olive green and papillae had orange discoloration. Kidneys contained multiple acute and subacute, 0.3 cm in diameter cortical infarcts characterized by wedge-shaped cortical foci which were either red and slightly raised or tan, slightly depressed and rimmed by hemorrhage. The bone marrow of femur, humerus, several vertebrae and ribs was diffusely yellow and fatty.

Histopathologic findings: Primary hepatic lesions were multifocal, periportal and centrilobular necrotizing hepatitis and necrotizing vasculitis. There were numerous intralesional 4-7 µm pseudohyphae and 3-5 µm blastospores. Necrotic foci with similar intralesional pseudohyphae and blastospores were present within mesenteric lymph nodes. Renal lesions included multiple septic cortical infarcts characterized by coagulation and liquefactive necrosis, hemorrhage, infiltration with viable and degenerated neutrophils and numerous intralesional small, gram positive, coccoid bacteria. The hypocellular bone marrow contained primarily adipose connective tissue and hemosiderin-laden macrophages. There was marked depletion of myeloid precursor cells and mild depletion of erythroid precursor cells and megakaryocytes.

Enterococcus spp. was isolated from liver, kidney and spleen. *Candida* (*Torulopsis) glabrata* was isolated from liver tissue.

Discussion: Fibrinous serositis and suppurative-embolic nephritis, together with isolation of *Enterococcus spp*. from liver, kidney and spleen are diagnostic for *Enterococcus spp*. septicemia. Lesions within liver and mesenteric lymph nodes, together with isolation of *Candida* (*Torulopsis) glabrata* are consistent with *Candida* fungemia. Portal of entry for *Enterococcus spp*. and *Candida glabrata* was likely the intestinal tract followed by hematogenous dissemination via the portal vein. *Candida spp*. and *Enterococcus spp*. are opportunistic pathogens, e.g. yeast, fungi, and/or bacteria which live on mucosal surfaces as commensal agents and gain

pathogenic properties in the case of immune suppression. In this case, marked immune suppression was caused by bone marrow aplasia of undetermined etiology.

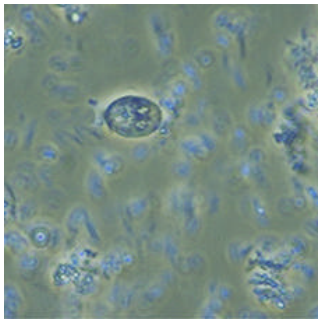
Hemorrhagic diathesis and icterus developed likely secondary to septicemia, liver damage, and/or bone marrow suppression.

In humans, *Enterococcus* septicemia and disseminated *Candida glabrata* infections are serious problems in patients with bone marrow hypoplasia or aplasia, most commonly related to chemotherapy and/or irradiation with cancer therapy, bone marrow transplantation or HIV infection. *Enterococcus spp*. are considered as important nosocomial pathogens in human hospitals, particularly because they are already resistant to many antibiotics and have a strong propensity to acquire additional antibiotic resistance determinants.

Reports about *Enterococcus* septicemia and *Candida* fungemia in dogs and cats are rare. One case of *Enterococcus faecalis* - associated discospondylitis was reported in a dog. Multiple antibiotic resistance was found in *Enterococcus faecium* strains isolated from surgical wounds of hospitalized cats.

Most *Candida spp*. isolated from dogs and cats have been identified as *Candida albicans*. Affected animals are almost always immune-suppressed due to cytotoxic chemotherapy and/or prolonged glucocorticoid treatment. Prolonged antibiotic treatment of some affected animals is also reported.

-by Sandra Schoeniger, ADDL Graduate Student



(photo from Provincial Lab website, www.provlab.ab.ca)

Intestinal Giardiasis

Giardia lamblia, often referred to simply as "Giardia", the causative agent of giardiasis, is a flagellated protozoan that was originally observed by Van Leeuwenhoek in 1681. The genus name of this parasite was named after French biologist Alfred Giard.

Giardia has an interesting morphology. Giardia exists in two forms, the trophozoite and the cyst. Trophozoites are motile due to their four pairs of flagella. This form is dorsoventrally flattened, piriform and has a unique internal structure. Giardia has a large adhesive disk that comprises the majority of the protozoan's ventral surface. Through the use of light microscopy, a pair of recurrent flagella that run longitudinally within the organism can be seen. These recurrent flagella are called axonemes. There are two nuclei, one on each side of the axonemes. The trophozoites measure 9-21 µm long x 5-15 µm wide x 2-4 µm thick. This form may be found attached to the epithelium of the duodenum and jejunum within an infected host.

The cyst form of *Giardia* is nonmotile. These are oval and have a thick, refractile wall. Two nuclei are in the recently formed cysts with four nuclei in the mature cysts. The cysts measure 8-12 µm long x 7-10 µm wide. This form is the infective form and may be found in the feces of infected animals.

Giardia has the ability to infect many mammals including the dog, cat, deer

mouse, ground squirrel, chinchilla, swine, pocket mouse, ox, guinea pig, and humans.

Transmission is by the fecal-oral route. Both humans and animals may become infected either by direct fecal ingestion or by the ingestion of contaminated water. Freshly passed cysts are immediately infective. The ingestion of a mere ten or fewer *Giardia* cysts is enough to cause infection.

Giardia has a direct life cycle. Once the cyst stage is ingested by a suitable host, excystation occurs within the duodenum. It is believed that excystation occurs as a result of exposure to the low gastric pH in addition to contact with pancreatic enzymes such as chymotrypsin and trypsin. During excystation, two binuclear trophozoites arise from each quadrinuclear cyst. The trophozoite form uses its large adhesive disk located on its ventral surface to attach to the epithelium of the duodenum and jejunum. The trophozoites reproduce asexually by binary fission. Some of these trophozoites encyst within the small intestine and pass out in the feces. Many theories have been proposed, but the exact mechanism by which *Giardia* causes diarrhea has not been established.

There are no pathognomonic clinical signs associated with giardiasis. The most common sign is chronic or intermittent foul-smelling bowel diarrhea. Diarrhea is usually lightly colored, greasy and mixed with mucus. Diarrhea is not usually watery and does not generally contain blood. Other common signs of giardiasis in dogs and cats include flatulence, weight loss, listlessness, malaise and growth retardation in immature animals. Weight loss usually occurs in the presence of good appetite and adequate food intake. Less commonly reported clinical signs include acute or chronic large bowel diarrhea with excess fecal mucus, tenesmus and hematochezia.

The only means by which a definitive diagnosis of giardiasis can be made is to demonstrate the actual parasitic agent. This diagnosis is established by identification of cysts and, less frequently, trophozoites in in fecal specimens. Trophozoites can be visualized by direct smears of diarrheal feces. Fecal flotation using zinc sulfate should be used to concentrate *Giardia* cysts. The passage of cysts is, to some extent, sporadic; therefore, a suspected patient should not be considered negative for *Giardia* until three consecutive negative examinations have been completed. Lugol's iodine solution can be used to stain both the trophozoites and cysts, making them easier to identify. *Giardia* antigens in the feces of an infected animal may be detected via indirect and direct immunofluorescent assays using monoclonal antibodies, and by direct fluorescent assays.

Treatment for giardiasis in humans includes quinacrine, metronidazole or furazolidone. Metronidazole is the drug of choice for treatment of giardiasis in dogs. Other drugs that may be used for canine infections are tinidazole and quinacrine. Metronidazole, febantel, fenebendazole or albendazole may be used to treat infected cats; however, optimal and efficacious drug treatment in cats has not been well established.

Determination of the immune response of dogs to *Giardia* has yet to be determined. Because most infections are usually self-limiting, many researchers suggest an acquired immunological resistance to the parasite. Epidemiologic research suggests that previous contact with *Giardia* may serve to increase resistance to re-infection. Although the exact mechanism of immunity is not completely understood, humoral immunity is considered to be important in the elimination of *Giardia* trophozoites from the host intestine. Immunologically naïve and immunocompromised hosts have been found to be more vulnerable and also suffer more severe and chronic infections. Research has shown, in experimentally infected humans and animals, that the immunocompetent host produces specific mucosal and serum antibodies against both cystosolic and surface *Giardia* antigens. The cellular immune system does not play a direct role in parasite clearance.

There is currently a commercially available vaccine against *Giardia* in the United States.

This vaccine has been demonstrated by researchers to be effective for prevention of clinical signs of giardiasis and reduction of cyst shedding in dogs and cats. Vaccination of companion and farm animals helps not only to reduce zoonotic transmission, but also to reduce both interspecies and intraspecies transmission.

Is *Giardia* a zoonotic concern? There is evidence that suggests that direct transmission from companion animals to humans does occur. Zoonosis is controversial regarding *Giardia*, but most researchers believe that its zoonotic potential merits adequate precaution when working with feces of animals that may be infected.

Control of *Giardia*, from a public health standpoint, should start with municipal drinking water. The prevalence of *Giardia* in humans within industrialized countries is 2-5%. The prevalence of *Giardia* in humans within developing countries is 20-30%. As many as 95% of human travelers to St. Petersburg, Russia have shown signs of giardiasis. In children that attend day care centers, the prevalence of *Giardia* has been found to be as high as 35%. Filtration can be quite effective for removing *Giardia* cysts from water. Since this parasite may be found in lakes, streams, and ponds, both hikers and backpackers must be warned to boil or filter drinking water prior to ingestion.

Giardia is a potential health concern for both man and animals alike. Correct measures should always be employed in order to properly diagnose, control and treat giardiasis. Much work has been done in the area of *Giardia* research, but there is still much to be done. Preventing and controlling giardiasis will require the joint efforts of both the human medical and veterinary medical professions.

-by Craig Hunt, Class of 2002

-edited by Randy White, DVM, PhD, ADDL Pathologist

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

September 2, 2002…… Labor Day
November 28-29, 2002……. Thanksgiving
December 25-26, 2002……. Christmas

ADDL NEWS

Our best wishes go with **Dr. Evan Janovitz**, former ADDL pathologist, who has recently begun a new career at Bristol Meyer Squibb in New Jersey. Dr. Janovitz received his PhD from Purdue's Veterinary Pathology program and has been a diagnostic pathologist at ADDL for the past 14 years.
We wish Dr. Janovitz and his family the best in their new venture.

Dr. Mika Tanabe joined the ADDL staff in July as visiting instructor. Dr. Tanabe completed her pathology residency at Cornell. In addition to her duties as ADDL pathologist, she will be teaching first year pre-med students.

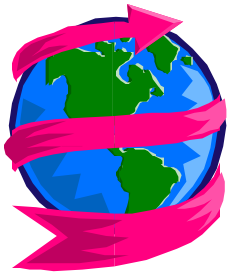
Drs. Kaori Sakamoto and Matt Renninger have completed their residency programs at ADDL. Dr. Sakamoto will continue her studies at Cornell in Ithaca, New York, while Dr. Renninger has accepted a position in the graduate program at Purdue's Veterinary Pathobiology department.

We also welcome our newest graduate student, **Dr. Phaedra Stiles**. Dr. Stiles received her DVM from Purdue in 2000 and has been in practice in Muncie, Indiana.

Mary Woodruff, Virology lab Supervisor, hosted the Heartland Chapter of the Association of Veterinary Microbiologists annual meeting in Lafayette, IN, April, 2002. Invited speakers were Dr. David Huxsoll, Plum Island USDA lab Interim Director, Scott LaPatra from Clear Springs Foods, Twin Falls, Idaho, Dr. Linda Saif, Ohio Agricultural Research and Development Center and Melissa Hills, IDEXX Laboratories.

Dr. Zheko Kounev, ADDL Food Safety Specialist and Avian Diagnostician, receives a plaque from USDA Under Secretary for Marketing and Regulatory Services Bill Hawks in recognition for his two year term as NPIP General Conference Committee Member. The plaque was presented to Dr. Kounev during the National Poultry Improvement Plan meeting in San Antonio, Texas, May, 2002.

Congratulations to **Drs. Ingeborg Langohr** and **Alok Sharma**, ADDL graduate students, for their recent awards at the North Central Conference of Veterinary Laboratory Diagnosticians. **Dr. Langohr's** presentation earned first place for graduate students presentations while **Dr. Sharma** received the third place award. The meeting was held in Reynoldsburg, Ohio in June, 2002.



ON THE ROAD

Dr. Leon Thacker spoke at the American Society of Microbiologists annual meeting in Salt Lake City, Utah, May, 2002.

Drs. Zheko Kounev and Ching Ching Wu attended the National Poultry Improvement Plan annual meeting in San Antonio, Texas, May, 2002.

Drs. Charles Kanitz, Bill VanAlstine, Sandra Schoeniger, and Ingeborg Langohr attended the North Central Conference of Veterinary Laboratory Diagnosticians in Reynoldsburg, Ohio, June, 2002.

Dr. Tom Bryan attended the Broiler Health Management School and Turkey Health Management School in Columbus, Ohio, May, 2002.

Drs. Sandra Schoeniger, Ingeborg Langohr, Theresa Boulineau and Alok Sharma presented cases at the Midwest Association of Veterinary Pathologists, August, 2002.

Dr. Leon Thacker and **Steve Vollmer,** Computer Sysems Manager**,** attended an ISO 17025 Assessor workshop in Las Vegas, NV, June, 2002.

New Tests at ADDL

Infection of cattle with bovine viral diarrhea (BVD) virus in the early stages of gestation can lead to the birth of calves that shed BVD throughout their lives. Serum samples can be tested, but they will require isolation of the virus. **Persistently infected BVD animals can be detected using the fluorescent antibody method on ear punch biopsies**. The Virology laboratory at Purdue ADDL is currently accepting such samples for testing.

A **PCR test for Porcine Reproductive and Respiratory Syndrome (PRRS)** is also available. The in-state charge for PRRS PCR is $25.00.

Additional PCR tests will be available in the near future.

Visit our website at
www.addl.purdue.edu

Online reports are available to veterinarians. Contact Steve Vollmer at 765-494-7440 or follow the instructions on the ADDL main page.

West Nile Encephalitis In Horses

West Nile Virus (WNV) encephalitis is a viral disease that is relatively new to the United States. The disease gets its name from the West Nile District of Uganda in Africa where it was first recognized in humans in 1937. The first cases of WNV infection in horses were recognized in France and Egypt in the early 1960's. WNV infection was known to occur in Africa, the Middle East and parts of southern Europe, but the virus was not isolated in the U.S. until 1999, when an epidemic of WNV encephalitis and aseptic meningitis was diagnosed in New York City. Concurrent epizootics occurred in horses on Long Island and in birds. The close genetic relationship between WNV isolates from Israel and New York suggests that the virus was imported into North America from the Middle East. It is not known how the virus came to the U.S. but, given the location of the first outbreak, it is likely that it arrived via airline or boat in an infected bird or mosquito.

WNV is a member of the Japanese encephalitis virus complex of the genus *Flavivirus*, family Flaviviridae. This genus includes nine viruses distributed around the world. In the U.S., the complex has two other representatives, Powassan and St. Louis Encephalitis viruses, both of which cause encephalitis in humans. In 1999, 9 of 25 (36.0%) horses from Long Island, NY with clinical signs of the disease died or were euthanized. In 2000, 60 cases of WNV were reported in horses from 7 states, and 23 (38.3%) horses either died or were subjected to euthanasia. In 2001, 640 confirmed cases of WNV infection in horses were reported from 20 states, and 156 (24.4%) horses either died or were euthanized.

Wild birds of many different species are the reservoir for WNV. The virus is transmitted by mosquitoes who acquire it from infected birds. Horses and humans are considered accidental or dead-end hosts. A mosquito that has fed on an infected bird can bite people or horses (or other mammals) and transmit WNV to them. Infected birds may die from the disease though most avian species are resistant. Consequently, birds may have the virus circulating in their bloodstream for some time (viremic stage) allowing further infection of other mosquitoes, but they do not develop symptoms of illness. Horses and humans only develop a low-level and transient viremia, thereby diminishing the importance of their role in the transmission cycle of the virus.

Persistence of the virus in migrating birds is considered to be one of the main factors in the spread of the disease. At least 17 species were affected in 1999 and 76 in 2000, particularly the American crow. The signs of illness in these birds include convulsions, tremors, head tilt, wing drop, paralysis, loss of balance, and circling. Less specific signs, such as weakness and lying on the chest, may also be observed. Although as many as 40 species of mosquitoes are potential hosts and 14 different species were identified as carriers of the virus in the U.S., the primary species involved are *Culex pipiens* and *Aedes vexans.*

The incubation period of WNV infection is usually 5-22 days. Clinical signs in affected horses may include both central and peripheral nervous system signs, consisting most frequently of ataxia, lack of interest in surroundings, weakness of limbs, muscle fasciculation, and loss of appetite. Horses may become recumbent and may be unable to get up without help. Some horses may develop fever. The target organ of the virus is the brain and spinal cord where it causes inflammatory reaction (encephalitis), manifested by variable neurological signs. Affected horses, which show no progression of gait abnormalities, usually recover completely with no consequences within 5-15 days.

The clinical signs of the disease may be indistinguishable from other equine encephalitides including rabies, equine herpes virus-1, equine protozoal myeloencephalitis, and Eastern, Western, or Venezuelan equine encephalomyelitides. Therefore, diagnostic tests are necessary for a definitive diagnosis. Whole blood, serum, and cerebrospinal fluid (CSF) should be submitted to the National Veterinary Services Laboratory, through Purdue ADDL, with a complete history of the case. If the animal presented rapidly progressive neurologic signs including recumbency, it should be submitted to ADDL requesting rabies and WNV testing. Field postmortem analysis should follow USDA guidelines.

There is no specific treatment for WNV encephalitis. General supportive care should be provided. Regardless of the treatment, horses that survive usually recover quickly. A new vaccine is available for prevention of WNV infection in horses. It is a killed vaccine which must be given in two doses initially, with an interval of three to six weeks. Both doses should be completed at least three weeks prior to mosquito season. Efficacy data of the vaccine are not available at this time; however, the vaccine is considered to be safe.

Since mosquitoes are associated with WNV transmission, the key for preventing or controlling future outbreaks of WNV among horses is to control mosquito populations and prevent horses from being exposed to these insects. Recommendations include reducing mosquito breeding sites, providing screened housing, using insect repellants, and reducing outside exposure of the animals. Horse owners also need to watch for dead birds, particularly crows, on their property. Presence of the virus in a given area may be first indicated by seeing dead birds in the vicinity. Crows are particularly susceptible. Diagnosis of WNV can be determined by submitting dead birds to the ADDL or the Indiana Department of Health.

Testing at this time is limited to crows,blue jays or raptors as these are the species most susceptible to the disease

-by Rajeev Nair, ECFVG Student

-edited by Dr. Ingeborg Langohr, ADDL Graduate student

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