PURDUE UNIVERSITY

DIAGNOSTIC FORUM

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A Quarterly Newsletter from the Indiana Animal Disease Diagnostic Laboratory
at Purdue University, West Lafayette, Indiana 47907 (765-494-7440)

From the Director
Dr. Stephen B. Hooser

Early September brought us a moment to celebrate. The Heeke ADDL at SIPAC, near Jasper, was started 40 years ago in 1969!

The original mission of the Heeke Lab was to support the poultry industry in southern Indiana. A 1977 lab addition allowed an expansion of services. In 1999, the lab was renamed for the late Dennis H. Heeke, a state legislator who helped establish the lab and promoted southern Indiana's poultry industry.

As early as 1974, then Director Merrill Ranck reported the first occurrence of Dactylaria infection in chickens. Then, in 1994, working with the turkey industry, ADDL diagnosticians Dr. Tom Bryan and Tom Hooper helped recognize turkey coronavirus and then developed diagnostic tests that assisted in the eradication of the disease from Indiana. In May 2005, Heeke ADDL pathologist Duane Murphy recognized and reported the occurrence of a foreign animal Disease, Rabbit Hemorrhagic Disease, in Indiana. As a result, the disease was controlled and eradicated from the state.

Thanks from all of us to the Heeke ADDL Staff!

Hot Topics

- **Heeke ADDL 40th anniversary celebration**
- **Accreditation site visit**
- **New tests and charges**
- **New User's Guides have been mailed. If you need one, contact the ADDL.**
- **Test results can be emailed to you if desired. To begin, contact us by emailing addl@purdue.edu or phoning us at 765-494-7440.**

Inside the Diagnostic Forum

Focus on Heeke ADDL

The Heeke Animal Disease Diagnostic Laboratory, a branch of the West Lafayette ADDL, is located on the grounds of the Southern Indiana Purdue Agricultural Center near Dubois, IN serving the poultry, livestock, wildlife, and companion animal owners in southern Indiana.

Heeke Staff includes:
Dr. Duane Murphy, Mammalian Pathologist, Co-Director(15 years at Heeke)
Dr. Tom Bryan, Avian Diagnostician, Co-Director (30 years)
Margaret Gelhausen, Technician (12 years)
Tom Hooper, Professional Assistant (30 years)
Denise Riley, Clerk (21 years)
Carl Allen, Building Services (1 year)

Front
Denise Riley
From left to right
Duane Murphy
Tom Bryan
Margaret Gelhausen
Tom Hooper

Abby Kempf Strong won third place in the American Board of Toxicology Student Competition 2009 for her poster and presentation "Presumptive Zinc Toxicosis in a Dog Following Ingestion of a Brassiere". Currently a junior in the Purdue School of Veterinary Medicine, Abby is pictured here with her ABVT Diplomate Sponsor Dr. Steve Hooser, Director of the Indiana Animal Disease Diagnostic Laboratory at Purdue.

Recently added tests and in-state charges

Bacteriology

• Aerobic counts	$20.00
• Anaerobic counts	$25.00

Molecular Diagnostics

• Real time PCR *Coxiella burnetii*	$25.00

Pathology

• Decalcification	$17.00
• Painted margins	$25.00
• Processing eyes	$20.00

Serology

• Influenza A ELISA	$5.00
• Toxoplasmosis ELISA	$5.00

Toxicology

• Chlorine	$15.00

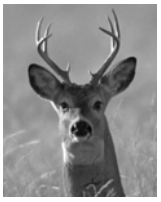
Virology

• Influenza virus	
-HA partial gene sequence	$100.00
-HA complete gene sequence	$200.00

Our congratulations to former ADDL graduate student Dr. Robert Johnson who recently passed the American College of Veterinary Pathologists certifying examination and is now a Diplomate of the ACVP. Dr. Johnson completed his anatomic pathology residency program at the Purdue ADDL in June, 2009.

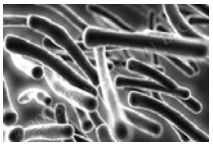
We are pleased to welcome our two new graduate students: Drs. Cindy Argue and Nozomi Shimonohara.

Hosted by Purdue and organized by ADDL pathologist Dr. Jose Ramos-Vara, the annual Midwest Association of Veterinary Pathologists meeting was held at Turkey Run State Park on August 13-14, 2009, bringing together veterinary pathologists and pathology graduate students from throughout the Midwest. Pictured above are former, current and future participants of the Purdue pathology training program.



Tuberculosis in Cervids

Tuberculosis is a chronic, granulomatous disease of wild and domestic ruminants that poses zoonotic risk. Eradication of the disease in cattle has been a federal goal since the early 20th century; however, complete eradication of tuberculosis has been thwarted by the presence of tuberculosis in wild populations of cervids in specific regions in the United States. With the increase in farming of deer and elk, tuberculosis is an important contemporary issue for farmers who raise cervids or domestic ruminants.



Tuberculosis in ruminants is caused by *Mycobacterium bovis. Mycobacterium bovis* is a gram positive, small, slightly curved rod. These bacteria are aerobic and acid-fast; thus, bacteria can only be visualized with stains such as Ziehl-Neelson. *Mycobacterium bovis* has a thick cell wall that contains a high content of waxes and glycolipids. The composition of the cell wall offers excellent protection against humoral defense mechanisms and disinfectants, and is responsible for the granulomatous response and long-term survival of organisms within macrophages. *Mycobacterium bovis* can remain active on a fomite in cooler climates up to 112 days.

The spread of *Mycobacterium bovis* organisms is primarily through respiratory secretions and saliva. Therefore, exchange of these secretions on feedstuffs is likely occurring between wild deer and cattle and/or captive cervids in Michigan. This proposed mode of exchange is further complicated by the fact that *Mycobacterium bovis* can live for an extended length of time in cooler climates; therefore, direct wild deer to cattle or captive cervid contact is not needed to spread the disease. The route of infection is usually indicated by the distribution of the lesions. In deer, infection is by inhalation or ingestion, with the bulk of the lesions found in retropharyngeal lymph nodes. One of the main risk factors for tuberculosis infection is overcrowding. Overcrowding increases the opportunity that a single infected animal can infect multiple animals. Supplemental feeding and baiting of wild deer, which brings large populations of animals into smaller areas, may also promote spread of the disease.

The typical clinical signs of tuberculosis in deer are quite variable. The type of clinical signs that are observed are due to the route of infection. The most consistent clinical sign is swelling of lymph nodes, particularly the medial retropharyngeal lymph node, which drains the nasal passage and mouth. Respiratory distress and lung lesions occur after inhalation of the bacteria. Emaciation can also occur commonly with chronic *Mycobacterium bovis* infection.

Grossly, lesions in deer are different from those observed in cattle. The main lesion seen in a deer is purulent lymphadenitis of the medial retropharyngeal lymph node. The abscess is filled with a pale white to tan, purulent material. In cattle, affected lymph nodes nodes contain caseous granulomas that can be surrounded by fibrosis. Cervids can exhibit granulomatous lymphadenitis, but at a much lower frequency than in cattle.

Lung lesions are concentrated in the dorsocaudal half of caudal lung lobes. The lung lesions appear as pale yellow to tan nodules that are seen throughout the lung parenchyma and pleural surface. These nodules can range from small miliary foci of 1mm in diameter to large abscesses greater than 30cm in diameter. When these lesions are cut, a pale white to tan purulent exudates is exuded. The abscesses in deer are indistinguishable grossly from abscesses caused by other bacteria such as *Staphylococcus* sp., *Streptococcus* sp., *Actinobacillus* sp., or *Actinomyces* sp.

Histopathologically, the lymph node contains a round, central area of caseous necrosis with a narrow mantle of mixed inflammatory cells. The leukocytes consist of neutrophils, epithelioid macrophages, lymphocytes, and plasma cells. The lung lesions usually have more neutrophils than the lymph nodes. In cattle, tuberculous lesions consist mainly of a central, irregular, coalescing area of caseous necrosis. The center is mineralized and is surrounded by macrophages, lymphocytes, plasma cells, and Langhans giant cells. In the cervid, mineralization is centrally and peripherally located, with foci of mineralization located in the cellular infiltrate. Acid-fast bacilli are difficult to detect in the lesions from cattle, as compared to lesions in cervids.

The standard protocol for identification of possible infected animals in the field is tuberculin testing. A single cervical skin test is used in most herds as a preliminary test. The single cervical skin test is done on cervids one year of age or older and any cervid younger than one year that was not born into the herd. The tuberculin test is performed in the neck as opposed to the caudal fold, as in cattle, due to the increased sensitivity of the cervical test in cervids. The test is performed by injecting 0.1 ml of tuberculin intradermally in the mid-cervical region. The test is then read 72 hours later. A positive reaction includes visualization and palpation of a mass of 2 mm in size or greater. The animals that are positive to a single cervical skin test are subjected to the comparative cervical skin test. The comparative cervical tuberculin test is administered by an approved state or federal veterinarian within 10 days of a positive single cervical skin test. In this test, an injection of bovine tuberculin will be injected

intradermally along with a separate injection of avian tuberculin in two separate areas of the cervical neck region. These two injections will be evaluated to determine whether the cervid is infected with *Mycobacterium bovis* or another species of *Mycobacterium*.

After a cervid is confirmed as a positive reactor, the whole herd is quarantined until the reactor is slaughtered or necropsied. At necropsy, if the reactor deer is found to have lesions consistent with tuberculosis, histopathology and isolation of *Mycobacterium bovis* is performed. If *Mycobacterium bovis* is isolated from the lesion, the herd is considered an affected herd. The affected herd is quarantined until the herd has tested negative on three whole herd tests. These tests should be given 90 days, 270 days, and 360 days after the reactor tested positive. If the herd tests negative those three times, the quarantine is lifted and the herd is considered an unclassified herd. The herd must then undergo five consecutive annual tests to be a classified herd. An alternative to testing of an affected herd is depopulation of a herd.

Prevention of the spread of tuberculosis is an active endeavor, especially with the concern of spread to cattle and interstate commerce. Nowhere is this more evident than in Michigan, where there is an active tuberculosis infection in the wild cervid population. The proposed methods of prevention of the spread of tuberculosis have met great opposition in Michigan. Cattle farmers have pushed for the complete eradication of deer to rid the area of tuberculosis. The hunters do not want to see the complete eradication of deer as that will rid the area of hunting, which is considered economically important in Michigan. So, Michigan has decided to decrease the population through increases in deer hunting permits in an attempt to lower the number of infected deer. This policy has resulted in a reduction in the prevalence of the disease to a constant level of 1-2% infection. Michigan has also banned the use of deer baiting in the area of tuberculosis positive deer. Some ways to prevent the spread of tuberculosis to cattle and captive cervids is feeding cattle and cervids away from wooded areas. Feeding them in open areas where deer would not have cover will theoretically keep deer from congregating in an area where cattle and cervids would be eating. Also, excluding deer from stored feed sources can help prevent the spread of tuberculosis through contaminated feed. Ultimately, the education of hunters, farmers, and veterinarians about clinical signs and whom to contact is the only way to prevent an outbreak in cervids and cattle.

-by Seth McDevitt, PUSVM Class of 2010
-edited by Dr. Grant Burcham, ADDL Graduate Student

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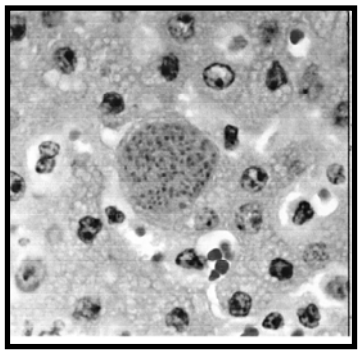
Final Diagnosis: Toxoplasmosis in a juvenile cat

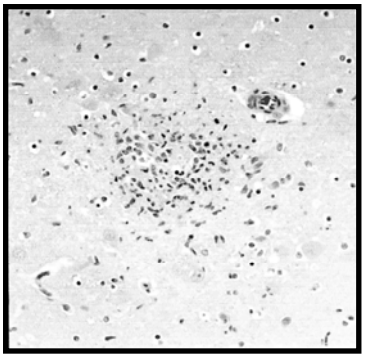
History: A reportedly six to seven-week-old domestic shorthair cat was submitted to the Animal Disease Diagnostic Laboratory for necropsy. The history reported that the kitten was found recumbent and nonresponsive in its cage, and was subsequently euthanized.

Gross findings: Grossly, the cat was in poor body condition and moderately dehydrated. The thymus was markedly atrophied. Mesenteric lymph nodes were markedly swollen and bulged on cut section.

Histologic findings: The hepatic parenchyma contained numerous, variably sized foci of necrosis. Affected foci contained anuclear and hypereosinophilic hepatocytes infiltrated by few macrophages and lymphocytes. Few hepatocytes contained 25-35 µm in diameter protozoal cysts, characterized by a thin cyst wall containing numerous 1-2 µm in length, punctuate to elongate bradyzoites.



An intrahepatocyte *Toxoplasma gondii* tissue cyst containing numerous punctuate to crescent-shaped bradyzoites (H&E, 100X). Note the lack of inflammation surrounding the tissue cyst.



A nodular aggregate of glial cells with central necrosis within the cerebrum (H&E, 20X). Inflammation is most likely centered on extracellular tachyzoites.

The cerebral, cerebellar, and brainstem parenchyma contained numerous small, nodular foci of necrosis infiltrated by moderate numbers of glial cells and few macrophages and lymphocytes. Rare protozoal cysts, not associated with the foci of necrosis, were observed within the cerebrum.

The adrenal gland cortices contain multiple foci of necrosis and few intracellular protozoal cysts.

Mesenteric lymph nodes contain multiple extensive foci of necrosis.

Pulmonary alveolar septa were diffusely, markedly expanded by histiocytes and fewer lymphocytes, plasma cells, and neutrophils. Alveoli were frequently lined by plump epithelial cells with large round nuclei and few prominent chromocenters (type II pneumocytes) and contained moderate numbers of histiocytes with abundant eosinophilic, vacuolated cytoplasm.

Ancillary testing: No bacteria or viruses were isolated by bacterial culture and virus isolation of the lung, liver, kidney, lymph node, or spleen.

Discussion: The presence of necrosis in multiple organs, including the liver, adrenal glands, lymph nodes, and brain, along with protozoal cysts within multiple organs supports a diagnosis of systemic toxoplasmosis in this cat. Although *Toxoplasma gondii* and *Neospora caninum* are difficult to differentiate histologically, systemic Toxoplasma gondii infection is by far the most common cause of systemic protozoal disease in juvenile cats *Toxoplasma gondii* is an intracellular coccidian parasite that has a wide host range that includes all domestic species, rodents, birds, primates, and humans. Domestic cats are the definitive hosts and the sexual stage of the parasite occurs within feline enterocytes. Transmission to cats is thought to occur most frequently by ingestion of infected tissues. Other routes of infection include congenital infection and ingestion of contaminated feces. Oocysts, which may be shed in the feces of infected cats, mature into sporozoites, the infectious stage. Once within the host, sporozoites divide and produce tachyzoites. From the gastrointestinal tract, Toxoplasma is transported to tissues either free within the plasma, or intracellularly by lymphocytes, macrophages, and granulocytes. Tachyzoites may infect almost any host cell to form a parasitophorus vacuole within the host cell membrane. Proliferation of tachyzoites results in destruction of the host cell and subsequent release of infectious zoites. These organisms may also encyst within cells to persist indefinitely as tissue cysts.

The characteristic lesion associated with systemic toxoplasmosis in cats is necrosis. Lesions may occur in almost any organ but are most commonly found in the brain, liver, lung, lymph nodes, heart, skeletal muscle (including the tongue), and eye. Additionally, a nonsuppurative and proliferative interstitial pneumonia, as was observed in this case, may occur in disseminated disease.

The majority of infected cats have clinically silent infection due to an appropriate humoral and cell-mediated immune response, and subsequent latency of the organism. Development of systemic disease may be the result of multiple variables including age, infectious dose, host species, and immune status. Immunosuppression secondary to stress, glucocorticoids, immunosuppressive drugs, or viral infection such as FIV, FeLV, and canine distemper virus in dogs, may predispose the animal to developing systemic disease. Acute infection of pregnant animals, including women, ewes and does, may result in parasitemia, placentitis, and abortion or infection of the fetus. *Toxoplasma gondii* should be considered as a potential cause of abortion in all domestic species.

Prenatal or neonatal infection may result in acute death, or nonspecific signs such as lethargy, depression, and hypothermia. Other clinical signs include fever, icterus, neurologic signs, pneumonia, lameness, and ocular abnormalities.

Antemortem diagnosis of systemic *Toxoplasma gondii* infection can be extremely difficult. Serology is frequently used despite obvious shortcomings. A positive antibody titer indicates exposure but not acute infection, and titers may persist in latent infection due to the humoral response to tissue cysts. Therefore, antemortem diagnosis should be based on a combination of history, serologic

testing (preferably acute and convalescent titers) and clinical signs.
-by Dr. Ryan Jennings, ADDL graduate student

References:

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

November 26-27……..Thanksgiving
December 24-25……Christmas
December 31, January 1, 2010……New Year
January 18……..Martin Luther King Day

Thanks to all of you who responded to our survey. We will use your responses as we strive to improve our service to you.

Please let us know what kinds of information/articles you would like to see in this newsletter by emailing us at addl@purdue.edu or phoning us 765-494-7440.

DIVERSE
EXCELLENT
ENGAGED

September 17, 2009

CALL FOR NOMINATIONS
FOR THE
SVM AWARD FOR EXCELLENCE IN SERVICE
FOR CALENDAR YEAR 2009

Dear Veterinary Colleague,

Please help us reward excellence in service! The SVM Award for Excellence in Service was established to honor our faculty and staff veterinarians for their distinguished service efforts. Please tell us about the excellent service that you received from the Purdue University School of Veterinary Medicine and/or the Animal Disease Diagnostic Laboratory. Nomination letters for the award should include the name of the nominee, the service unit, and a description (no more than 1 page) of the exceptional services provided by this individual. The exceptional service must be delivered through the PUSVM or ADDL and not as a private consultant.

Previous winners of the award shall be ineligible for nomination for a period of three years following the award. The last three years' recipients are Dr. Diane Bevier in 2006, Dr. Mark Hilton in 2007, and Dr. Catharine Scott-Moncrieff in 2008.

Please either email nominations to: svmengaged@purdue.edu

or mail them to Dr. Sandy Amass, Purdue University School of Veterinary Medicine, 625 Harrison Street, West Lafayette, Indiana, 47907-2026

Nominations are due by **February 1, 2010.**

Sincerely,

Sandy Amass, DVM, PhD, Dipl. ABVP
Associate Dean for Engagement

PURDUE UNIVERSITY SCHOOL OF VETERINARY MEDICINE — OFFICE OF ENGAGEMENT

Lynn Hall of Veterinary Medicine, 625 Harrison Street ■ West Lafayette, IN 47907-2026
(765) 494-8052 ■ Fax: (765) 496-2608 ■ Email: svmengaged@purdue.edu

Heeke ADDL

Southern Indiana Purdue
Ag Center Agriculture Building

The 40th anniversary of the Heeke Animal Disease Diagnostic laboratory's service to the animal industry of southern Indiana was recognized at a celebration held on Friday, September 11, 2009 on the grounds of the Southern Indiana Purdue Agricultural Center. The event included the opening of a new College of Agriculture building located near the Heeke ADDL. Guest speakers included Dr. Randy Woodson, Purdue University Provost, Dean Jay Akridge, Purdue School of Agriculture, Dean Willie Reed, Purdue School of Veterinary Medicine, Anne Hazlett, Director of the Indiana State Department of Agriculture, Dr. Bret Marsh, Indiana State Veterinarian and Ted Seger, President of Farbest Foods.

Lunch included turkey tenders provided by Farbest Foods and the Indiana Turkey Market Development Council and prepared by Sanders Catering, eggs donated by the Indiana State Poultry Association and prepared by Purdue Chef Emeritus Hubert Schmieder and Ruth Spitznagel, and butter donated by Holland Dairy and Prairie Farms.

Following the Heeke ADDL program, guests were invited across the road for dessert to honor the opening of the new Purdue Agriculture building.

Guest speakers from left to right
-Dr. Willie Reed
-Anne Hazlett,
-Dr. Jay Akridge
-Dr. Stephen Hooser, Director, ADDL
-Dr. Randy Woodson
-Ted Seger
-Dr. Bret Marsh

More photos can be viewed on the Purdue SVM Engagement website.

www.purdue.edu/svmengaged

Click on Awards and Events

DIAGNOSTIC FORUM

Diagnostic Forum is a quarterly newsletter of current services, regulations and research projects involving the ADDL which may be of interest to Indiana veterinarians and animal owners. It is our intention that the information provided will serve you. Please send your comments, suggestions, requests and questions to: Diagnostic Forum Editor, Purdue ADDL, 406 S. University St., West Lafayette, IN 47907 or email to addl@purdue.edu.

ADDL SECTION HEADS

Director: Steve Hooser, DVM, PhD
Assistant to Director: Linda Hendrickson, BS, MA
Avian: Tsang Long Lin, DVM, PhD
Avian: Pat Wakenell, DVM, PhD
Bacteriology: Ching Ching Wu, DVM, PhD
Business Manager: Tonya Byrd, BS
Computer Services: Steve Vollmer, BS
Histology: José Ramos-Vara, DVM, PhD
Molecular Diagnostics: Ramesh Vemulapalli, DVM, PhD
Pathology: Steve Lenz, DVM, PhD
Serology/Virology: Roman Pogranichniy, DVM, PhD
Toxicology: Christina Wilson, PhD
Heeke ADDL Co-Directors: Tom Bryan, DVM
Duane Murphy, DVM, PhD

VETERINARY PATHOLOGISTS:
Christine Holland, DVM, PhD
Steve Lenz, DVM, PhD
Tsang Long Lin, DVM, PhD
Peg Miller, DVM, PhD
Duane Murphy, DVM, PhD
José Ramos-Vara, DVM, PhD
Leon Thacker, DVM, PhD
Pat Wakenell, DVM, PhD