

Volume 20 No. 1  
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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

A HUGE thanks to all of the clients of the ADDL during the past year and our best wishes for a safe and successful 2010!

An equally huge thanks to the dedicated faculty and staff of the ADDL whose hard work has made all of those diagnoses possible!

Please let us know of anything that we can do to help.

## Hot Topics

- In-state fees if veterinarian or owner reside in Indiana! P.6
- Mycotoxins, p.5
- Pathology in Practice, new JAVMA feature, p. 2
- Tests added in 2009, p. 6
- Test results can be emailed, p. 2

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## Focus on Accessioning

The Accessioning group, supervised by Linda Hendrickson, is responsible for receiving, accessioning, and distributing cases to the appropriate laboratory within the ADDL. In addition to maintaining case records, they are the main communications link between the ADDL and its users.

The Accessioning staff includes

- Louann Albregts, 5 years at ADDL
- Lorraine Fox, 10½ years
- Mary Kay Presley, 9 years
- Shellie Rodarmel, 10 years
- Lance Mosley, Student Staff



Seated, left to right  
Shellie Rodarmel  
Mary Kay Presley  
Standing, left to right  
Lance Mosley  
Lorraine Fox  
Louann Albregts

## Pathology in Practice premieres in JAVMA

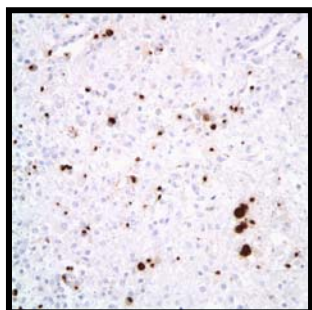
The *Journal of the American Veterinary Medical Association* (JAVMA) has a new feature called **Pathology in Practice**, published in cooperation with the American College of Veterinary Pathologists. The first article appeared in April 15, 2009. Since then, **two articles have featured diagnostic cases evaluated at the Purdue Animal Disease Diagnostic Laboratory**. Josh Webster, Larry Horstman, and Peg Miller reported a case of white snakeroot poisoning in a heifer (JAVMA 235:827-29, 2009) Pam Mouser, José Ramos-Vara, Ramesh Vemulapalli, and Catherine Scott-Moncrief published a case of protozoal encephalitis in a dog (JAVMA 235:1153-55, 2009).

The objectives of Pathology in Practice ([http://www.avma.org/journals/avma/ifa\\_pathology.asp](http://www.avma.org/journals/avma/ifa_pathology.asp)) are

- To provide education and continuing education in diagnostic pathology
- To strengthen the partnership between diagnostic pathologists and clinicians
- To increase awareness of pathology as a career opportunity

A popular format for Pathology in Practice is a diagnostic challenge with the case presented as an unknown on page 1 and resolved on page 2. Such case presentations, often classic examples of well-documented diseases, have been confined to pathology seminars or meetings. Pathology in Practice takes these presentations, with their inherent educational value, beyond pathology residents and pathologists to a broader audience. Check out this feature in the Veterinary Medicine Today section of JAVMA, test your diagnostic prowess, and watch for forthcoming reports of intriguing cases from the Purdue ADDL and elsewhere.

Mouser PJ, Ramos-Vara JA, Vemulapalli R, Scott-Moncrief C. Pathology in Practice. JAVMA 2009 235(10):1153-55.



**Subject: Protozoal encephalitis in a dog**  
Cerebrum, dog. Encephalitis.  
Immunohistochemical detection of *Sarcocystis neurona* merozoites (individual organisms and clusters) within the inflammatory infiltrate.

**.Summary.** An adult dog developed thrombocytopenia, anemia, pyrexia and intermittent

seizures that did not improve with symptomatic treatment. The animal was submitted to the Purdue ADDL for necropsy. The most significant changes were present in the cerebrum, which presented asymmetric dilatation of the lateral ventricles (hydrocephalus). Microscopically, the cerebrum, cerebellum, and brainstem had multiple foci of inflammation characterized by neutrophils, macrophages, lymphocytes, plasma cells, and fewer eosinophils. Within the inflammatory foci there were numerous intra- and extracellular crescent-shaped merozoites and rare schizonts. The tentative diagnosis was protozoal encephalitis, most likely produced by *Sarcocystis* spp. To confirm this diagnosis, immunohistochemistry for *Sarcocystis neurona*, *Neospora caninum*, and *Toxoplasma gondii* was performed. Immunohistochemical results were consistent with a diagnosis of *Sarcocystis* encephalitis. Molecular analysis (PCR), using paraffin embedded sections of the affected brain, was performed to confirm this diagnosis. The amplified genome matched *Sarcocystis neurona* genome and therefore this diagnosis was confirmed. *Sarcocystis neurona* is the cause of equine protozoal myeloencephalitis. It has been associated with cases of encephalitis in dogs. Due to the similarities of microscopic lesions produced by *Toxoplasma*, *Neospora*, and *Sarcocystis* in the brain, immunohistochemistry and molecular techniques are necessary to determine the specific causal agent.

Entire article available at

<http://avmajournals.avma.org/doi/full/10.2460/javma.235.10.1153>



White snakeroot was growing in the pasture where a heifer died with heart and skeletal muscle necrosis.

This entire article, written by Drs. Joshua Webster, Larry Horstman, and Margaret Miller, can be read in JAVMA 235(7):827-29 or on the JAVMA website at

<http://avmajournals.avma.org/doi/full/10.2460/javma.235.7.827>

### ADDL Lab Results by

1. Email (Call ADDL with email address)
2. Fax
3. Internet/Web

Laboratory results are available on the Internet. Call us to set up an account or go to our web page [WWW.ADDL.PURDUE.EDU](http://WWW.ADDL.PURDUE.EDU)  
- Click on Online Reports tab  
- Click on Request Info and follow instructions





## Contagious Equine Metritis “A Current Perspective”

### Introduction and History:

Contagious Equine Metritis (CEM) is a transmissible venereal disease of equids caused by the gram negative bacterium, *Taylorella equigenitalis*. CEM was first diagnosed in Europe in 1977 but, over the following year, several other countries including the United States (Kentucky) reported outbreaks. Shortly thereafter, the disease was eradicated from the U.S. and, until December of 1998, was classified as a foreign animal disease.

**Transmission:** *Taylorella equigenitalis* can be transmitted directly during breeding of a carrier stallion or mare or indirectly through artificial insemination or fomites. The highly effective transmission of CEM can be attributed, at least in part, to unrecognized stallions carrying the bacteria on their external genitalia. As such, multiple mares may become infected before the disease is even suspected.

**Clinical Signs:** There are no reports of stallions infected with CEM showing clinical signs; however, clinical disease in mares is localized to the reproductive tract resulting in temporary infertility. Mares can demonstrate three patterns of infection: acutely infected, chronically affected, or asymptomatic carriers. Whereas acute infections present with copious, thick, gray vaginal discharge 10-14 days post breeding, chronic infections have less vaginal discharge and milder uterine inflammation, but are more difficult to eliminate. After recovering from acute infections, mares can carry the bacterium asymptotically for months. CEM rarely causes permanent infertility or abortions.

**Gross, Histological and Cytological Findings:** During the initial two to three weeks of infection, the endometrium and cervix are swollen, edematous, and covered with cloudy gray to white exudates. The superficial (stratum compactum) and deep (stratum spongiosum) layers of the endometrium are thickened by edema and infiltrated by neutrophils and fewer eosinophils which transmigrate into the endometrial epithelium and uterine lumen. By 14 days post-infection, the edema subsides and the inflammatory infiltrate consists of lymphocytes, plasma cells, and fewer neutrophils until day 21 when the inflammation becomes a mixed mononuclear cell population. Inconsistent and less severe microscopic lesions include suppurative salpingitis and vaginitis. No lesions are found in the clitoral fossa or clitoral sinus; however, organisms tend to persist in these locations for longer periods of time and are therefore common culture sites.

**Diagnostics:** Reproductive losses associated with CEM could significantly impact the economic status of the equine industry in the U.S.; therefore, great efforts have been taken to learn more about diagnosing CEM. There are currently three tests used to determine if a horse is infected with *T. equigenitalis*. These include bacterial culture, serological testing, and test mating.

Bacterial culture is the most reliable and accurate way to arrive at a diagnosis; however, challenges exist with respect to sample handling. A swab of the genitourinary tract may be taken from the clitoral sinus, clitoral fossa, endometrium, and cervix, or the preputial folds, urethral fossa, urethra, skin of the penis, and pre-ejaculatory fluid of the mare and stallion, respectively. Samples are then placed in liquid Amie's Charcoal medium and refrigerated until it is plated on chocolate agar. False negatives occur if transit time is prolonged or the sample is warmed. Despite its reliability, culture is time consuming and technically demanding, thus newer methods are being developed.

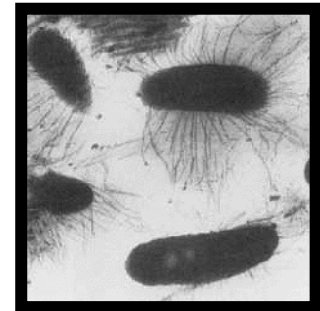
Serological testing protocols have been surfacing since the emergence of CEM. The most commonly implemented test at this time is complement fixation. However, this test is limited to mares that have produced detectable antibodies to *T. equigenitalis*. Polymerase chain reaction (PCR) tests are also used and have improved efficiency and reliability. In addition, an Enzyme-Linked Immunosorbent Assay (ELISA) has been designed for detection, but is less commonly employed. Test mating combines technology from the tests detailed above. In test mating, a stallion is bred to two CEM-negative mares. The mares are then tested by culture and serology to check for infection. The testing procedure requires 35 days before a stallion is declared negative.

Current acceptable protocols for diagnosis include test mating with culture for stallions and culture combined with the complement fixation test for mares.

**Current status:** On December 15, 2008, a quarter horse stallion in central Kentucky was confirmed to be infected with *T. equigenitalis*. Fifteen days later, three stallions in Indiana also tested positive for the bacteria. Epidemiological investigations have failed to identify the source of the outbreak. As of April, 2009, 21 stallions and 5 mares in the United States have been confirmed positive for *T. equigenitalis*. From these 26 positive animals, another 960 have been exposed making a total of 986 affected horses (270 exposed or positive stallions and 708 exposed or positive mares), covering a range of 48 states (Hawaii and Rhode Island have no links to the disease). All of the positive and exposed horses were put in quarantine and underwent testing and treatment protocols. Thus far, 823 (83.5%) of the 986 horses, including all horses from Indiana, completed testing and treatment protocols and were free of disease.

-by Seth Lundquist, Class of 2010

-edited by Dr. Chad Frank, ADDL Graduate Student



## References:

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### Turkey coronaviral enteritis in Indiana turkey flocks in 2009

Since 1995, Tom Hooper, ADDL-Heeke, has performed serological testing against turkey coronavirus (TCoV) using direct

and/or indirect immunofluorescent antibody assays for more than 180,000 turkeys from U.S. turkey flocks. More than 32,000 serum samples were tested in 2001 from various turkey producing states. Requests for TCoV serology have diminished since 2001; 7,848 tests were performed in 2008.

TCoV has been quiet in southern Indiana turkeys until September 2009. An 8-week-old commercial male turkey flock was showing increased water consumption and wetter litter. No increased mortality was seen. Virus was isolated in embryonated turkey eggs. The flock was marketed at 20 weeks with below average body weights but normal livability. Serological tests against TCoV have been run on turkey flocks with clinical enteritis as well as those in the surrounding areas. Flock supervisors have been diligent about submitting six serum samples twice in two-week intervals for detection of antibodies to TCoV in

order to confirm infection. Tom Hooper has provided Drs. Tsang Long Lin and Ching Ching Wu TCoV isolates for comparison of genetic uniformity among TCoV isolates over the years because chicken coronaviruses (infectious bronchitis virus) tend to vary. TCoV isolates from various geographic locations in the U.S., including those from Indiana, have been found to possess more than 90% genetic similarity and share close antigenicity over the last 15 years. Nevertheless, genetic analysis of Indiana TCoV isolated in 2009 is being conducted by Drs. Lin and Wu.

**Clinical summary :** In discussion with submitters for TCoV serology over the years, Tom Hooper and Dr. Tom Bryan have felt that the virus can show very few signs in some flocks, but persists quietly until feed conversions are noted and testing commenced. Routine serological profiles against TCoV have been helpful to the turkey industry for those subclinical flocks mentioned above as well as the clinical flocks that need turkey coronavirus infection controlled by biosecurity measures and eradication programs.

-by Dr. Tom Bryan, Heeke ADDL Avian Diagnostician

## References

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Purdue ADDL and Heeke ADDL will be closed on the following University holidays in 2010.

May 31.....Memorial Day  
July 5.....Independence Day  
September 6.....Labor Day  
November 25-26.....Thanksgiving  
December 23-24.....Christmas  
December 30-31.....New Year





## Mycotoxins in Animal Feeds

**Background:** Due to the unseasonably wet weather and delayed harvest of grain, conditions

have been favorable for mold growth in corn crops throughout the state. Corn ear molds, particularly *Giberella* and *Fusarium* molds, have been reported throughout Indiana at levels that have not been observed in the state for decades. The persistence of these molds in the corn crop is a concern due to the potential of these fungi to produce mycotoxins. Mycotoxins are naturally-occurring, secondary metabolites produced by a variety of molds and can be toxic to animals and livestock. In Indiana, the mycotoxins of major importance include aflatoxin, deoxynivalenol (also known as DON or vomitoxin), fumonisin, and zearalenone. *Giberella* and *Fusarium* molds can appear red or pink in color (and occasionally white) and can potentially produce deoxynivalenol, zearalenone, and fumonisin. *Aspergillus* molds are typically yellow-green in color and can produce aflatoxin.

Since August 2009, the Indiana Animal Disease Diagnostic Laboratory has detected significant amounts of deoxynivalenol, fumonisin, and zearalenone in corn/grain samples. The approximate concentrations detected for these mycotoxins range from 1-40 ppm for deoxynivalenol, 2-70 ppm for fumonisin, and 1-10 ppm for zearalenone. For information on the action/guidance levels for these mycotoxins in livestock feed, see tables 1-4 below. It is important to remember that observation of mold on corn or other grain does not necessarily mean that mycotoxins are present. Therefore, it is important that the corn or grain is submitted for laboratory testing in order to assess the presence of mycotoxins in suspect samples.

-by Dr. Christina Wilson, Head of Toxicology/Analytical Chemistry

**Table 1. Action Levels for Total Aflatoxins in Livestock Feed**

Class of Animals	Feed	Aflatoxin Level
Finishing beef cattle	Corn and peanut products	300 ppb
Beef cattle, swine, poultry	Cottonseed meal	300 ppb
Finishing swine >100 lb	Corn and peanut products	200 ppb
Breeding cattle, breeding swine and mature poultry	Corn and peanut products	100 ppb
Immature animals	Animal feeds and ingredients, excluding cottonseed meal	20 ppb
Dairy animals, animals not listed above, or unknown use	Animal feeds and ingredients	20 ppb

**Table 2. Guidance Levels for Total Fumonisin in Animal Feeds**

Class of Animal	Feed Ingredients & Portion of Diet	Levels in Corn & Corn by-products	Levels in finished feeds
Equids and rabbits	Corn and corn by-products not to exceed 20% of diet**	5 ppm	1 ppm
Swine and catfish	Corn and corn by-products not to exceed 50% of diet**	20 ppm	10 ppm
Breeding ruminants, breeding poultry and breeding mink*	Corn and corn by-products not to exceed 50% of diet**	30 ppm	15 ppm
Ruminants ≤ 3 months old being raised for slaughter and mink being raised for pelt production	Corn and corn by-products not to exceed 50% of diet**	60 ppm	30 ppm
Poultry being raised for slaughter	Corn and corn by-products not to exceed 50% of diet**	100 ppm	50 ppm
All other species or classes of livestock and pet animals	Corn and corn by-products not to exceed 50% of diet**	10 ppm	5 ppm

\*Includes lactating dairy cattle and hens laying eggs for human consumption

\*\*Dry weight basis

**Table 3. Advisory Levels for Vomitoxin (DON) in Livestock Feed**

Class of Animal	Feed Ingredients & Portion of Diet	DON Levels in Grains & Grain By-products and (Finished Feed)
Ruminating beef and feedlot cattle >4 months old	Grain and grain by-products not to exceed 50% of the diet	10 ppm (5 ppm)
Chickens	Grain and grain by-products not to exceed 50% of the diet	10 ppm (5 ppm)
Swine	Grain and grain by-products not to exceed 20% of the diet	5 ppm (1 ppm)
All other animals	Grain and grain by-products not to exceed 40% of the diet	5 ppm (2 ppm)

**Table 4. Minimum Levels of Zearalenone Associated with Clinical Signs in Animals**

Class of Animal	Feed Ingredients	Zearalenone Levels
Prepubertal gilts	Diet	1-3 ppm
Sexually mature cows	Diet	3-10 ppm
Bred sows	Diet	>15 ppm
Young boars	Diet	>20 ppm
Mature cows	Diet	>25 ppm
Virgin heifers	Diet	>10 ppm

## References:

1. Wise K and Woloshuk K: 2009. Corn Ear Rots Widespread in Indiana. *Pest & Crop* 26:1-2.
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## USER'S GUIDE, FEES, INFORMATION at

[www.addl.purdue.edu](http://www.addl.purdue.edu)

Email: [addl@purdue.edu](mailto:addl@purdue.edu)

**Following is a comprehensive list of new tests and/or charges initiated in 2009.**

**In state charges apply if either the submitting veterinarian or the owner reside in Indiana.**

Laboratory	Instate charge
<b>Bacteriology</b>	
Aerobic counts.....	\$20.00
Anaerobic counts.....	\$25.00
Drag swab.....	\$15.00
<b>Molecular</b>	
<i>Coxiella burnetii</i> real time.....	\$25.00
Encephalitozoon RT-PCR.....	\$35.00
<i>Mycoplasma hyopneumoniae</i> RT-PCR.....	\$25.00
<i>Chlamydia psittaci</i> RT-PCR.....	\$25.00
<b>Pathology</b>	
Process eyes.....	\$20.00
Decalcification.....	\$17.00
Painted margins.....	\$25.00
<b>Serology</b>	
Bovine coronavirus IFA .....	\$3.00
Bovine coronavirus IFA titration.....	\$3.00
Influenza A ELISA.....	\$5.00
Toxoplasmosis ELISA.....	\$5.00
<b>Toxicology/Analytical Chemistry</b>	
Cyanide.....	\$35.00
<b>Virology</b>	
Influenza virus partial HA Gene Sequencing..	\$100.00
Influenza virus H Gene Sequencing.....	\$200.00

### Out of state fees

Out of state fees are applied only when both the submitting veterinarian and owner are out of state and is based on the information provided on your submission paperwork.

If you have questions when you receive your bill, please notify us at 765-494-7440.

## Final Diagnosis:

### *Heterobilharzia americana*

**Clinical History:** A 5-year-old intact female Shih-Tzu presented for evaluation of vomiting, diarrhea, and decreased appetite. She was in heat approximately one month previous to onset of clinical signs. Radiographs of the abdomen revealed mildly distended bowel loops. A presumptive clinical diagnosis of pyometra was made and an exploratory laparotomy was performed.

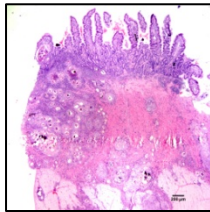
**Surgical Findings:** Laparotomy revealed a mildly turbid fluid within the peritoneum. Multiple firm nodules (approximately 0.5 cm in diameter) were present in the right lobe of the pancreas, small intestinal serosa and enlarged mesenteric lymph nodes. The uterus was mildly distended by fluid. Biopsy specimens from the pancreas, lymph node, and jejunum were submitted for histopathology. An ovariohysterectomy was performed after the biopsies and uterus and ovaries were also submitted for histologic evaluation.

**Histologic Findings:** The lymph node contained numerous round to oval, approximately 80 µm wide ova with a yellow to tan, thick hyalinized wall distributed primarily in the paracortex. Many of the ova contained developing miracidium, although a few were empty. A marked inflammatory reaction surrounded individual ova and consisted of many epithelioid macrophages with fibrosis, eosinophils, neutrophils, lymphocytes, plasma cells, and few multinucleated giant cells.

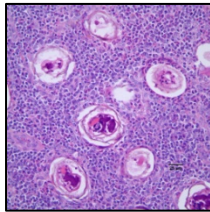
The small intestine (jejunum) contained ova and granulomatous inflammation distributed transmurally and near venules, likely arborizing from the mesenteric veins. Similar ova and granulomatous inflammation was observed in the pancreas.

Uterus and ovaries were histologically unremarkable.

**Ancillary testing:** Fecal sedimentation in saline revealed miracidia-laden eggs.

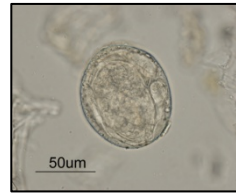


Small intestine with transmural parasitic ova containing miracidium and surrounded by granulomatous inflammation. Eggs are consistent with *Heterobilharzia americana*.



Mesenteric lymph node with numerous parasitic ova and surrounded by granulomatous inflammation. Eggs are consistent with *Heterobilharzia americana*.

**Ancillary testing:** Fecal sedimentation in saline revealed miracidia-laden eggs.



Miracidia-laden trematode egg consistent with *Heterobilharzia americana* from fecal sedimentation. Note the miracidium within a thick refractile capsule and no operculum.

**Discussion:** *Heterobilharzia americana*, classified as a schistosome, is a blood fluke of wild and domestic carnivores and is widespread in southern Atlantic and gulf coast states (Florida, Louisiana, Mississippi, Texas, Georgia, North Carolina, South Carolina) as well as east central states and southeast Kansas. The life cycle of *H. americana* is indirect, involving an aquatic snail (*Lymnaea cubensis* and *Pseudocuccinea columella*) as the intermediate host. Cercariae that are released from the snail intermediate host infect dogs and wildlife through direct skin penetration. Clinically significant *H. americana* infection includes massive inflammation leading to intestinal disorders, dehydration, pancreatic insufficiency, and systemic dissemination. Antemortem diagnosis requires fecal sedimentation in 0.9% NaCl to identify characteristic trematode eggs. Schistosome ova are large (70-90µm), non-operculated, and contain miracidium. Unfortunately, schistosome larvae were not identified in multiple tissue sections. Diagnosis was based on fecal sedimentation by the Clinical Parasitology Laboratory at Purdue University as well as histopathologic evaluation of ova. The dog was presumably infected while vacationing in Florida in July and subsequently became ill in August of the same year. Treatment with fenbendazole for 14 days was initiated and repeated after three weeks. One month following administration of high doses of fenbendazole, no ova were detected on sedimentation, feces were formed, and appetite and activity were normal.

-by Dr. Abigail Durkes, ADDL Graduate Student

## References:

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2. Goff W and Ronald J: 1982. American Journal of Veterinary Research 42:1775-76.
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## 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. 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](mailto:addl@purdue.edu).

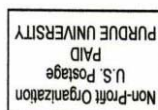
### ADDL SECTION HEADS

Director: Steve Hooser, DVM, PhD  
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Avian: Pat Wakenell, DVM, PhD  
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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:

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Peg Miller, DVM, PhD  
Duane Murphy, DVM, PhD  
José Ramos-Vara, DVM, PhD  
Leon Thacker, DVM, PhD  
Pat Wakenell, DVM, PhD  
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ANIMAL DISEASE DIAGNOSTIC LABORATORY  
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