

Diagnostic Forum

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A Quarterly Newsletter from the Indiana Animal Disease Diagnostic Laboratory at Purdue University



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From the Director.....

The long winter of 2013-2014 is over and it's time for a couple of days of Spring before Summer arrives in a few weeks! In this issue of the ADDL Diagnostic Forum, we welcome Dr. Grant Burcham to the Heeke Lab at SIPAC and Congratulate Drs. Durkes, Charney, Beck, and Broman on their awards. Check out the photos from the cover of *Viral Immunology* that are part of Dr. Pogradichniy, Dr. Lenz, and colleague's article on, "DNA-vaccine platform development against H1N1 subtype of swine influenza A viruses."

Parasitology services are now available at the ADDL. In the fall, the Clinical Parasitology Laboratory in the Department of Comparative Anatomy moved to the ADDL under the direction of our Head of Microbiology, Dr. Hammac. Many thanks to Dr. Joe Camp, for helping set up the lab, train the staff in parasitology techniques, and continue helping with parasite identifications, and to Elaine Baird, Manny Benitez and Leonie Leduc for learning and performing the parasitology tests.

Updates from the Bacteriology Lab provide the latest on urine and feces, while the Toxicology Lab warns us to be on the lookout for strychnine poisoning.

Dr. Grant Burcham to start at Heeke ADDL in June

Dr. Burcham will begin his work as the head of the Heeke ADDL lab in Dubois County on June 2nd, 2014. "I am looking forward to working with the producers and animal owners in southern Indiana to help address animal health needs there. I am also excited about working alongside the excellent staff currently at the Heeke lab," Burcham said.

Dr. Burcham earned a Master of Science in Veterinary Pathology in 2010 and a Doctorate of Veterinary Medicine in 2007 from Purdue, and a Bachelor's degree in Biology in 2003 from Hanover College. He is a diplomate of the American College of Veterinary Pathologists.

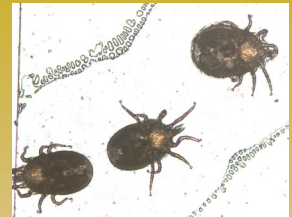
Dr. Burcham succeeds Dr. Duane Murphy, who retired from Purdue after nearly 20 years of service to the university.



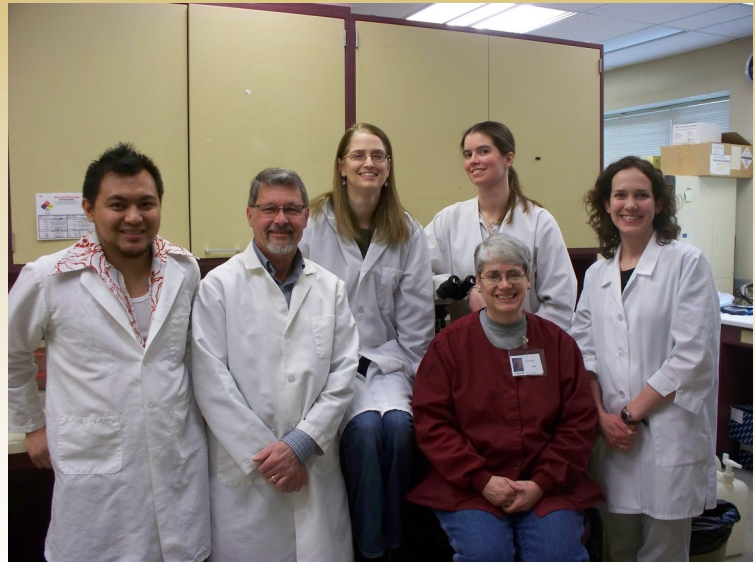
Please join us in welcoming Dr. Burcham to the Indiana ADDL team!



Trichodectes canis



Dermanyssus



Parasitology Team from left to right: Manny Benitez, Dr. Joe Camp, Kathi Burke, Elaine Baird (sitting front), Leonie Leduc, Dr. Kenitra Hammac

After nearly 35 years in the Department of Comparative Pathobiology, the diagnostic parasitology laboratory has relocated to the ADDL as of November 1, 2013. The laboratory is under the direction of Dr. Kenitra Hammac with Dr. Joe Camp serving as the consulting parasitologist and Elaine Baird as the lead technician.

We offer qualitative fecal flotation (both Sheather's sugar solution and ZnSO₄), quantitative fecal flotation, acid fast stain for *Cryptosporidium* sp., direct fecal exam, fecal sedimentation, Baermann technique, parasite identification, Knott's test, canine heartworm antigen test and feline heartworm antibody test.

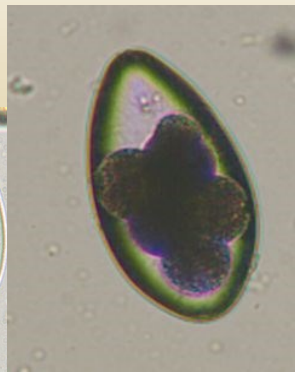
Please visit the parasitology page of the ADDL website for a detailed list of available tests .

(<https://www.addl.purdue.edu/TestsFees/BySection.aspx>) and pages 4 and 5 of the PDF test and fee guide at

<https://www.addl.purdue.edu/TestsFeesFeeSchedule2011.pdf>.

Accessions requesting only parasitology tests will not be subject to the standard accession fee.

Mullerius capillaris



Nematodirus sp.

Parasitology Comes to ADDL

ACVP Awards

Dr. Virginia Charney,
shown with presenter

PVM Graduate Student Wins Comparative Pathology Scholarship Award

Congratulations to ADDL's pathology resident Abby Durkes (PU DVM '08), who was named as Purdue University's recipient of the 2013 Student Scholarship Award given by the CL Davis Foundation for the Advancement of Veterinary and Comparative Pathology. The annual award recognizes one pathology resident or graduate student at each of the approximately 20 North American veterinary pathology training programs. Dr. Durkes was selected by the veterinary pathologists in Comparative Pathobiology to be Purdue's recipient. The award was given at the CL Davis Foundation Awards Reception at the annual meeting of the American College of Veterinary Pathologists (ACVP) November 18 in Montreal, Canada.



Dr. Abby Durkes



PVM Graduate Student Wins ICPI Trainee Travel Award

ADDL pathology resident Virginia Charney (TAMU DVM '09) received a Trainee Travel Award from the Intersociety Council for Pathology Information to attend the annual meeting of the American College of Veterinary Pathologists (ACVP) November 16-20. Awardees were selected on the basis of their submitted abstracts and CVs. Dr. Charney's poster, entitled, "Characterization of Bone Metastases from Canine Transitional Cell Carcinoma", was co-authored by Drs. Debbie Knapp, Hock Gan Heng, and Peg Miller.

PVM Graduate Students Win Young Investigator Awards

Congratulations are in order for ADDL's pathology residents Jessica Beck (UGA DVM '13), NIH Graduate Partnerships Program Fellow, and Meaghan Broman (Wisc DVM '10), who received second and third place Young Investigator Awards in the Diagnostic Pathology category at the annual meeting of the American College of Veterinary Pathologists (ACVP) in November. Awardees were selected by a team of judges on the basis of their poster presentations. Dr. Beck's poster, entitled, "Multifocal idiopathic nodular bone formation in the dermis and subcutis of a cynomolgus macaque (*Macaca fascicularis*)" was based on a study at the Mannheimer Foundation in Homestead, Fla., and co-authored by Drs. J.F. Lane, R.C. Smedley, S.M. Howell, P.R. Morales, and J.L. Wagner. Dr. Broman's poster, entitled, "Small intestinal muscularis thickening in dogs: correlation of histologic and ultrasonographic findings", was co-authored by PVM veterinarians Peg Miller, Jeff Ruth, and Hock Gan Heng.

Congratulations!



Dr. Jessica Beck (center) and Dr. Meaghan Broman (right)

Strychnine Poisoning in Animals

By Dr. Christina Wilson, Jonathan Butz, and Mary Mengel



Strychnine-treated grain

Strychnine, an alkaloid derived from the seeds, tree, and bark of the *Strychnos nux-vomica* plant, has historically been used as a potent rodenticide.¹ Although once a restricted use pesticide, strychnine-containing baits are now commercially available over-the-counter as bait blocks or treated grain formulations (red or blue-green in color) and typically contain 0.5% (5,000 ppm) strychnine.² These baits are commonly used to control moles, gophers, ground squirrels, coyotes, and wolves. Unfortunately, malicious and accidental poisonings in small animals are becoming more common due to the resurgence in availability and use of strychnine as a pesticide/rodenticide.

Strychnine blocks the inhibitory neurotransmitter glycine and is therefore a potent CNS stimulant. It is rapidly absorbed from the gastrointestinal tract with onset of clinical signs occurring within 10 minutes to 2 hours, depending on the dose.² The most common clinical sign observed is an acute onset of convulsions or seizures in the exposed animal. Other clinical signs can include hypersensitivity to external stimuli, extensor rigidity or muscle stiffness, uncontrollable muscle spasms, metabolic acidosis, respiratory distress, tachycardia, and death. Strychnine can also cause elevations in the serum enzymes glutamic oxaloacetic acid (SGOT), creatinine kinase (CK), and lactate dehydrogenase (LDH).¹

Diagnosis of strychnine poisoning is based on case history and detection of strychnine in biological samples or source material. In suspect cases of poisoning, the following samples can be submitted to the diagnostic laboratory for testing: vomitus, gastric lavage washings, stomach contents, liver, urine, serum, bait, or suspect source material. Differential diagnoses to consider are toxicants that cause CNS stimulation/seizures. These can include exposure to bromethalin, metaldehyde, tremorgenic mycotoxins, organophosphate/carbamate insecticides, pyrethrins/pyrethroids, nicotine, sodium monofluoroacetate, caffeine/theobromine, organochlorine pesticides, neurotoxic cyanobacteria, antidepressant medications, or illicit drugs (LSD, methamphetamine, e.g.). Given the increased use and availability of strychnine-containing baits, it is important to consider strychnine as a differential in cases in which animals have an acute onset of convulsions or seizures.

References

1. Gupta, RC (2012) Non-Anticoagulant Rodenticides. *In: Veterinary Toxicology: Basic and Clinical Principles*. R. Gupta (editor). Elsevier, Inc., San Diego, CA, pp. 548-550.
2. Hall, JO (2011) Strychnine. *In: Blackwell's Five-Minute Veterinary Consult, Clinical Companion, Small Animal Toxicology*. G. Osweiler (editor) Wiley-Blackwell, Ames, IA, pp. 791-797.

Updates from the Bacteriology Lab

Semi-quantitative urine cultures: Urine submissions are now cultured by a semi-quantitative method in order to provide more useful information to the submitter. A standard comment accompanies all urine culture results to assist interpretation according to guidelines set forth by the International Society for Companion Animal Infectious Diseases (ISCAID). A minimum of 0.5mL urine is required for semi-quantitative culture. Urine swabs and samples of insufficient volume will be cultured by standard, non-quantitative methods.

Johne's PCR

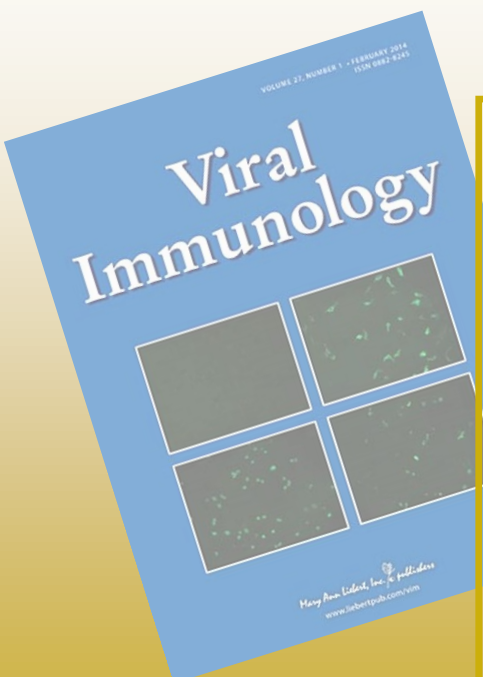
By Dr. Roman Pogranichniy and Dr. Kenitra Hammac

Johne's disease, a chronic intestinal disease of ruminants caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP), can be difficult to diagnose due to a delay of years between exposure and the development of clinical signs. Delayed antibody production and intermittent shedding in feces compound diagnostic difficulties. ADDL is now recommending PCR over culture as a means to detect MAP shedding in feces based on recent studies* and the performance of our laboratory and other MAP testing laboratories on the National Veterinary Service Laboratory's (NVSL) Johne's Disease Proficiency Test which have demonstrated that PCR is at least as sensitive as culture. In the past, culture was considered the gold standard due to inhibitors in fecal samples that interfered with the PCR tests, but new extraction methods have overcome the problem of PCR inhibitors. The benefit to our clients is the quick turn-around time of 48 hours for PCR as compared to the 7 weeks that it takes for culture. Culture will no longer be less expensive, as we have raised the price of Johne's culture to \$35 per sample to meet the rising costs of test materials and procedures, which, unbeknownst to our clients, also used PCR to confirm any suspect cultures. Keep in mind that the same interpretive problems associated with negative cultures also apply to negative PCR tests. A culture or PCR test on feces may be negative because the animal is not infected, or because the animal is infected but was not shedding the organism in feces at the time of sample collection. The difficulties of interpreting negative fecal samples for sheep and goats is amplified by the fact that small ruminants tend to shed less organism than cattle. Johne's culture is still available by request, and serologic testing remains the preferred method for herd screening. Johne's PCR will be run in the ADDL on Wednesdays and Fridays of every week except official holidays at Purdue University to accommodate everyone. Cost for each PCR test is \$35 per sample or \$40 for pooled samples.

*Alinovi. "Real-time PCR, compared to liquid and solid culture media and ELISA for the detection of *Mycobacterium avium* ssp. *paratuberculosis*." *Veterinary Microbiology*. 2009

*Logar. "Evaluation of combined high-efficiency DNA extraction and real-time PCR for detection of *Mycobacterium avium* ssp. *paratuberculosis* in subclinically infected dairy cattle: comparison with fecal culture, milk real-time PCR and milk ELISA." *BMC Veterinary Research*. 2012.

Research at the ADDL



Dr. Pogranichniy and his research team are working on developing an effective universal DNA vaccine against influenza A virus. His recent research work was published in the journal *Viral Immunology* and pictures from the article were featured on the cover of the journal. The highlight of the research is: "The DNA vaccine administered intramuscularly stimulated epitope-specific immunity against two T-cell epitopes in all ten mice before the virus challenge. All vaccinated mice survived a lethal dose of virus challenge, while all mice in the challenge control group died. The DNA vaccine delivering epitopes in this study showed promising protection against influenza virus in an animal model; however, more work needs to be done in developing a universal DNA vaccine against influenza A virus."

Currently Dr. Pogranichniy is looking for additional private or federal funding to continue his research in this area.

Huiling Wei, Stephen D. Lenz, David H. Thompson, and Roman M. Pogranichniy. *Viral Immunology*. August 2012, 25(4): 297-305

Questions? Comments? Concerns?

**We value your opinion. Please
contacts us at:**

Phone 765-494-7440

Email ahighlan@purdue.edu

ADDL is pleased to offer new submission forms
now tailored to fit your specific diagnostic needs.

They can be found at:

<https://www.addl.purdue.edu/Forms/Forms.aspx>

Now offering: PEDv PCR

Cost is \$30.00 plus accession fee

Submit samples within the first 24 hours of diarrhea:

- ◆ Small intestine
- ◆ Rectal swabs
- ◆ Feces/colonic contents (fresh-chilled for PCR)

For more information please visit our website at:
www.addl.purdue.edu

ADDL Lab Results are available by:

- ◆ Email (call ADDL with your email address)
- ◆ Fax
- ◆ Internet/Web