New Tests/Fees

**Nocardiarom Placentitis**

**Haemonchus contortus** in sheep and goats

Yew Plant Toxicity

New Graduate Students

Immunohistochemistry in a Diagnostic Setting

ADDL Schedule

UPS/GlobalVetLink/Lab Results

### Changes to Fee Schedule

Enclosed with this newsletter is a current fee schedule effective July, 2011.

New tests/fees are indicated below.

#### Pathology

**Parasitology processing**

**Abortion panel**

#### Bacteriology

**Aerobic enrichment**

**Milk culture, aerobic**

**Volume discount > 10 samples**

**Mycoplasma culture**

**Salmonella series (up to 5)**

#### Molecular Diagnostics

**Brucella real time**

**Multiplex C3B**

**Multiplex LTN real time**

**Johnes**

**Mycoplasma bovis**

**Novel N1 Subtype real time**

#### Serology

**Anaplasmosis ELISA**

**Volume discount >45 samples**

**Bluetongue ELISA**

**Volume discount >45 samples**

**Bovine leukosis virus ELISA**

**Volume discount >45 samples**

**CAE ELISA**

**Volume discount >45 samples**

**H1N1 Pfizer HI**

**H3N2 Pfizer HI**

**Neospora ELISA**

**Volume discount >45 samples**

### From the Director

**Stephen Hooser**

Labor Day has come and gone, so Fall has unofficially come to the great state of Indiana. Many thanks to Dr. Tom Bryan, Poultry Diagnostician at the Heeke ADDL, who retired on June 30 after 30 years of service to Indiana and the poultry industry! Dr. Duane Murphy, Tom Hooper, Margaret Gelhausen and Denise Riley, remain hard at work, devoted to serving animal clients, veterinarians and the poultry and livestock industries of southern Indiana, Monday through Friday, 8-5.

Many, many thanks to the ADDL staff who helped with testing for the 2011 Indiana State Fair! Special thanks to Barb Million in Molecular Diagnostics, Lorraine Fox in Accessioning and, in Analytical Toxicology, Kim Meyerholtz, Assistant Chemist and Dr. Christina Wilson, Head Analytical Chemist/Head of Toxicology for the efforts that they put in!

As always at both the Heeke and West Lafayette ADDL, the technical and quality management of ADDL sections are committed to good professional practice and compliance to the requirements for accreditation as defined by AAVLD. For example, the ADDL Analytical Toxicology Section (no affiliation with the Indiana State Toxicology Lab in Indianapolis) performs all of its testing according to well-established protocols with documentation according to the ADDL Quality System. All data are scientifically reviewed to ensure that established acceptance criteria have been met before releasing data to our clients.

The State Fair is over. The last corndog is eaten. It is time for Fall.

### Nocardiarom Placentitis in a Mare

by Dr. Duane Murphy, Veterinary Pathologist, Heeke ADDL

Abstract

An equine fetus and placenta were submitted to Heeke ADDL with a history of abortion 5 weeks prematurely, preceded by premature udder development. Otherwise, the mare appeared normal. No gross or histologic lesions were found in the fetus. Placenta had a large mucosal lesion located near the bifurcation of the uterine horns. Mucosa in the center of this lesion was thin and pale and, at the margins, was thick, gray and friable, surrounded by an area of hyperemia. Histologically, the placenta had extensive epithelial necrosis and neutrophilic infiltration, and was covered by a thick layer of necrotic debris containing "sulfur granules". Warthin-Starry stain revealed small branching filamentous bacilli within these granules. Bacilli were not acid fast and did not stain with Brown & Brenn's gram stain. A mix of bacteria was isolated from placental mucosa, including an "actinomycete-like organism" that could not be further identified. No bacteria were isolated from fetal tissues. The case was given a final diagnosis of placental actinomycosis.

The findings in this case are similar to those described by Hong et al (1993) for nocardiform actinomycete-associated placentitis. An unusually high incidence of this disease occurred in the spring of 2011, both in Kentucky and southern Indiana. No specific factors have been associated with this increased incidence. We were unable to definitively identify the organism in our case, but Crossiella equi is usually cited as the causative agent. The characteristic location of the lesion near the uterine bifurcation (far from the cervix) is unusual for bacterial placentitis and, when seen, the practitioner should strongly consider nocardial infection. Nocardiform infection is limited to the placenta with sparing of the fetus, and the placenta must be included in the submission for a satisfactory diagnosis. Even though postpartum placentas are frequently contaminated, the practitioner should swab the lesion and submit it for bacterial culture with the specific request that the lab look for nocardia-like organisms.

The entire article with references can be viewed on our web page.
Haemonchus contortus in sheep and goats: An insidious killer

By Rose Paddock, Class of 2012

Edited and illustrated by Dr. Peg Miller, ADDL Pathologist

Haemonchus contortus causes decreased productivity and death in sheep and goats. Most animals succumb in the spring, when larvae emerge from hypobiosis. The periparturient rise in egg shedding contaminates the pasture just when young animals are most susceptible. Haemonchus contortus eggs hatch in the feces and become infective larvae in as little as 3-4 days. Common clinical signs of haemonchosis, failure to thrive and weight loss, may be accompanied by anemia, hypoprothrombinemia, and submandibular edema. However, diarrhea seldom develops, and animals may die “suddenly without overt illness.” Regular assessment of ocular mucous membrane pallor by the FAMACHA system is useful to monitor animals for anemia. Haemonchus eggs can be detected, but not distinguished from those of other strongyloids, by fecal flotation. A recently developed fluorescent-labeled peanut agglutinin test for rapid identification and speciation of Haemonchus eggs may obviate the need for larval culture. Diagnosis is usually straightforward at necropsy. The carcass is pale. Edema may be most striking in submandibular subcutis, producing the so-called “bottle jaw.” The abomasum has red-brown contents with the “barber-pole” worms. Control of haemonchosis entails early diagnosis, pasture management, strategic de-worming, and avoiding anthelmintic resistance. Larval antigen is a target for vaccine development studies. Genetic selection is also promising as certain familial lines have heritable resistance to parasitism.

The entire article can be seen on our website www.addl.purdue.edu

Articles can be faxed or mailed to you at your request.
Immunohistochemistry (IHC) uses immunologic and histologic techniques to detect antigens in tissues. The antigen is recognized by a specific antibody that is added to the section. The immunologic reaction is visualized under the microscope by adding an enzyme, a substrate to the enzyme and a chromogen, producing a colored reaction. IHC is a very sensitive and specific technique. Advantages of IHC in diagnostics include: 1) Retrospective and prospective studies can be done on a variety of samples, 2) Antigen detection can be correlated with morphologic changes, 3) Stained slides can be stored for a long time, 4) Routine fixation and processing of samples is acceptable, 5) Tissues in paraffin blocks can be stored for years and still be suitable for immunohistochemistry.

Uses of IHC in veterinary diagnostics
Neoplastic and infectious diseases are the main focus of IHC in veterinary medicine. The Purdue ADDL IHC service offers a variety of tests for both infectious and neoplastic diseases of a variety of animal species. Please contact the ADDL for current tests available and fees or check the tests offered online (http://www.addl.purdue.edu/SampleSubmission/Pathology.aspx). Following are several examples in which IHC has practical application.

1. **Diagnosis of neoplasia.** Often, the tissue origin of a tumor cannot be determined with routine histology. Using specific antibodies for different tissues or cells (e.g., cytokeratin for epithelium, vimentin for mesenchymal cells, lymphoid markers, etc.), the origin of many tumors can be determined with IHC. Immunohistochemistry is commonly requested for lymphoma (B vs T cells), histiocytic proliferations (e.g., histiocytic sarcoma), endocrine neoplasms, and undifferentiated neoplasms.

2. **Detection of micrometastases.** Early metastasis can be difficult to detect using conventional histology. IHC highlights the presence of single or small groups of neoplastic cells in metastatic sites. Early detection of micrometastases increases the chances of survival with surgical removal of affected nodes or by modification of the treatment protocol.

3. **Prognostic markers.** Some proteins are expressed in neoplastic, but not in normal mature cells (e.g., embryonal proteins), expressed in neoplastic cells in larger amounts than in normal cells (e.g. cycle-related proteins), or structurally modified in neoplastic cells (mutant p53 protein). These changes may have prognostic significance in certain tumor types. For instance, the immunohistochemical detection of KIT protein in mast cell tumors of dogs has prognostic significance.

4. **Diagnosis of infectious diseases.** Detection of antigens of an infectious agent with IHC has etiologic significance. The advantage of IHC over microbiologic techniques is that antigen detection can be correlated with histopathologic changes and thus can confirm the significance of a particular bacterial or viral isolate obtained by other methods. Importantly, IHC does not require fresh or frozen tissues because it uses the same procedure as routine histology (formalin-fixed, paraffin-embedded tissues).

**How to submit samples for immunohistochemical testing**
We test samples that have been fixed in formalin, so you do not have to do anything special. Just submit the sample as you would for routine histopathology. Please, do not hold fixed samples in your office longer than 2 days, as prolonged fixation may destroy antigens. As soon as you place your sample in formalin, send it to ADDL. You are welcome to request specific tests that are available at ADDL. If not available at Purdue ADDL, we will try to find another laboratory performing that test. Alternatively, the diagnostician will suggest which test(s) are more appropriate after examining the HE sections on a given case. Currently, IHC tests are not included in the regular histopathology fee (IHC tests will accrue an additional cost), so we will contact you before performing IHC.

**Interpretation of results**
Immunohistochemistry facilitates diagnosis of infections and determination of the histogenesis and prognosis of neoplasms. Immunohistochemical results should be interpreted by the diagnostician provided that he/she has all the information pertaining to the case. For IHC, the diagnostician will send you a report indicating Positive (detected) or Negative (not detected) for a given marker (antigen) and, if pertinent, the percentage of positive cells and/or the antigen localization. Whether this result is significant must be interpreted in the context of the case, as is true for other diagnostic techniques. A careful assessment of the clinical history, lesions and all test results should be made before formulating a definitive diagnosis. Conversely, a negative result by immunohistochemistry does not eliminate the possibility of presence of a particular infectious agent/protein or its potential significance to the case. Sometimes infectious agents in subacute or chronic infections are in too low concentration to be detected by IHC. Due to mutations or other mechanisms, neoplastic cells may modify (upregulate/downregulate) the expression of proteins resulting in unexpected results.

In summary, immunohistochemistry is a valuable technique for the diagnosis of infectious and neoplastic diseases of animals. It is sensitive, specific, economical and relatively easy to perform. Although not always considered the “gold standard”, it can be as specific as bacterial and virus isolation, provided adequate controls are used.

**References**
ADDL Schedule
Purdue ADDL and Heeke ADDL will be closed on the following University holidays in 2011.
November 24-25.........................Thanksgiving
December 23-26..............................Christmas
December 30-January 2, 2012..............New Year

ADDL Lab Results by
Email (call ADDL with your email address)
Fax
Internet/Web
Laboratory results are available on the Internet. Call to set up an account or go to our web page (addl.purdue.edu), then
1. Click on Online Reports tab
2. Click on Request Info and follow instructions.

Reduced UPS shipping rates for ADDL clients
• ADDL has reached an agreement with UPS for submitters to send samples to the West Lafayette Lab at a reduced rate using its Authorized Return Service. Packages will arrive at ADDL the following morning.
• Pre-addressed labels will be provided to you by ADDL.
• Submitter will be billed $7.00 per package.
• Call us at 765-494-7440 or visit our website at www.addl.purdue.edu to request labels.
• If multiple cases are submitted in a single shipment, the UPS charge will be added to one case.

GlobalVetLink is now available for electronically requesting and reporting Coggins tests (Equine Infectious Anemia): ELISA and AGID at 8.50/test with no accession fee. In order to have access to a Global VetLink account, contact the company directly at www.globalvetlink.com or phone 515-296-0860.

If you are currently using our histopathology mailers (via U.S. mail) and would prefer taking advantage of the UPS option with its guaranteed delivery time, we will provide you the formalin-filled jars without an address label at $15.00/box of 12.