

Diagnostic Forum

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

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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..... \$2.00
 Abortion panel..... \$135.00

Bacteriology
 Aerobic enrichment..... \$5.00
 Milk culture, aerobic.....\$25.00
 Volume discount > 10 samples...\$10.00
 Mycoplasma culture.....\$25.00
 Salmonella series (up to 5).....\$100.00

Molecular Diagnostics
 Brucella real time..... \$25.00
 Multiplex C3B.....\$50.00
 Multiplex LTN real time.....\$50.00
 Johnes.....\$25.00
 Mycoplasma bovis.....\$25.00
 Novel N1 Subtype real time.....\$35.00

Serology
 Anaplasmosis ELISA.....\$8.50
 Volume discount >45 samples.....\$5.00
 Bluetongue ELISA.....\$6.00
 Volume discount >45 samples.....\$5.50
 Bovine leukosis virus ELISA.....\$7.00
 Volume discount >45 samples.....\$5.50
 CAE ELISA.....\$8.00
 Volume discount >45 samples.....\$4.50
 H1N1 Pfizer HI.....\$3.00
 H3N2 Pfizer HI.....\$3.00
 Neospora ELISA.....\$10.00
 Volume discount >45 samples.....\$5.00

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 AAFLD. 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



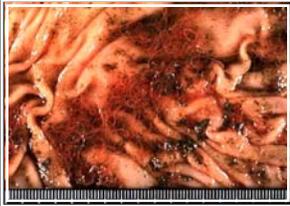
Abstract

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.



Weanling Suffolk lamb, found dead without observed clinical signs. Note tissue pallor, translucent water blood, and submandibular subcutaneous edema ("bottle jaw").



Opened abomasums with pale mucosa, scanty red-brown content, and tangles of the barber-pole worm, *Haemonchus contortus*



Drs. Katharine (Katie) Horzmann (left) and Virginia Reynolds (right) have joined the CPB department and ADDL as first year anatomic pathology residents/graduate students. Dr. Horzmann received the BS degree in Animal Science, Masters of Public Health, and DVM degrees from the University of Missouri in 2007, 2010, and 2011, respectively. Included in her education was an internship at the Missouri Department of Agriculture, elective clinical rotations in lab animal medicine and food animal/theriogenology, a Select Sires Artificial Insemination internship (Missouri and South Dakota), an exotic nutrition internship at the St. Louis Zoo, and a research experience in the Veterinary Research Scholars program. She enjoys fencing, hiking, training pigs to run on a treadmill, and international travel. Her career goals beyond the residency include doctoral research and an academic position in pathology.

Dr. Reynolds graduated from the University of San Diego with a BA in Environmental Studies. She received her DVM from Texas A&M in 2009. During her education, she melded her interests in the environment and veterinary medicine through studies abroad in Australia, Mexico, and South Africa, as well as the southeastern U.S.. She has two years of practice in a rotating small animal internship in Greenwich, RI, and as an emergency veterinarian in Round Rock, TX. While practicing in Texas, she spent one day/week in the fall semester of 2010 participating in histopathology rounds and necropsy rotations with TAMU pathologists and residents. In her "spare" time, she trained for and ran her first (and last, so she says) marathon. Dr. Reynolds plans to pursue a PhD degree and has a special interest in infectious diseases and their impact on the environment.

Haemonchus contortus in sheep and goats: An insidious killer

By Rose Paddock, Class of 2012

Edited and illustrated by Dr. Peg Miller, ADDL Pathologist

Haemonchosis 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, hypoproteinemia, 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 strongylids, by fecal flotation. A recently developed fluorescein-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.

Yew Plant Toxicity

by Dr. Christina Wilson, Head of Toxicology/Analytical Chemistry and Kimberly Meyerholtz, Assistant Chemist

Yews (*Taxus* spp.) are evergreen plants commonly used for ornamental landscaping throughout the Midwest. The most common varieties of this plant that are found in Indiana include English yew (*Taxus baccata*), Japanese Yew (*Taxus cuspidata*) and Canada Yew (*Taxus canadensis*). These plants can be highly toxic and have been implicated in numerous animal and livestock poisonings. In the majority of the clinical cases reported, yew poisoning is frequently due to accidental exposure as a result of animals being unwittingly fed clippings from yew bushes. In a 500 pound animal, it may take as little as 0.5 pounds of yew clippings to be potentially fatal.

The poisonous nature of the yew plant is attributed to the presence of cardiotoxic taxine alkaloids, which are present in the leaves, bark, and seeds of the plant. Toxic amounts of these alkaloids remain in the plant throughout the year and are not significantly degraded or decreased by drying. These toxins cause cardiac dysrhythmias and a rapid onset of clinical signs. Clinical signs in animals can vary depending on the amount of yew ingested. However, in most cases of yew poisoning, animals are often found dead in 24 hours or less after ingestion without showing adverse clinical signs of toxicity. Other clinical signs that may be observed can include ataxia, bradycardia, dyspnea, muscle tremors, recumbency, and convulsions leading to collapse and death.

Diagnosis of yew poisoning is primarily based on history of exposure or macroscopic identification of yew plant fragments in the stomach, rumen, or other parts of the digestive tract. The Toxicology Section of the Indiana Animal Disease Diagnostic Laboratory handles several cases of yew poisoning each year. In most of these cases, livestock deaths could have been prevented had the animals not been given access to the plant. Therefore, ensuring that animals and livestock do not have access to yew clippings or plants is vital.

References

1. Alden CL, Fosnaugh CJ et al:1977. Japanese yew poisoning in large domestic animals in the Midwest. JAVMA 170(3) 314-316.
2. Bryan-Brown T:1932. The Pharmacological Actions of Taxine. OJ Pharm and Pharmacol 5:205-19.
3. Casteel SW, Cook WO:1985. Japanese yew poisoning in ruminants. Mod Vet Practice 66:875-76.
4. Evers RA, Link RP:1972. Yews, *Taxus* species. IN: Poisonous Plants of the Midwest and Their Effects on Livestock. College of Agriculture, University of Illinois at Urbana-Champaign, IL. pp 81-82.
5. Kingsbury JM:1964. Taxaceae. In: *Poisonous Plants of the United States and Canada*. Prentice-Hall Inc., Englewood Cliffs,NJ. pp121-23.
6. Suffness M: 1995. Taxol:Science and Applications. CRC Press. Boca Raton, FL. pp 7-8, 311-12.
7. Tyler VE:1960. Note on the occurrence of taxine in *Taxus brevifolia*. J Am Pharm Assoc 683-84.
8. United States Department of Agriculture, Natural Resources Conservation Service, <http://plants.usda.gov>
9. Veatch JK, Reid FM, Kennedy GA: 1988. Differentiating yew poisoning from other toxicoses. Vet Med 83:298-300.
10. Watt JM, Breyer-Brandwijk MG: 1962. Taxaceae. In: *The Medicinal and Poisoning Plants of Southern and Eastern Africa*. E. & S. Livingstone, Edinburgh. Pp 1019-22.
11. Wilson CR and Hooser SB:2007. *Taxus* Poisoning. In: *Veterinary Toxicology: Basic and Clinical Principles*. Ed. R. Gupta. Elsevier, Inc., San Diego, CA. pp 929-35.



Immunohistochemistry in a Diagnostic Setting

By Dr. Jose Ramos-Vara, ADDL Pathologist, Head of Histology

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 etiological 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

- Ramos-Vara JA: 2005. Technical aspects of immunohistochemistry. *Veterinary Pathology* 42:409-430.
- Ramos-Vara JA et al: 2008. Suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories. *Journal of Veterinary Diagnostic Investigation* 20:393-413.

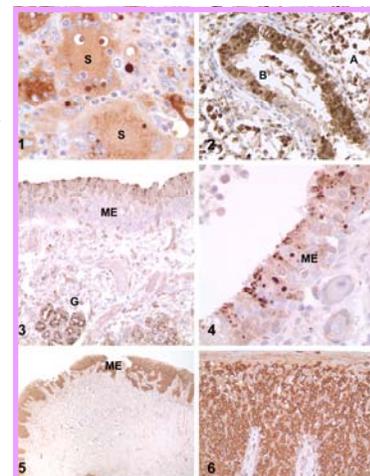


Fig. 1. Lymph node, pig. Porcine circovirus 2 infection. The antigen (brown color) is in the cytoplasm of syncytial (S) cells and histiocytes.
 Fig. 2. Lung, equine fetus. Equine herpesvirus 1 infection. Intranuclear and intracytoplasmic labeling (brown color) of numerous cells of the bronchiolar (B) epithelium and alveolar (A) cells and macrophages.
 Fig. 3. Nasal mucosa, dog. Canine distemper virus (CDV) infection. Numerous mucosal epithelial cells (ME) and the epithelium of the nasal glands (G) have CDV antigen (brown stain).
 Fig. 4. Nasal mucosa, dog. Canine distemper virus (CDV) infection. Detail of CDV antigen in mucosal epithelial cells (ME).
 Fig. 5. Lip, dog. Mycosis fungoides (epitheliotropic T-cell lymphoma). Neoplastic lymphocytes in the mucosal epithelium contain abundant CD3 antigen. CD3 is the marker for T lymphocytes.
 Fig. 6. Lip, dog. Mycosis fungoides (epitheliotropic T-cell lymphoma).. Detail of the mucosal epithelium infiltrated by neoplastic CD3-positive lymphocytes.

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.

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.

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.

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