PURDUE UNIVERSITY

DIAGNOSTIC FORUM

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Volume 20 No. 3
Summer 2010

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

Hot Topics

- **New fees for some tests on July 1, 2010.**
- **Abortion diagnostic screen now available. Details on our website**
- **GlobalVetLink for Coggins testing now available. See p. 4**
- **UPS for overnight sample submission. See p. 4**
- **Developing a new PCR test for *Mycoplasma bovis*. More information to follow.**

Thanks for your feedback? In response to requests from Indiana veterinarians:

- Global VetLink for Coggins testing instituted
- UPS labels for fixed-cost delivery to ADDL available
- New and improved tests
- ADDL personnel continue to work hard and pass proficiency testing
- Would you like the ease of having a screen for small animal diarrhea? If so, please let us know at addl@purdue.edu

Inside Diagnostic Forum

Focus on Bacteriology

Bacteriology/Mycology technicians are responsible for isolating and identifying bacterial and/or fungal agents and providing sensitivity panels to submitters. Supervised by Dr. Ching Ching Wu who has been Head of Bacteriology/ Mycology at ADDL for 17 years, lab technicians are

- Bonnie Vera, 23 years at ADDL
- Sharon Young, 23 years
- Ron Gillespie, 10 years
- Nathan Simpson, 9 years
- Liu-ying Ni, 6 years
- Catherine Harlan, 9 months
- Samantha Wall, 5 months
- Manny Benitez, student staff
- *Not pictured*, Renu Bajaj, 9 years

Seated, left to right
Ching Ching Wu
Samantha Wall
Bonnie Vera

Standing, left to right
Katie Harlan
Nathan Simpson
Liu-ying Ni
Ron Gillespie
Sharon Young
Manny Benitez

Chronic Diarrhea in Small Animals

Diagnostic Profiles

Chronic diarrhea is a common presenting complaint for small animal patients. The causes include infectious etiologies as well as noninfectious conditions involving dietary factors, idiopathic inflammatory enteropathies and neoplasia. Initial diagnostic efforts should differentiate between small intestinal and large intestine disease and rule out extra-intestinal diseases. Physical examination and clinical signs generally can distinguish small bowel from large bowel diarrhea. Baseline data from a complete blood count, serum chemistry panel and urinalysis are used to identify underlying extra-intestinal disease. Primary diseases in other organ systems that can result in diarrhea to be ruled out include liver failure, renal failure, and endocrinopathies such as hyperthyroidism in cats, hypoadrenocorticism and exocrine pancreatic insufficienty. Ruling out thyroid, adrenal or pancreatic disease would require thyroid panel, ACTH stimulation test and serum trypsin-like immunoreactivity (TLI) tests, respectively.

A fecal examination is most valuable for identification of parasitic agents. Microscopic examination of serial fecal samples concentrated by flotation can identify nematode eggs, oocysts of coccidian and cryptosporidia, as well as the trophozoites of *Giardia* sp. and *Tritrichomonas* sp. Giardia is more readily detected when zinc sulfate flotation is used. Both *Giardia* and *Cryptosporidia* can be detected by more sensitive methods such as ELISA, PCR and immunofluorescent antibody staining.

Fecal culture for bacterial agents is often part of the diagnostic approach to diarrhea. The bacteria most often associated with diarrhea in dogs include *Clostridium perfringens, Cl difficile, Campylobacter* sp., pathogenic *E. coli* and *Salmonella* sp. Isolation of these organisms from clinically normal animals is not uncommon and so, interpretation of laboratory findings is not without controversy. These organisms can be demonstrated by fecal culture as well as by more sensitive techniques. *Cl. perfringens* enterotoxin (CPE), a specific toxin associated with canine disease, can be demonstrated by ELISA tests and the toxin associated genes can be demonstrated by PCR testing. As with positive culture for *Cl. perfringens*, CPE can also be demonstrated in dogs without diarrhea. The pathogenicity of *E. coli* isolates requires molecular testing by PCR. Many questions remain concerning bacteria-associated diarrhea and the interpretation of laboratory demonstration of these organisms and their toxins.

Cytologic examination of stained rectal or colonic mucosal scrapings can be used to determine if leukocytes are present, indicating an inflammatory or infectious etiology, and to identify etiologic agents. This is especially useful for cases of large bowel diarrhea.

Determination of serum folate and cobalamin concentrations can be used to localize intestinal damage to either the proximal or distal small intestine. Low serum folate suggests a proximal lesion and is sometimes associated with dietary sensitivity. Low levels of cobalamin suggest a distal lesion and have been associated with small intestinal bacterial overgrowth (antibiotic responsive diarrhea), as well as exocrine pancreatic insufficiency. Increased folate levels also suggest bacterial overgrowth. Decreased levels of both folate and cobalamin suggest a diffuse lesion. In cats with intestinal disease, determination of serum cobalamin concentrations is particularly important in that response to therapy is optimized only when cobalamin status is corrected. Generally, folate and cobalamin tests are combined with TLI testing as a panel.

Analysis of feces for α1-proteinase inhibitor is rarely used in suspected cases of protein losing enteropathy. This serum protein can leak into the intestinal tract but is resistant to digestion and can be detected in feces. This is a specialized test which has limited availability and is technically difficult to perform.

The final and most invasive diagnostic technique for chronic diarrhea is intestine biopsy, obtained either by endoscopy, laparoscopy or laparotomy. Endoscopic biopsy is the least invasive technique and allows multiple biopsies, but its use limits sampling to the stomach, duodenum distal ileum (dogs only), and colon and only mucosal specimens can be obtained for histologic examination. Full thickness biopsies from any segment of the gastrointestinal tract can be obtained by laparoscopy and laparotomy. Full thickness biopsy specimens allow diagnosis of conditions where characteristic changes are most evident in the deep aspect of the lamina propria, submucosa, or even the tunica muscularis. This often includes gastrointestinal neoplasia. Biopsy may allow visualization of etiologic agents such as fungi and parasites or characteristic histologic changes that are diagnostic, but the technique has limitations. Some gastrointestinal diseases have similar histologic changes and cannot be differentiated on the basis of pathology alone. Final diagnosis may be dependent upon interpretation of a combination of clinical impressions and laboratory results as well as response to therapy.

New PRRS Serology Test

The ADDL Serology Laboratory, supervised by Dr. Roman Pogranichniy, is pleased to offer a new serological PRRSV test from IDEXX (HerdCheck*X3 Antibody ELISA). This test is available beginning July 6, 2010 at a cost of $5.00/sample and is Designed to detect PRRS antibodies in serum or Plasma with quick turnaround time.

Call us at 765-494-7440 if you have questions.

ADDL Schedule

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

September 6.....	Labor Day
November 25-26.....	
Thanksgiving	
December 23-24.....	Christmas
December 30-31.....	New Year

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. Call us at 765-494-7440 or log on to our website www.addl.purdue.edu to request labels.

Submitter will be billed $6.00/package for shipments up to 15 pounds. If cases from multiple owners are submitted in the same shipment, the $6.00 charge will be added to one case.

If you currently use 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 jar without an address label for $15.00/box of 12.

Global VetLink Now Available

In ADDL, we have added new reporting capability of test results for Equine Infectious Anemia Virus (Coggins test: ELISA and AGID - $8.50/sample with no accession fee). These tests can be requested and reported via Global VetLink. In order to have access to a Global VetLink account, contact the company directly at

info@globalvetlink.com or
www.globalvetlink.com or
515-296-0860

ADDL lab results are available by……

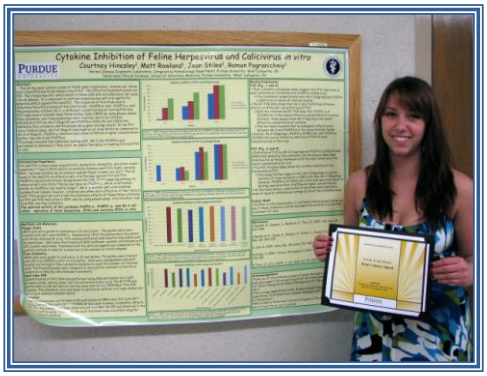
-Email (call ADDL with email address)

-Fax

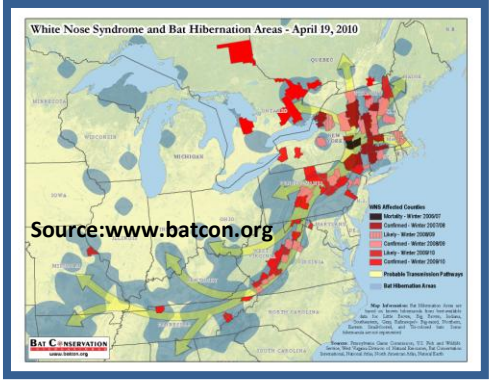
-Internet/Web (Call us to set up an account or go to our web page addl.purdue.edu, click on Online reports, then "Request Info" and follow the instructions.

RECOGNITION

Former ADDL Anatomic Pathology Graduate Student, Dr. Josh Webster, was presented the Applied Research Award at the recent Purdue Phi Zeta Day and has been nominated for the national award in Applied Research. His paper "Effects of formalin fixation on diagnostic immunohistochemistry in domestic animals" was published in the Journal of Histochemistry and Cytochemistry Vol 57:753-761, 2009



In collaboration with ADDL Head of Virology/Serology Dr. Roman Pogranichniy, Dr. Jean Stiles (Veterinary Clinical Sciences, and Matt Rowland (University of Michigan), **Courtney Hinesley,** Purdue School of Agriculture, won the Dean's Choice Award for her project "Cytokine Inhibition of Feline Herpesvirus and Calicivirus *in vitro.* Courtney presented her poster at the Undergraduate Research and Poster Symposium on April 7, 2010.



Indiana's Endangered Bat, *Myotis sodalis*, Threatened by White-Nose Syndrome

In February 2006, White-nose Syndrome (WNS) was first documented at Howe's Cave, New York. White-nose syndrome is a lethal dermatophytic disease of hibernating bats associated with *Geomyces destructans sp. nov.* As of March, 2010, WNS has been confirmed in caves and mines throughout the eastern United States, including Alabama, Connecticut, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Tennessee, Vermont, Virginia, and West Virginia. More than a million bats of six different species have perished from this deadly disease with mortality rates approaching 100% at many sites. In March, biologists in Ontario reported the first detected case of WNS in Canada. White-nose syndrome continues to spread rapidly across North America, seriously threatening the remaining colonies of hibernating bats.

Geomyces destructans is a novel *Geomyces* fungal species. Geomyces sp. are slow-growing, opportunistic fungi found primarily in the soil of colder regions worldwide. The fungus produces hyphae and arthroconidia. Unlike conidia of other species, *Geomyces destructans* are asymmetrically curved. Optimal growth of *G. destructans* occurs at temperatures found in winter bat hibernacula. The body temperature of bats in torpor (temporary hibernation) drops to within a few degrees of the ambient temperature of their hibernaculum, usually 36° to 50°F. During torpor, the bat's metabolic rate drops by approximately 96% and the immune response is down-regulated, providing ideal conditions for growth of *G. destructans.*

The primary route of transmission of WNS is bat to bat contact with fungal spores. Fungal spores of *G. destructans* originated from European caves. These spores were inadvertently spread to caves and mines in the United States by fomites, the contaminated equipment and clothing of cavers.

The classical presentation of WNS in hibernating bats includes profuse white delicate hyphae and conidia covering the surface of the muzzle, wing membranes and/or the pinnae. Animals suffering from WNS emerge prematurely from their hibernaculum, are emaciated with depletion of fat reserves, and wings are damaged or scarred due to fungal invasion.

Visible fungus on the skin of bats is often not observed once the bat emerges from the hibernaculum making gross disease difficult to detect. Definitive diagnosis of WNS requires histopathology with preferred tissue samples from the rostral muzzle, nose and wing membrane.

Histologically, affected tissues have extensive fungal invasion of the dermis with an absence of inflammatory response. The diameter and shape of hyphae vary. Hyphae can have parallel or non-parallel walls measuring 2-5µm. Curve-shaped conidia measuring 2.5µm wide and 7.5 µm in length have uni- or bi-lateral blunted ends, and a basophilic center. Fungal hyphae fill hair follicles and sebaceous and aprocrine glands of the muzzle with invasion of underlying tissues. In the wing membrane and pinna, fungal hyphae are associated with epidermal erosions and ulcers which extend into underlying connective tissue. Bats collected shortly after hibernation also have small quiescent packets of fungal hyphae within the dermis surrounded by a thin layer of acellular material.

Currently, surveillance of White-nose Syndrome is being conducted by the United States Geological Survey National Wildlife Health Center (Madison, WI) and the United States Fish and Wildlife Service with the collaboration of State wildlife specialists and biologists.

Federal agencies recommend adherence to cave advisories and closures to help prevent the transmission of WNS. The U.S. Fish and Wildlife Services outline decontamination protocols to be used before entering a cave. Updated protocols can be found at http://www.fws.gov/northeast/wnsresearchmonitoring.html
Protocols for bat submission can be found at http://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/USGS_NWHC_Bat_WNS_submission_protocol.pdf
For the most current information on White-nose Syndrome, visit the websites of the United States Geological survey National Wildlife Health Center at http://www.nwhc.usgs.gov or Bat Conservation International www.batcon.org/wns

-by Hillary Hart, Clinical Year Student
-edited by Dr. Abby Durkes, ADDL Graduate Student

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