Diagnostic Forum

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

In this issue:

www.addl.purdue.edu

765-494-7440

From the Director…….

The most important part of any laboratory is its staff. The ADDL is fortunate to have an energetic and knowledgeable staff who are dedicated to helping veterinarians and animal owners throughout the state. In this issue we celebrate the awarding of the PVM Outstanding Staff Award to Karen Crane, the Serology Section Laboratory Supervisor.

In October of 1978 Karen first started at the ADDL numbering blood samples. From filing on index cards to the front desk to Virology to Serology, Karen has had a profound impact on the day to day workings of the ADDL. She has always placed the clients needs first and foremost. Her ability to communicate with such a diverse group of individuals from students, staff, and veterinarians, to farmers and state officials is truly a gift. Karen is always ready to engage in conversation and is always looking for ways to improve the ADDL as a whole. Her dedication to the Indiana ADDL is second to none!

Many thanks to Karen for her devoted service to the veterinarians and animal owners of Indiana!

Feline Infectious Peritonitis: A Confusing Diagnosis

By Alexandra Myers Class of 2015

Edited by Dr. Margaret Miller

Abstract

Feline infectious peritonitis (FIP) is a dreaded and deadly viral illness of young cats. The disease is complex and still poorly understood which has made diagnostics and treatment a veterinarian's nightmare since the discovery of the disease in the mid-1900s. Researchers are still actively searching for the exact mutation or mutations that allow the relatively benign feline enteric coronavirus (FECV) to become so virulent so quickly. Once the mutation is found, it should be much easier to test for the virulent strain and to develop an effective vaccine. Until then, veterinarians are left with several confusing diagnostic tests, none of which are totally accurate nor reliable. Currently, when a veterinarian can collect an effusion or lymph node aspirate, the fluorescent antibody test for FIP is one of the best, least invasive ways to obtain a convincing diagnosis. When a biopsy can be obtained or post-mortem examination performed, immunohistochemistry is considered to be the most definitive test. Having a firm diagnosis allows the veterinarian to feel more comfortable when giving owners the bad news about this difficult disease.

Read the complete article at: https://www.addl.purdue.edu/

PEDv Pooling Now Available at the ADDL

After analyzing the data from our recent PEDv Pooling Study, pooling of up to 5 fecal samples from a clinically affected swine herd for PEDv PCR has been approved in the ADDL.

When submitting samples for PEDv, please indicate on your submission form if you wish to have fecal samples pooled or tested individually. All samples will be tested individually unless pooling is requested.

Samples from pools testing Positive or Suspect must be tested individually to identify Positive or Suspect animals.

The cost for PEDv PCR is $30.00 plus a $10.00 accession fee.

Swabs will be tested individually only, as pooling may result in false negative results.

Pooling is not available for PDCoV at this time.

Please call the ADDL at 765-494-7440 if you have any questions.

Immunohistochemistry at ADDL

By Dr. José A. Ramos-Vara, and Dee DuSold

Immunohistochemistry (IHC) is a diagnostic technique that entails the detection of a target antigen (usually a protein) on tissue sections using antibodies. The tissue antigen is recognized by a specific antibody added to the tissue section. The antigen-antibody reaction is then visualized using a histochemical (enzyme-substrate-chromogen) reaction. The visualization of the antigen in a particular tissue or cell is the basis for diagnoses that are not possible with routine histopathology.

Uses of IHC in veterinary medicine:

1. Diagnosis of neoplasia. The use of IHC in neoplasms is based on the unique antigens expressed by different types of neoplastic cells and by their nonneoplastic cells of origin. Exploiting this feature, diagnosticians can classify leukocytic tumors (e.g., histiocytic sarcoma, lymphoma, mast cell tumor). Tumors that are morphologically similar (tumor mimics) but have a very different prognosis (e.g., epitheliotropic lymphoma and cutaneous histiocytoma in dogs) can be distinguished with immunohistochemistry. The improved specificity in the diagnosis of neoplasia with IHC can improve cancer treatment. Unfortunately, the use of only one IHC test is not helpful in many cases due to "antigen sharing" among different cell types/tumors. Therefore, a panel of IHC tests is typically required for definitive diagnosis.

2. Diagnosis of carcinomas of unknown primary site (CUPS). One of the main challenges a diagnostician faces is the evaluation of tumors simultaneously present in several organs, making determination of the primary site difficult with routine histopathology, particularly with biopsy samples. IHC may aid in the determination of the primary site.

3. *Prognosis and theranostics*[1]. Although the use of IHC for these purposes is well established for many human tumors, advances have also been made in small animal oncology. Examples are prognostic markers used for canine melanoma, mast cell tumor, osteosarcoma, and mammary tumors. Proliferation index is the most common prognostic marker used in a wide variety of animal cancers. An example of biomarkers used in animal theranostics is KIT. This antigen has variable intracellular expression depending on the mast cell tumor grade, which is used to customize therapy for a given tumor.

4. *Diagnosis of infectious diseases*. IHC can identify microorganisms (e.g., virus, bacteria, protozoa, prions) in tissue sections. Although the use of IHC in infectious diseases has been largely replaced by more sensitive and specific techniques (e.g., PCR), the ADDL offers IHC tests for some microorganisms. IHC is less sensitive than PCR, but allows the co-localization of microorganisms in specific cell types or within a lesion, which is not possible with routine PCR methodologies.

Interpretation of results:

IHC results are part of the final diagnosis and only exceptionally considered a stand-alone technique. Therefore, a pathologist must evaluate not only the IHC sections but also the routine histopathology and any other ancillary tests in a given case. In other words, IHC is complementary to the other techniques available to a pathologist.

Immunohistochemistry at ADDL:

IHC has been offered at ADDL for more than 10 years. To search for available tests, visit (http://www.addl.purdue.edu/TestsFees/BySection.aspx). In most cases, IHC is performed after the pathologist reviews routine histopathology sections. IHC tests are not included in the standard pathology (necropsy, mail-in, biopsy) fee, so clients are contacted for permission to run these additional tests. Samples for IHC are processed in the same way as for routine histopathology so there are no special submission procedures other than prompt immersion of the sample in an adequate volume of formalin fixative.

The ADDL has recently purchased a new IHC stainer that has improved the turnaround time of results. Keep in mind that the list of available IHC tests may change over time depending on our clients' needs. Please contact the ADDL to confirm that the requested test is available. Your feedback is very much appreciated. Email us at addl@purdue.edu.

[1] The use of diagnostic tests in therapeutic decisions.

Purdue Veterinary Medicine Outstanding Staff Award

Congratulations to ***Karen Crane***, Serology Laboratory Supervisor on receiving the 2014 PVM Outstanding Staff Award!!

Dean Willie Reed and Karen

Turkey Coronavirus PCR Now Offered at ADDL

The ADDL can now diagnose the presence of Turkey Coronavirus (TCV) by PCR. Samples to submit from affected turkeys are cloacal swabs, intestinal tissues, or feces.

The cost for this test is $30.00 plus $10.00 accession fee.

Please call the ADDL at 765-494-7440 if you have any questions.

Porcine Delta Coronavirus PCR Now Offered at ADDL

The ADDL can now diagnose the presence of Porcine Delta Coronavirus (PDCoV) by PCR in any age pigs or environmental samples.

Samples to submit are:

- Jejunum and ileum, 6-8" segments, fresh (for PCR) and formalin-fixed

Spiral colon, fresh (for PCR – package separately from jejunum/ileum)
Other samples amenable to testing for PDCoV and PEDv include oral fluids, rectal swabs and feces/colonic contents (fresh chilled for PCR).

Please call the ADDL at 765-494-7440 if you have any questions.

For more information on Swine Enteric Coronavirus Diseases (SECD), including Porcine Epidemic Diarrhea virus (PEDv) and Porcine Delta Coronavirus (PDCoV) please see the USDA SECD site: http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/animalhealth and click on, "Porcine Epidemic Diarrhea Virus".

MALDI—TOF

For Rapid ID of Bacteria

By Dr. Kenitra Hammac

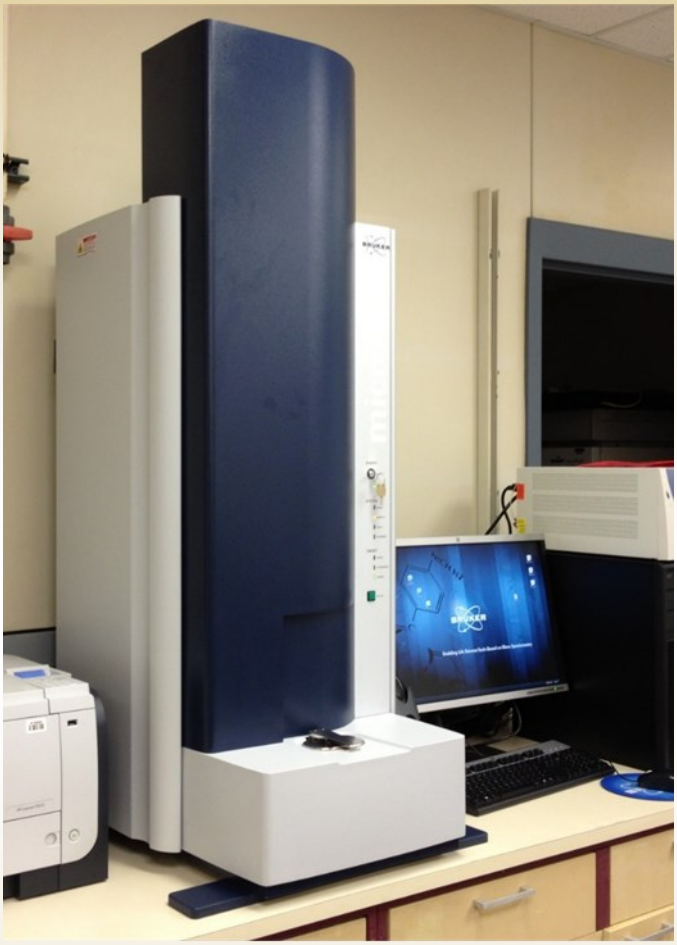
Bacteriology Section Head

As we approach the 1 year anniversary of the MALDI-TOF in the ADDL, we're excited to update you on its use. What's the MALDI-TOF? It is a mass spectrometer that uses a laser to blast proteins into small pieces that then travel through a vacuum tube with a detector waiting at the other end. The detector captures a spectrum which can be used to analyze the sample.

The bacteriology lab uses the MALDI-TOF to directly analyze bacterial colonies. A software program maps the spectra to a proprietary database, and a match results in identification of the organism. This entire process occurs within just a few minutes. Because of our quality standards, we perform in-lab verification prior to reporting results generated through new methods. During the past year, we have verified 27 bacterial identifications on the MALDI-TOF for reporting without further biochemical testing, and we are continually testing more bacterial species. One MALDI-TOF verification/methods-development study was presented by one of our bacteriology technicians, Manny Benitez, at the National AAVLD annual meeting this fall.

What does this mean for our clients? Because the MALDI-TOF can identify bacteria directly from a colony, we are often able to report results within 24 hours of receiving a sample in the lab. Compare that to using standard biochemical tests like you learned in school which at best can give you a result within 48 hours of the sample's arrival in the lab. Antimicrobial susceptibility testing requires an overnight incubation, so MIC results are usually available the day after the species identification. Faster results allow you to make management decisions sooner.

In the next issue we'll update you on what's happening with the MALDI-TOF in the toxicology lab.



The MALDI-TOF

Bacteriology fee updates for **milk cultures**:

Our testing protocols and fees have been restructured to provide better service to our clients. Please call the laboratory if you have any questions.

Aerobic Milk Culture Cultures with three or more isolates are reported as "contaminated" according to National Mastitis Council guidelines.	$12
Volume rate per sample for 20 or more samples:	$10
Mycoplasma Culture, milk	$10
Volume rate per sample for 20 or more samples:	$8
Aerobic Bulk Tank culture Quantitative aerobic culture + mycoplasma culture.	$40

Pesticide Poisoning: Methomyl Toxicity

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

Methomyl is a broad-spectrum carbamate insecticide sold as a restricted-use, crop insecticide or as a non-crop, "fly bait" insecticide for residential use. Methomyl is available over-the-counter and is sold under the trade names *Starbar Golden Malrin® Fly Bait*, *DeoSect™ II Fly Bait*, and *Stimukil® Fly Bait*. These products are formulated with 1% (10,000 ppm) methomyl and the granules have a characteristic turquoise blue color (see figure 1). These bait formulations are often scattered on the ground or mixed with a liquid (such as soda or other sweet liquid) to attract and kill flies in animal or poultry houses and to control insects on fruits and vegetables. Unfortunately, these products are illegally being promoted to control coyotes, raccoons, and opossums. As a result, accidental ingestion of this insecticide has resulted in acute poisoning in non-target species, such as dogs and cats. Over the past two years, the ADDL has observed an increase in cases of accidental methomyl poisoning in animals. These cases were due to animals unwittingly ingesting the poison when it was being used residentially to control insects near fruit trees or when the product was misused to control nuisance wildlife and rodents.

Methomyl inhibits acetylcholinesterase in nervous tissue causing acetylcholine to accumulate at the muscarinic and nicotinic receptors at neuromuscular junctions, ultimately resulting in overstimulation of the sympathetic and parasympathetic nervous system.[1] Onset of clinical signs may occur within 15 minutes to 1 hour post-exposure, depending on the dose. Muscarinic signs can include salivation, lacrimation, urination, defecation, dyspnea, and/or emesis (SLUDDE signs). Miosis and bradycardia may also be observed.[2] Nicotinic clinical signs are often associated with seizures, muscle fasciculations, tachycardia, ataxia and/or paralysis in exposed animals.[2] Death occurs due to respiratory failure.

Methomyl exposure in animals is diagnosed at the ADDL based on clinical signs/case history and diagnostic testing of biological samples from the animal. Methomyl can be detected in whole blood (submitted in EDTA or heparin), liver, stomach/rumen contents, vomitus, bait, or contaminated water or foodstuffs. Testing acetylcholinesterase activity in brain tissue or whole blood (submitted in EDTA or heparin) can also suggest exposure to methomyl (or other carbamate and organophosphate insecticides). Brain or blood acetylcholinesterase levels less than 25% of normal are considered diagnostic, while levels less than 50% are considered suspicious for exposure.[2] Caution must be taken when collecting samples for diagnostic testing as methomyl and other carbamate and organophosphate insecticides can be dermally absorbed.

Differential diagnoses to consider in these cases include other CNS stimulants such as carbamates/ organophosphates, metaldehyde, sodium monofluoroacetate, caffeine, antidepressant medications, chocolate, illicit drugs (cocaine, LSD, methamphetamine), lead, nicotine, 4-aminopyridine, strychnine, organochlorine insecticides, pyrethrins/ pyrethroids, tremorgenic mycotoxins, zinc phosphide, or systemic diseases.

Prevention is key for limiting exposure to methomyl or other insecticides. Ensure that animals and pets do not have access to the insecticide and make sure pet or livestock owners follow labeled instructions on the proper use of the product. Please feel free to contact the ADDL Toxicology Section if you have a suspect case or need a consult with regard to specimen submission or direction on diagnostic testing.

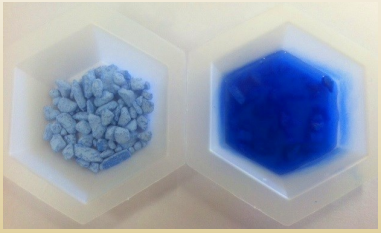


Figure 1.

Methomyl bait granules (left) and methomyl granules in water (right)

References

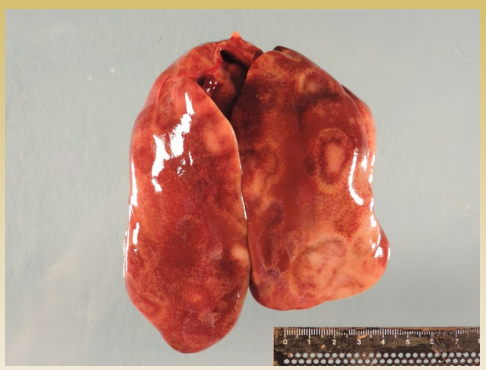
1. Gupta, RC (2012) Organophosphates and Carbamates. *In*: Veterinary Toxicology: Basic and Clinical Principles. R. Gupta (*editor*). Elsevier, Inc., San Diego, CA, pp. 573-585.

2. Gualtieri, J (2011) Organophosphate and Carbamate Insecticides. *In:* Blackwell's Five-Minute Veterinary Consult Clinical Companion Small Animal Toxicology. GD Osweiler, LR Hovda, AG Brutlag, JA Lee (*eds*). Wiley-Blackwell, Ames, IA, pp. 628-635.

Technical report: Histomoniasis (blackhead) in commercial and backyard poultry

By: Dr. Yuko Sato

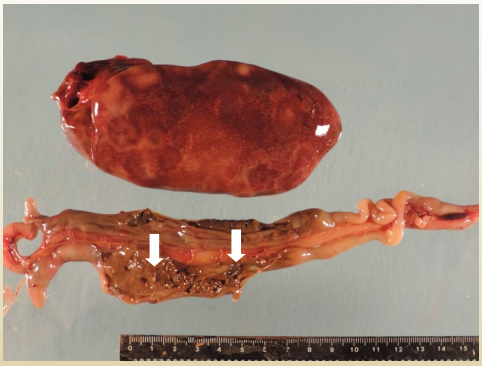
Edited by: Dr. Pat Wakenell

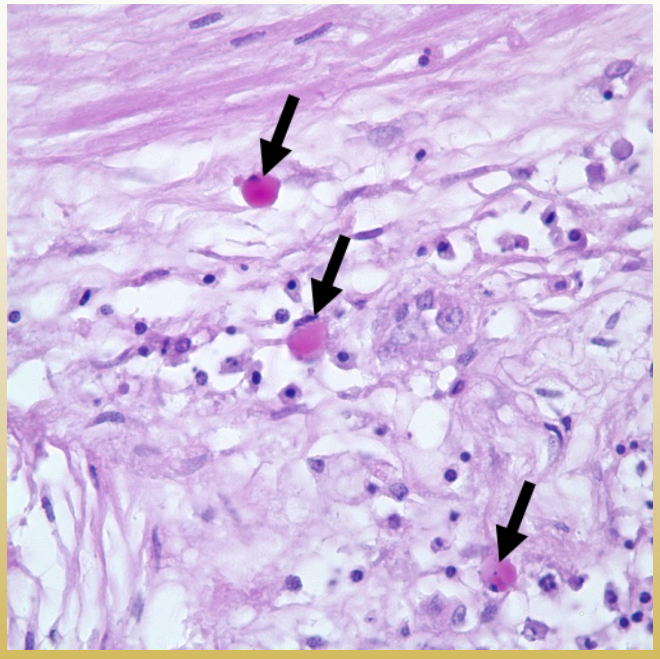


Multifocal to coalescing necrotic target lesions in the liver of a turkey with histomoniasis.

Histomoniasis (blackhead) is a protozoal disease caused by *Histomonas meleagridis* that primarily affects the liver and ceca of turkeys and other gallinaceous birds, including chickens, peafowl, and quail. Transmission of blackhead occurs by direct contact with infected birds or infected feces, as well as indirect infection via ingestion of infected cecal worms (*Heterakis gallinarum*) or earthworms that contain infected cecal worms. Clinical signs develop in 6-12 days, and occur most commonly at 11 days post-infection. They include listlessness, emaciation, unkempt feathers, and yellow, sulfur-colored droppings. The name, "blackhead" is somewhat of a misnomer, as typical presentations of diseased turkeys do not include cyanosis of the head. Gross lesions are characterized by pathognomonic, necrotic target lesions in the liver and caseous cecal cores. Histopathologic lesions are characterized by numerous round to ovoid, faintly eosinophilic to golden-brown PAS-positive bodies, ranging from 10-20 µm in diameter, surrounded by a halo, within macrophages or inflammatory cellular debris in H&E stained tissue sections. Since there are no chemotherapeutic products that are approved and available for treatment of histomoniasis in the U.S., control strategies are focused on prevention. Routine deworming, cleaning of premises, and practicing good biosecurity, such as avoiding multiple species of poultry in a flock (especially chickens and turkeys), are key measures for prevention and control.

Multiple pale whitish cecal worms (arrows) within a necrotic cecal lumen





PAS-positive histomonads (arrows) in the cecum; bar = 50 µm, 100x magnification, Periodic acid-Schiff (PAS) stain.

FOR THE ENTIRE ARTICLE, VISIT:

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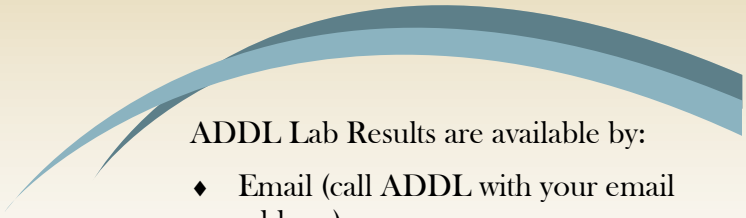
Questions? Comments? Concerns?

We value your opinion. Please contacts us at:

Phone 765-494-7440

Email ahighlan@purdue.edu



ADDL Lab Results are available by:

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

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