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A Quarterly Newsletter from the Indiana Animal Disease Diagnostic Laboratory
at Purdue University, West Lafayette, Indiana 47907 (765-494-7440)



From the Director

Stephen B. Hooser

With the rains, springtime has returned to Indiana and, in the seasonal spirit of renewal, we will welcome the accreditation team from the American Association of Veterinary Laboratory Diagnosticians for a site visit that will be part of our application to renew the accreditation of the ADDL. Every five years, the AAVLD re-evaluates all state veterinary diagnostic laboratories as part of its accreditation process. An important part of our preparation is the Laboratory Quality System that was instituted under the leadership of Dr. Thacker. This Quality System helps guide the laboratory in its quest for continual improvement in its services to Indiana. Another source to guide the laboratory will be the development of a five year Strategic Plan. We intend to develop this plan, in part, with input from clients of the ADDL from across the state. So, within the next few months, we will begin asking for your input on this plan. In the meantime, please let us know if you have any suggestions. In these difficult economic times, we can't always guarantee that we will be able to honor your requests, but we will always listen for ways to improve upon our mission to aid in the prevention, control, and eradication of animal diseases for the state of Indiana, to provide prompt and accurate diagnostic services, and to add to the wealth of the state by working with local, state, federal, and international partners to meet current and future needs.

- **Classical swine fever surveillance program – veterinarians can be paid \$50.00/sample for submitting tonsils from clinically ill swine.....see p. 2 for details**
- **Encephalitozoon real time PCR now available – see p. 2 for cost and preferred specimens**
- **Change in charge for EIA – see p. 2**
- **Sequence analysis of influenza A viral isolates now available - see p. 2**
- **Dr. Steve Lenz now coordinating all swine mail-in cases – see p. 2**
- **Charges instituted for decalcification of histopathology samples and evaluation of painted margins – see p. 2**

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Focus on... Serology

Supervised by Virology/Serology Section Head Dr. Roman Pogranichniy, the Serology lab technicians perform testing on serum samples for a wide variety of infectious agents for both diagnostic and regulatory purposes.

Serology laboratory staff:

- Karen Crane, Laboratory Supervisor, 30 years at ADDL
- Cheryl Chapple, Laboratory Records Clerk and Technician, 30 years at ADDL
- Alice Hardebeck, Technician, 9 years at ADDL
- Cheryl Parker, Technician, 31 years at ADDL
- Brenda Turner, Technician, 18 years at ADDL



Left front: Alice Hardebeck
Left center: Cheryl Parker
Left back: Cheryl Chapple

Right front: Dr. Roman Pogranichniy
Right center: Karen Crane
Right back: Brenda Turner

New Tests

1. Sequence analysis of influenza A viral isolates is now offered through the Virology Section at ADDL
 - Sequencing of influenza A virus hemagglutinin region and partial gene analysis is offered at a cost of **\$100/isolate**. Test is performed only on viral isolates
 - Full length analysis of the hemagglutinin gene is offered at **\$200/isolate**. Test is performed only on viral isolates
2. Real time PCR is now available for the detection of Encephalitozoon species in tissue samples. Samples may be fresh, frozen, fixed or paraffin-embedded (brain, kidney, lung, liver, placenta). The cost is \$25/sample.

Information on Charges

- The standard \$7.00 accessioning fee (to cover processing, IT, etc) is now being added to all cases submitted to the ADDL, including Serology cases.
- Sequencing of influenza A virus hemagglutinin region and partial gene analysis \$100.00/isolate
- Sequencing of influenza A virus full length analysis of the hemagglutinin gene \$200.00/isolate
- EIA \$3.00 + \$7.00 accessioning fee \$10.00/animal
(cost of EIA test reduced to partially offset the addition of accession fee)
- Encephalitozoon real time PCR \$25.00/sample
- Decalcification of samples submitted for histopathology additional \$17.00
- Evaluation of painted margins additional \$25.00

New Procedure for Swine Cases

In an effort to more fully meet the needs of Indiana's swine veterinarians, the ADDL has implemented the following change for swine cases. All swine mail-in cases that include a request for histopathology will be evaluated and coordinated by **Dr. Steve Lenz** who will be your point of contact. You can reach him at 765-494-7440.

Swine submitted for complete necropsy examination will be overseen by the senior pathologist on duty that day.

Classical Swine Fever

USDA/APHIS Veterinary Services, in cooperation with the Indiana Animal Disease Diagnostic Laboratory at Purdue University, and other network laboratories across the United States, has initiated a Classical Swine Fever surveillance program. The objective of this program is the rapid detection if CSF virus is introduced into U.S. swine. One of the specific tasks is testing tonsil samples submitted from sick pigs.

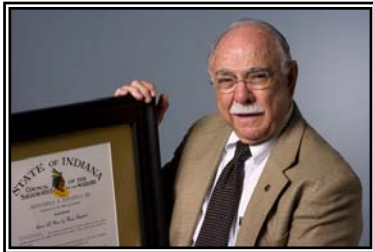
Veterinarians will be paid \$50.00 per eligible tonsil sample received at the ADDL for CSF testing.

Samples must be from clinically ill swine and shipped to ADDL cooled. **DO NOT FREEZE OR FIX SPECIMENS.** Please indicate on the accession form that the tonsils are being submitted for the CSF survey.



Leon Thacker Honored

Dr. Leon Thacker, former Director of the Purdue ADDL and current pathologist, annual Dr. Leon Thacker, former Director of the Indiana Animal Disease Laboratory at Purdue was the recipient of two coveted awards at the recent Indiana Veterinary Medical Association meeting in Indianapolis. The IVMA presented him their Veterinarian of the Year Award. Former Indiana State Senator Bob Jackman (pictured) presented him the Sagamore of the Wabash award on behalf of Governor Daniels. In addition, Dr. Thacker was presented the Distinguished Service Award by the American Association of Veterinary Laboratory Diagnosticians. Please join us in congratulating Dr. Thacker for these outstanding achievements.



Atypical Mycobacteriosis in Swine



Atypical mycobacteriosis is a chronic infectious disease caused by atypical mycobacteria (AM), which are mycobacteria other than those belonging to the *Mycobacterium tuberculosis* complex that includes *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti* and *M. canettii*. Additionally, AM usually do not include *M. leprae* and *M. avium* subsp *paratuberculosis*. AM are widely distributed in nature, and are potentially pathogenic to a variety of species. The main pathogenic AM belong to the *M. avium* complex (MAC). These are most prevalent among birds and pigs. MAC infection in pigs occurs worldwide and sometimes leads to serious economic losses at slaughter. The incidence of disease caused by AM is also increasing among immunodeficient patients such as those with Acquired Immunodeficiency Syndrome (AIDS).

Etiology: AM are gram-positive, aerobic, slow-growing, acid-fast rods. They are also known as mycobacteria other than tuberculous mycobacteria (MOTT) or non-tuberculous mycobacteria (NTB). They are divided into 4 groups (I through IV) by Runyon based on differences in growth rate, colony pigmentation and other characteristics. MAC, which belong to group III, can be

further subdivided into 28 serovars. The latest classification divided MAC into two species: *M. avium* and *M. intracellulare*. *M. avium* contains 4 subspecies: *avium*, *paratuberculosis*, *silvaticum* and *hominisuis*. In the U.S., where more than 15 MAC serovars have been isolated, serovars 1, 2, 4 and 8 are the most commonly isolated from lesions in infected pigs.

AM are found in soil, salt and fresh water, insects, earthworms, sawdust and peat. Pigs are likely most commonly infected through exposure to contaminated environment (such as sawdust and peat used for bedding) and infected wild birds; sows that excrete MAC are an additional source of infection.

The infection almost exclusively occurs by ingestion. An experimental study in which 5-week-old pigs were orally exposed to the bacteria demonstrated the occurrence of gross lesions in mesenteric lymph nodes 10 days post infection. Respiratory and wound infections are uncommon. Transmission of the bacteria between humans or from pigs to humans has not been reported to date; therefore, humans are thought to be infected from the environment as well.

Clinical findings: Most of the infected pigs do not show any specific clinical signs. Gross lesions are often restricted to lymph nodes, and are incidental findings when pigs are slaughtered or die due to another, unrelated disease. Generalized lesions, including severe granulomatous enteritis causing chronic diarrhea and wasting, have been reported; however, such cases are rare.

Pathologic findings: It is thought that MAC usually enter through the mucosa of the pharynx and/or the small intestine, with gross lesions limited to the tonsil and the mandibular, retropharyngeal and mesenteric lymph nodes, particularly the jejunal lymph node. These lesions are characterized by well-circumscribed, yellowish white nodules. They range in size from very small (<1mm) up to relatively large, with eventual involvement of the entire node. Uncommonly, generalized lesions are seen, characterized by small, white, smooth-surfaced nodules in the liver, spleen, lung, kidney and many lymph nodes, indicating hematogenous spread. These lesions are usually not accompanied by any clinical signs. In rare cases, the wall of the small intestine may be thickened due to infiltration of the lamina propria by inflammatory cells.

Histologically, lesions are primarily characterized by granulomatous inflammation, composed of epithelioid macrophages and multinucleate giant cells, sometimes with central caseation necrosis, but without distinct encapsulation. Special stains such as Ziehl-Neelsen are used to demonstrate the presence of low numbers of intralésional acid-fast bacilli.

Diagnosis: Intradermal injection of purified protein derivative (PPD) tuberculin into the dorsal surface of the ear is the diagnostic test recommended on a herd basis. The reading is performed 48 hours later. Since MAC infection does not cause any specific clinical signs, even if a

pig has generalized disease, it is difficult to establish a clinical diagnosis. Since some tuberculous pigs may fail to react to the tuberculin test, this should be repeated in a herd that previously had animals with positive reactions. An enzyme-linked immunosorbent assay (ELISA) has also been developed in order to detect antibodies in pigs infected with MAC. This assay is useful to test replacement animals. At postmortem examination, the presence of intralosomal acid-fast bacilli is usually demonstrated by using an acid-fast stain on smears or sections from tissues with lesions. Careful interpretation is necessary since other acid-fast bacteria such as *Rhodococcus equi* can cause tuberculosis-like lymphadenitis in pigs. Bacterial culture is the gold standard test. It takes 4-6 weeks to grow visible colonies if solid media are used for culture; if liquid or broth media are used, the growth rate is slightly shorter. MAC are identified through biochemical and seroagglutination tests and through molecular techniques. PCR assays can be used to amplify regions of the 16S rRNA gene from MAC. Insertion sequences (IS), which are species specific, are also used for bacterial identification. IS elements are identified as IS901, IS1110, IS1245 and IS1311 for *M. avium* and IS1141 for *M. intracellulare*. Finally, restriction fragment length polymorphism (RFLP) analysis of IS elements can be used for strain genotyping.

Treatment and Prevention: Since infected pigs do not normally have specific clinical signs, it is uncommon to treat pigs. Thorough cleaning of infected premises using heat and disinfectants such as 3% formalin, 2% Lysol or 2.5% phenol are reportedly effective in eliminating the bacteria, as is the removal of animals that are PPD tuberculin test positive. It is unknown whether BCG vaccination can protect pigs from MAC infection. From a public health perspective, MAC infection is a serious problem for immunodeficient patients since MAC is a common opportunistic infectious agent. The disease tends to be disseminated in these patients, whereas AM in immunocompetent patients affects primarily the lungs. In the U.S. and Europe, the incidence of tuberculosis in the general population is low; the incidence of AM disease is, however, high in AIDS patients. Since AM are ubiquitous in nature, it is difficult to control disease caused by these bacteria. Nonetheless, there is a need to determine potential wildlife and environmental reservoirs in order to reduce the exposure of human and domestic animals to the mycobacteria.

-by Dr. Nozomi Shimonohara, ADDL extern

-edited by Dr. Ingeborg Langohr, former ADDL graduate student

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ADDL Schedule

The Indiana ADDL at Purdue University and Heeke ADDL will be closed on the following University holidays in 2009.

May 25.....Memorial Day
 July 3.....Independence Day
 September 7.....Labor Day
 November 26-27.....Thanksgiving
 December 24-25.....Christmas
 December 31- January 1, 2010.....New Year

Final Diagnosis

Rhodococcus equi in a horse



History: A 4-month-old Tennessee walking horse colt was submitted to the Indiana Animal Disease Diagnostic Lab at Purdue University for necropsy. The submitter reported an approximately 1-1/2 month history of pneumonia and osteolytic lesions in the right distal cannon bone and fetlock joint. The foal was treated with antibiotics and had two arthroscopic surgeries on the affected joint, but did not show significant signs of improvement and was subsequently euthanized.

Gross findings: The right fetlock joint contained moderate amounts of cloudy tan to white fluid, with numerous

strands of fibrin. The synovial membranes were thickened and covered by tan to yellow exudates that extended to and infiltrated into the deep digital flexor and common digital extensor tendons as well as the interosseous ligament. The distal physis of the cannon bone was lytic, had roughed irregular surfaces, and was coated by tan to pink, thick, creamy material.



Cranial ventral aspect of left lung lobe : pulmonary abscess



Right fetlock joint: suppurative tenosynovitis and osteomyelitis

The lungs were diffusely mottled heavy, and oozed red froth on cut section. Numerous (10-15) firm, tan to red, raised, round to irregularly shaped nodules ranging in size from 2X2X1.5 – 8X8X6 cm were scattered throughout all lung fields. The nodules contained abundant thick, tan to yellow, creamy exudate. The tracheobronchial lymph nodes ranged in size from 3-7 cm in diameter and were tan to red, slightly firm and, when incised, contained copious amounts of creamy tan material.

Histologic findings: The distal physis of the cannon bone was segmentally separated from the epiphysis. At the interface with the epiphysis, the physis was necrotic with a fibrillated surface; there were decreased numbers of cells with few small clusters of degenerate chondrocytes, and it was segmentally infiltrated by numerous neutrophils and fewer macrophages. Marrow spaces of the metaphysis and epiphysis were infiltrated largely by neutrophils with fewer macrophages and a loose meshwork of fibrous connective tissue. Macrophages had abundant foamy eosinophilic cytoplasm that was stippled with basophilic rod shaped bacteria. Bony trabeculae had a scalloped surface, were multifocally fragmented, and segmentally lacked a peripheral rim of osteoblasts. There were increased numbers of osteoclasts along the peripheral trabeculae. Inflammatory cells extended through the periosteum and into the adjacent soft tissues forming pyogranulomas.

Pulmonary airways and adjacent alveolar spaces were multifocally effaced by large aggregates of neutrophils, macrophages, and multinucleated giant cells. The mononuclear cells contained variable numbers of round, eosinophilic, intracytoplasmic bacteria (bacilli). Surrounding and coursing through the inflammatory aggregates were thick mats of mature fibrous connective tissue. An area of coagulative necrosis was focally circumscribed by clusters of neutrophils and fibrous tissue,

consistent with an abscess. Within the less severely affected segments of lungs, the alveolar spaces contained numerous macrophages and fewer neutrophils. Alveolar septa were segmentally lined by type II pneumocytes.

Ancillary findings: A swab from the fetlock joint and sections of lung, spleen, lymph node, and small intestine were submitted for aerobic bacterial culture. *Rhodococcus equi* was isolated from the lungs, fetlock joint, spleen, and lymph node. A goodpasture gram stain was applied to sections of cannon bone and lung and revealed numerous, intracytoplasmic, gram positive bacilli within macrophages.

Discussion: Characteristic gross and microscopic lesions, coupled with positive bacterial cultures and identification of intracytoplasmic, gram positive bacilli, were consistent with *Rhodococcus equi* infection in this foal. *R. equi* is a common pathogen in foals that is predominantly seen in animals less than six months of age. The primary target organs are the lungs, intestines, and associated lymph nodes with resultant pyogranulomatous bronchopneumonia, ulcerative typhlocolitis, and pyogranulomatous lymphadenitis, respectively. Once the infection is established, hematogenous dissemination may occur to the liver, spleen, bone/joint, and skin. Additionally, *R. equi* has been documented to cause abscesses in swine, sheep, cats, cattle, llamas.

R. equi is a gram positive facultative intracellular bacteria that is found in soil. Most infections are acquired through inhalation or ingestion of soil-borne organisms and virulence is attributed to at least two predominant mechanisms. Replication occurs within the cytoplasm of macrophages and is associated, at least in part, to the expression of the plasmid-encoded, virulence-associated protein, VapA. Additionally, virulence is achieved by preventing lysis through inhibiting the conversion of phagosomes to phagolysosomes.

Antemortem diagnosis is based on clinical signs, evidence of pulmonary abscess on thoracic radiographs, and aerobic bacterial cultures of transtracheal wash fluid, abdominal fluid, or synovial fluid.

-by Dr. Chad Frank, ADDL Graduate Student

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ADDL laboratory results are available via our web page. To set up an account, call 765-494-7440 and ask for the Computer Systems Manager

Or

Request via our web page.

- **Addl.purdue.edu**
- **Online reports tab**
- **Request info and follow instructions**

DIAGNOSTIC FORUM

Diagnostic Forum is a quarterly newsletter of current services, regulations and research projects involving the ADDL which may be of interest to Indiana veterinarians and animal owners. It is our intention that the information provided will serve you. Please send your comments, suggestions, requests and questions to: Diagnostic Forum Editor, Purdue ADDL, 406 S. University St., West Lafayette, IN 47907 or email to addl@purdue.edu.

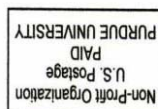
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Assistant to Director: Linda Hendrickson,, MA
Avian: Tsang Long Lin, DVM, PhD
Avian: Pat Wakenell, DVM, PhD
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Business Manager: Tonya Byrd, BS

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