Fall 2004

FROM THE DIRECTOR
H. Leon Thacker, DVM, PhD

Good day from ADDL. Again, fall is a wonderful time of the year. Changing colors, harvest time, chill in the air, shortened days and football. In their own ways, good things. At the ADDL some items of interest include: testing of animals at the Indiana State Fair this year revealed more animals positive for having received unauthorized drugs than at any time since we started testing. I believe that this should be publicized to the State Fair exhibitors as a deterrent to exhibitors in future years. An incident occurred with one of the champion animals in 2003, but there was not much publicity given to it. Perhaps more publicity would have been beneficial to abandonment of this practice by others this year.

Later this year we will be performing testing for Chronic Wasting Disease on samples from approximately 1000 hunter killed deer from the ’04 hunting season. Up to now, we have tested over 4,000 deer and found no evidence of CWD among deer taken in Indiana.

We have recently received acceptance of an offer we extended to Dr. Roman Pogranichniy who will be joining our faculty as diagnostic virologist next year. The diagnostic virology position of the ADDL has been vacant for a few years and we are excited about having Dr. Pogranichniy join us to provide the valuable services of a most capable veterinary virologist to our diagnostic efforts.

A measure of the suitability, capability, operating procedures, and standards of our laboratory will be under examination in late November when our laboratory will be visited by a site visit team to evaluate us for re-accreditation of our laboratory by the American Association of Veterinary Laboratory Diagnosticians. We are preparing for the visit and look forward to the feedback provided us by the accreditation process.

An old adage is that “the rewards for providing good diagnostic services are requests for more diagnostic services”. This has been demonstrated by the requests received by our molecular diagnostics section for same day turn-around testing of swine semen samples for PRRS. The number of requests has risen to the point that it will be necessary for practitioners or swine owners to contact the molecular diagnostics section to reserve time for semen submissions 2-3 days ahead of time unless you are among the routine submitters for service on Mondays and Thursdays. At this time, Tuesdays, Wednesdays, and Fridays have available slots for testing.(See page 6 of this newsletter.

I hope this finds each of you enjoying beautiful fall weather and glad we’re not in Florida. Our thoughts and best wishes go out to those who have lost so much to the recent unstable weather there.
In each issue, we will feature a case submitted to ADDL that we hope will be of interest to you.

**FINAL DIAGNOSIS:**
Feline Panleukopenia and *Bordetella bronchiseptica* bronchopneumonia

**History:** A male domestic shorthaired cat, reportedly 3 months of age, was submitted for necropsy to the Purdue Animal Disease Diagnostic Laboratory. Upon presentation to the referring veterinarian, the kitten was recumbent and severely dehydrated with pale mucus membranes and a temperature of 96° F. The owner reported a one day history of vomiting and anorexia.

**Gross findings:** The small and large intestinal serosa was roughened and pale grey. Both the small and large intestine had thickened walls and contained a small amount of yellow mucoid material. Cranioventrally, the lungs were mottled dark red and firm. The thymus was reduced in size.

**Histopathologic findings:** The small intestinal villi were severely blunted or absent, and there was destruction of crypts. The majority of crypts that remained contained either no crypt epithelium or epithelial cells that were polyhedral with a large nucleus (hypertrophy). Peyer’s patches contained reduced numbers of lymphoid cells. In the lungs, there was infiltration of macrophages and neutrophils into alveolar spaces and pulmonary interstitium. Eosinophilic fibrinoid material, cell debris, and numerous finely basophilic coccobacilli filled many air spaces. Both the spleen and examined lymph nodes contained reduced numbers of lymphoid follicles. The morphologic diagnoses were necrotizing enteritis, lymphoid depletion, and bronchopneumonia.

**Discussion:** Feline panleukopenia virus, a parvovirus, is cytolytic and targets rapidly dividing cells such as lymphoid cells and crypt epithelium. The virus is also capable of altering the differentiation of the layers of the cerebellum during fetal development, producing cerebellar hypoplasia.

Panleukopenia virus is highly contagious and transmission is commonly fecal-oral. However, fomites are also an important source of transmission. The virus is very resistant to extreme temperatures and conventional cleaning agents. All of these factors contribute to the common occurrence of feline panleukopenia virus infection in animal shelters and humane societies.

Early in the course of the disease, the virus infects the bone marrow, lymphoid tissue, and thymus, resulting in lymphoid depletion, thymic involution, leukopenia, and enteritis. Grossly, intestinal serosa may be roughened and intestinal walls may be segmentally thickened and hemorrhagic. Peyer’s patches may be depressed. Thymic atrophy and lymph node edema may also be present.

As the virus destroys the rapidly dividing crypt cells, villi are lost to attrition and nonabsorption results. This contributes to the diarrhea often noted in feline panleukopenia patients. Histologically, villi may be shortened or absent. Crypt epithelium may be hypertrophic, attenuated, or absent and crypts may be dilated with mucus and necrotic cell debris. Peyer’s patches may be hypocellular. Enterocytes and lymphocytes may contain basophilic intranuclear inclusion bodies, although these are not commonly seen.

As the name suggests, panleukopenia virus infection causes depletion of all lymphoid cells. The resulting immune compromise increases susceptibility to other infections, such as respiratory disease or bacterial enteritis. Also, loss of the gastrointestinal mucosal barrier can increase susceptibility to bacterial infections. In this case, the gross and histopathologic findings of bronchopneumonia correlate with the culture of *Bordetella bronchiseptica* from the lung. Panleukopenia virus infection may have...
contributed to the incidence and/or severity of this respiratory infection by causing immune compromise.

Common postmortem ancillary tests performed when feline panleukopenia virus infection is suspected include virus isolation and fluorescent antibody (FA) testing or immunohistochemistry of the ileum, distal jejunum, spleen, lung, and tongue. These results, in addition to clinical history and gross and histopathologic findings, aid in a diagnosis of feline panleukopenia virus infection. In this case, the tongue and small intestine were positive for feline panleukopenia virus by virus isolation and FA. The final diagnosis is feline panleukopenia and *Bordetella bronchiseptica* bronchopneumonia.

From July 29, 2004 to August 31, 2004, there have been at least 19 diagnosed cases of feline panleukopenia at the ADDL. This is one of the largest outbreaks of panleukopenia in recent ADDL history. It is important for area clinicians to be aware of the increasing number of cats diagnosed with this disease.

-by Dr. Sarah Janke, ADDL Graduate Student

References:

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**Hemangiosarcoma: A Malignancy of Cats and Dogs**

Hemangiosarcoma, also known as malignant hemangiothelioma or angiosarcoma, is a malignant neoplasm that arises from vascular endothelial cells. The canine is the most frequently affected species, but the incidence in cats appears to be rising. As might be expected of a tumor originating in the blood system, they are highly malignant and can be found almost anywhere on the body.

In canines, they occur predominantly in older males, with an average age of 8-10 years. Breeds that are most frequently affected include the German shepherd and the Golden retriever. The spleen, right atrium, and subcutis are the common sites of involvement. In felines, they are usually solitary tumors with a predilection for the head (especially eyelids), ear tips, nasal planum, and non-pigmented skin. Feline, as well as some canine, cutaneous hemangiosarcomas are similar to squamous cell carcinoma in that they can be actinic or sunlight-induced. The etiology of hemangiosarcomas is unknown, but reports in human cases suggest a correlation between hemangiosarcomas and exposure to carbon dioxide, arsenicals, or vinyl chloride.

In general, the biological behavior of this neoplasm is highly aggressive with most forms of the tumor metastasizing early in the disease process. Visceral hemangiosarcomas are highly aggressive tumors with a poor prognosis. Death is often associated with rupture of nodules or masses and resultant hemoabdomen or hemo-pericardium. Cutaneous hemangiosarcomas are less aggressive than their visceral counterparts with lower metastatic potential and longer survival times.
Clinical presentation and history:
Clinical signs depend on the tumor size, location, presence of metastasis, and associated secondary complication (i.e., DIC or nodule rupture). More than half of dogs with hemangiosarcoma are evaluated because of acute collapse after spontaneous rupture of the primary tumor or a metastatic lesion. In addition, dogs with splenic hemangiosarcoma often are seen because of abdominal distension secondary to tumor growth or hemoabdomen. Other common presenting signs include visible bleeding (typically epistaxis), exercise intolerance, episodes of weakness, pale mucous membranes, increased respiratory rate, and depression and lethargy.

Dogs and cats with cutaneous or subcutaneous hemangiosarcoma are usually evaluated for a nodule that is generally a single, well-defined mass which is red/brown to black, soft to firm, and may exude blood when cut. The blood disorder that commonly accompanies hemangiosarcoma is disseminated intravascular coagulopathy (DIC). This process involves blood clotting that occurs inappropriately inside the blood vessels. In DIC, blood clotting factors are consumed rapidly resulting in platelet deficiencies, increased clotting times, decreased fibrin content, and increased fibrin degradation products. DIC can commonly be the cause of death in many cases of hemangiosarcoma.

Diagnosis: Hemangiosarcomas can be diagnosed cytologically on the basis of the appearance of fine-needle aspirates or impression smears. The neoplastic cells are similar to those in other sarcomas, as they are often spindle-shaped, but vary considerably in shape. These spindyloid cells often have large nuclei with a lacey chromatin pattern, one or more nucleoli, blue-grey vacuolated cytoplasm, and an increase in mitotic figures. Breed and clinical signs may also suggest a diagnosis of hemangiosarcoma. Histopathology is confirmatory.

Clinical diagnostic points:
- CBC: mild to severe anemia, leukocytosis, thrombocytopenia
- Chemistry: increased hepatic enzymes
- Abdominal radiographs: Appearance of an intra-abdominal mass
- Thoracic radiographs: pulmonary nodules, right atrial enlargement
- Abdominal ultrasound: splenic mass, peritoneal effusion, hepatic nodules, enlarged mesenteric lymph nodes

Treatment and prognosis: Surgical excision is the preferred choice of treatment for dermal or subcutaneous hemangiosarcomas. Various chemotherapeutic regimes have been attempted on dogs with multicentric visceral with little success. Survival times vary with the location and stage of the tumor but, in general (with exception of dermal hemangiosarcomas), are quite short. Studies have shown survival times of 20-60 days following detection of the tumor, with a one year survival rate in less than 10% of patients.

References

**Mycoplasma Disease in Cattle**

In recent years, more than 20 species of *Mycoplasma, Ureaplasma and Acholeplasma* have been isolated from cattle with different diseases. All of the 20 aforementioned species have been referred to as the Mycoplasmas. It is generally believed that Mycoplasmas play a secondary role in infections, most often exacerbating pre-existing disease; but it has been shown that *Mycoplasma bovis* (*M. bovis*) can play a primary role. *M. bovis* is considered one of the more pathogenic species and is the most frequent *Mycoplasma* pathogen of pneumonia, mastitis, and arthritis in cattle. Meningitis, otitis media, keratoconjunctivitis, decubital abscesses, vaginitis and/or abortion are other conditions which may be caused by *M. bovis*. In general, treatment of *Mycoplasma* diseases is difficult since *Mycoplasma spp.* lack a cell wall, which differentiates them from bacteria and are thus resistant to some commonly used antibiotics. Methods used for definitive diagnosis of *Mycoplasma* infection within an individual animal or herd include culture, fluorescent antibody test (FA-test), and/or serology. In addition, polymerase chain reaction (PCR) is a sensitive method which can be used. Selective media such as Friis or Hayflick’s T-mycoplasma media are necessary for *Mycoplasma* culture and cultures must be kept at 10% CO₂. To detect acute infection, paired serum samples are recommended, since rises in antibody titers occur 10-14 days after acute infection with certain *Mycoplasma spp.* PCR is an extremely sensitive method which can be used to confirm *Mycoplasma* infection.

**Pneumonia:** Two types of pneumonia are associated with *Mycoplasma* infections: contagious bovine pleuropneumonia and enzootic pneumonia of calves. Contagious bovine pleuropneumonia is caused by infection with *Mycoplasma mycoides* subsp. *mycoides* and is classified as a foreign animal disease. In “calf pneumonia”, (enzootic pneumonia), *Mycoplasma spp.* are seldom the only pathogens isolated. Most commonly, there is primary and/or concurrent viral and/or bacterial infection. Common involved respiratory viruses are Parainfluenza 3 virus (PI3), Infectious bovine rhinotracheitis virus (IBR) and/or Bovine respiratory syncytial virus (BRSV). Common involved bacteria are *Histophilus somni* (*H. somni*), *Pasteurella multocida*, (*P. multocida*), and/or *Mannheimia haemolytica* (*M. haemolytica*). Most commonly, respiratory viruses are primary pathogens. However, *M. dispar, M. bovis* and *Ureaplasma* may also act as primary pathogens. Several species of *Mycoplasma* may be isolated from calves with pneumonia, but only a few of these species are considered pathogenic. Respiratory pathogenic *Mycoplasma* spp. include *M. dispar, M. bovis, M. bovirhinus, M. bovigenitalium, Ureaplasma diversum*. With the exception of *M. bovis*, these mycoplasmas can be found as normal flora of the upper respiratory tract. Pneumonia develops after their
introduction into the lower respiratory tract, which is commonly preceded by impairment of mucociliary clearance and/or immune defense. Mycoplasmas can be introduced in a herd by subclinical Mycoplasma carriers. These cattle shed the organism through nasal discharge for months to years without showing clinical signs. Clinical signs observed in cattle with pure Mycoplasma pneumonia are coughing, induced by stress or movement, slight tachypnea, low grade fever and mild depression. Affected cattle usually retain a good appetite, which distinguishes this type of pneumonia from other viral and/or bacterial pneumonias. At necropsy, cranioventral areas of lungs are red-blue, firm and ooze purulent material on cut section. Histologically, there is chronic bronchointerstitial pneumonia characterized by peribronchiolar and perivasculater lymphocytic cuffings, purulent bronchiolitis, accumulation of neutrophils and macrophages within alveolar lumens, epithelialization of alveolar septae and atelectasis. Due to prominent lymphocytic cuffings, this type of pneumonia is also called “cuffing pneumonia”. Definitive diagnosis can be obtained through culture of a tracheal lavage fluid and/or tissue samples of lungs. In addition, fluorescent antibody tests for detection of antigen of distinct Mycoplasma spp. can be performed. Intramuscular application of oxytetracycline, erythromycin, or tylosin is recommended. In mixed infections with Mycoplasma spp. and bacteria, antibacterial therapy must be also effective against involved bacteria. Since “enzootic pneumonia” is a multifactorial disease associated with impaired pulmonary defense, proper management is also very important (i.e., adequate ventilation, prevention of overcrowding).

**Mastitis.** Although several Mycoplasma spp. (M. canadense, M. bovigenitalium, M. californicum) may cause mastitis, Mycoplasma bovis is the most prevalent. The disease spreads rapidly within a herd, thus the usual history in a farm with Mycoplasma mastitis is that several cows within a short period of time have acute mastitis in one or more quarters. All quarters are usually affected in lactating animals. In addition, the same farm may also have problems with lameness, reproductive problems, calf pneumonia, and adult cow respiratory disease. Cows affected with acute Mycoplasma mastitis have a dramatic drop in milk production. Affected quarters will be warm, swollen and light brown. On palpation, the parenchyma may be firm and often fine nodular. Drawn milk appears normal initially, but separates rapidly in a floccular deposit and a clear supernatant. Acute mastitis may be followed by chronic mastitis, intermittent acute flare-ups, or subclinical infection. Cows with subclinical infection can return to normal milk production, but they may continue to shed Mycoplasma spp. within milk. Mycoplasma spp. and, thus, Mycoplasma mastitis can be transmitted mechanically via handling, milking machines, and common udder wash solutions. Microscopically, acute infection causes neutrophilic mastitis characterized by neutrophilic infiltration of lobular interstitium, degeneration, and necrosis of alveolar epithelium and neutrophilic accumulation within alveoli, which is often followed by abscess formation. In subacute stages, macrophages predominate as inflammatory cells. Chronic Myco-plasma mastitis is characterized by hyperplasia of alveolar and ductular epithelium, aggregation of lymphocytes within interstitium and around ducts, interstitial fibrosis and lobular atrophy. For definitive diagnosis of Mycoplasma mastitis, culture of milk and/or udder parenchyma may be pursued. Immunoblot performed on milk samples is another method which can be used for diagnosis. As with other Mycoplasma diseases, Mycoplasma mastitis is difficult to treat. Thus, prevention by teat dipping, dry/lactating cow therapy, and proper maintenance of milking machine equipment is recommended. Once Mycoplasma
mastitis is detected within a herd, identification of infected cattle and their strict separation or culling may be necessary, since the disease is highly contagious and often therapy resistant.

Arthritis: The incidence of *Mycoplasma* arthritis is increased in cattle herds with enzootic *Mycoplasma* mastitis and/or pneumonia, since *Mycoplasma* arthritis occurs commonly secondary to bovine affected with *Mycoplasma* pneumonia or mastitis due to hematogenous spread. Oral infection of calves from dams with *Mycoplasma* mastitis may also occur. Lameness caused by *M. bovis* is typically a result of polyarthritis and/or tendosynovitis. One clinical sign of *Mycoplasma* arthritis is marked lameness. Animals have pain during movement and/or at palpation of affected joints. Capsules of affected joints are warm, distended and fluctuant on palpation. Gross examination of the affected joints will show fibrinosuppurative synovitis and tenosynovitis with cartilage erosions. Affected joint capsules are distended by opaque, cream colored exudates, which often contains fibrin flakes. Eroded cartilage may be replaced by polypoid granulation tissue. The synovium is often reddened and edematous. Microscopically, there may be ulceration of synovial membranes and infiltration of synovia and joint capsule with neutrophils, macrophages, plasma cells and/or lymphocytes. Neutrophils may accumulate within the joint space and there may be hyperplasia of synovial cells and villi. Synovial vessels are often congested and occasionally thrombosed. *Mycoplasma* culture of tissue samples and/or synovial fluid may be pursued for definitive diagnosis. Treatment follows the protocol of any septic arthritis. Recommended is lavage of affected joints with a through-and-through flushing method, most likely on a daily basis over the next 1-2 weeks. Local antibiotic therapy may be used. Systemic antibiotic therapy is recommended, since there is commonly concurrent *Mycoplasma* mastitis and/or pneumonia. It must be considered that *Mycoplasma* spp. have a poor response to antibiotic therapy. Antibiotics effective in *Mycoplasma*-induced lameness include danofloxacin, enrofloxacin, and tylosin. Aspirin can be given for pain management.

-by Suzanne Brishard, Class of 2003

-edited by Dr. Sandra Schoeniger,
ADDL Graduate Student

References:


From Molecular Diagnostics:

Due to the increasing number of semen samples submitted for PRRS testing and the limited number of samples that can be tested in 1 day, please notify Molecular Diagnostics/Barb (765-494-7451) 2-3 days in advance to schedule testing. We can no longer guarantee same day results unless testing is pre-arranged. Samples must be in ADDL by 10:00am on any given test day.
1. **What is immunohistochemistry?**
Immunohistochemistry (IHC) uses immunologic and histologic techniques to detect antigens in tissues. The antigen is recognized by a specific antibody (Fig. 1) 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 (Fig. 2). IHC is a very sensitive and specific technique. For diagnosticians, it is important to colocalize antigens and lesions.

2. **Uses of IHC in veterinary diagnostics**
Neoplastic and infectious diseases are the main focus of IHC in veterinary medicine. The ADDL IHC Service offers a variety of tests for both infectious and neoplastic diseases. Please contact the ADDL for current tests available and fees.

2.1 **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.

2.2 **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.

2.3 **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 specific tumor types. It has been reported recently that the immuno-histochemical detection of KIT protein in mast cell tumors of dogs has prognostic significance. We are testing some of these markers to determine their significance in veterinary cancers.

2.4 **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. The ADDL offers immunohistochemical tests for infectious diseases of small (feline herpesvirus, *Leptospira*, canine parvovirus, canine adenovirus, feline leukemia virus, canine distemper virus, etc) and food animals (IBR, BVD virus, TGE virus, *Listeria, Cryptosporidium, Neospora*, etc).

3. **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.

4. **Interpretation of results.**
Immunohistochemistry facilitates diagnosis of infections and determining the histogenesis and prognosis of neoplasms. A colored reaction (provided that it is specific according to the controls used), indicates the presence of components of the antigen (infectious agent, neoplasm, prognostic marker) sought. 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 a particular infectious agent or its potential significance to the case. Due to mutations or other mechanisms, neoplastic cells may modify (upregulate/downregulate) the expression of proteins resulting in unexpected results. It is important to re-emphasize that immunohistochemistry results, like those obtained by other diagnostic methods, must be supported by clinicopathologic data. Immunohistochemical results should be interpreted by the diagnostician provided that he/she has all the information pertaining to the case.

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.

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

About terminology:

**Histology:** Study of tissues and cells with general stains such as hematoxylin-eosin. It distinguishes cells and tissues by morphologic and tinctorial characteristics.

**Histochemistry:** Uses chemical reactions to demonstrate substances (e.g. PAS detects glycoproteins, trichrome stains distinguish muscle from collagen). It helps in distinguishing among morphologically similar cells.

**Immunohistochemistry:** Detects antigens or sequences of antigens (4-10 amino acids) that are characteristic of an infectious agent or a type of cell. **The recognition of an antigen is the result of an immune reaction, NOT a chemical reaction.** It is usually much more specific than general histology or histochemistry.

**Immunocytochemistry.** Similar to immunohistochemistry but done on cell smears or cytopreps. Some people use the terms interchangeably.
Joan Oaks, Laboratory Records Clerk and Manager of the shipping area, retired from ADDL on September 30. She joined the ADDL staff in 1997. We wish Joan and husband Floyd the best as they travel, camp and garden during retirement.

Drs. Ching Ching Wu and Tsang Long Lin attended the American Association of Avian Pathologists meeting in conjunction with the American Veterinary Medical Association meeting in Philadelphia, July, 2004.

Dr. Steve Hooser was an invited speaker at the American Veterinary Medical Association meeting in Philadelphia, July, 2004.

Margaret Gelhausen, Heeke ADDL bacteriology technician attended the Association of Veterinary Microbiologists meeting in Athens, Georgia, August, 2004.


Dr. Leon Thacker attended the 9 States Veterinary Conference in Lexington, Kentucky, August, 2004.

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

November 25-26, 2004......................... Thanksgiving
December 23-24............................... Christmas
December 30-31............................... New Year's

ADDL test results are available on the Internet. To set up an account, call 765-494-7440 and ask for the Computer Systems manager or log on to www.addl.purdue.edu

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