PURDUE UNIVERSITY

DIAGNOSTIC FORUM

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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
Dr. Stephen B. Hooser

Spring is here and, with it, the Spring edition of the ADDL Quarterly Newsletter!

The ADDL is very pleased to announce that we can now offer **overnight UPS service from Indiana veterinary practices to the West Lafayette ADDL at $6.00 for packages up to 15 pounds**. Please see p. 2 for more details.

The ADDL has recently been informed that it has been granted full accreditation for five years by the American Association of Veterinary Laboratory Diagnosticians (AAVLD)! In November, 2009, a four member site visit team from the AAVLD conducted an extremely thorough and detailed evaluation of the procedures, personnel, facilities and Quality System for both the West Lafayette and Heeke/SIPAC laboratories. Full accreditation for five years is the highest accreditation status offered and the ADDL is pleased to be able to offer the services of a fully-accredited lab to the Citizens of Indiana. Many thanks to Buffie Vaught, the ADDL Quality Manager, for overseeing the preparations.

Hot Topics

- **Fee changes, p. 2**
- ***Reduced rate UPS service now available. Overnight delivery to ADDL-WL for $6.00, p. 2***
- **Diagnostic Profiles, new feature, p. 3**
- **Abortion panels now in place, p.5**

Inside the Diagnostic Forum

Focus on Toxicology/Analytical Chemistry

Forensic toxicology takes place every day in the Toxicology/Analytical Chemistry Section. In this laboratory, tissues, fluids, feed, water and other samples are examined for the presence of toxic chemical compounds or essential vitamins and elements.

The lab at ADDL is staffed by Dr. Christina Wilson, Head of Toxicology and Head Chemist, and Kim Meyerholtz, Assistant Chemist.

Dr. Christina Wilson Kim Meyerholtz

**********PLEASE NOTE**********

The following price adjustments took effect on April 1, 2010. New prices are as follows:

Accession fee	$10.00 (applied to all cases except EIAs)
Necropsy	$88.00
Necropsy with photodocumentation	$188.00
Spinal cord removal	$50.00 additional
Histopathology	$28.00
Virus isolation	$25.00
Mycotoxin Screen I	$50.00
EIA	$8.50 (no accession fee applied)

New Test (effective June 1, 2010)

Abortion Screen	$145.00 (see pages 3-5)

American Board of Veterinary Toxicology Video Profiles Series

The American Board of Veterinary Toxicology has released a televised video documentary which explains its relationship with veterinary medicine, toxicology and human health. The ABVT strives to inform and educate practicing veterinarians, veterinary medical students and the public about toxicological hazards to pets, livestock and wildlife. Dr. Steve Hooser, Purdue ADDL Director, is currently serving as Vice President of the ABVT.

The documentary is part of the Profiles Series airing on a variety of cable networks, such as CNBC and Bravo. The future broadcast schedule is currently in production; however, you can view the video now on ABVT's website at http://www.abvt.org/public/video.

Information on the Profiles Series can be found at http://profilesseries.com

UPS -OVERNIGHT DELIVERY TO ADDL-WL FOR $6.00

- **ADDL has reached an agreement with UPS for submitters to send samples 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.**
- **Cost will be a flat rate of $6.00 for packages up to 15 pounds.**
- **Client will be billed $6.00/package. If cases from multiple owners are submitted in the same shipment, the $6.00 charge will be added to only one accession.**
- **Call us at 765-494-7440 to request labels.**

Our congratulations to Dr. Ching Ching Wu who recently was honored with the Meritorious Service Award from the North Central Avian Disease Council. This award recognizes an individual who has made outstanding contributions in the area of poultry health service and was presented to Dr. Wu based on her years of service to the poultry industry, diagnostic innovations, and her leadership roles in professional organizations.

The ADDL Diagnostic Forum introduces a new feature entitled "Diagnostic Profiles" in this issue. The intent will be to suggest a panel of diagnostics which will be appropriate and helpful in defining the cause of non-specific disease syndromes. Practitioners are often presented with patients having clinical signs which fall into a general category of disease that may represent one of a number of specific conditions and may result from various etiologies. Approaching a differential diagnosis simultaneously with multiple testing modalities should expedite determination of a definitive diagnosis. This approach will provide the most useful, timely and complete information needed for consideration in therapeutic and disease control decisions.

As we introduce Diagnostic Profiles, let's consider **abortion** as an example of a timely "syndrome". Abortion in all species, including small animals, horses and food animals, may be caused by both infectious and non-infectious factors. A multifaceted approach is necessary for diagnostic investigation into this type of syndrome and, even then, the underlying cause may remain unknown. Successful diagnosis of a specific etiology is achieved about 50% of the time.

When an aborted fetus, preferably accompanied by the placenta and serum from the dam, is submitted to ADDL, multiple laboratories become involved in searching for the cause of the problem. The protocol starts with a necropsy and then proceeds with the array of tests that are listed on the ADDL web site. The reported success rate for determining a specific diagnosis is over four times greater when the fetus, placenta and serum are submitted than when only fetal tissues are submitted for laboratory examination.

If submission of the entire fetus to ADDL is not possible, then performing the necropsy in the field and submitting specimens is the next best approach. Gross lesions in aborted fetuses are rare, non-specific and often limited to diffuse congestion and excessive fluid within body cavities. When observed, gross lesions are most common in lung and placenta. Microscopic lesions are most often observed in lung, placenta, heart, brain, liver or kidney. Consequently, a routine set of tissues, both fixed and fresh, should be collected for ancillary laboratory testing. These are detailed in the following tables.

Infectious etiologies of abortion include viruses, bacteria, fungi, protozoa and *Mycoplasma*. Some of these agents are easy to isolate or demonstrate by traditional methods while others are not. Molecular diagnostic techniques are now employed to demonstrate the presence of those agents that cannot be readily isolated.

Certain abortifacient agents have historically posed a problem for laboratory diagnosis. Agents such as *Leptospira sp*. and *Brucella sp*. require extended incubation time. Agents such as *Coxiella burnetii* and *Chlamydophila abortus* pose a health risk to laboratory technicians. Protozoal agents such as *Neospora* sp. and *Toxoplasma gondii* cannot be cultured. Employing the polymerase chain reaction (PCR) method provides a rapid, safe and accurate means of demonstrating these agents.

Fresh tissues to be submitted should include placenta, stomach/abomasal content, lung, kidney, spleen, heart and brain. These tissues are used for culture of bacteria and fungi as well as virus isolation and molecular diagnostics. Packing fresh chilled (NOT frozen) tissues separately in a well-chilled insulated container and shipment via an overnight delivery service is recommended for best diagnostic results.

Tissues for histopathology should include placenta, brain, eyelids, thymus, lung, heart, liver, spleen, kidney, skeletal muscle, abomasums (stomach), small and large intestine. For best results, specimens submitted for histopathology should be no thicker than 0.5 cm and should be fixed in 10 times their volume of 10% neutral buffered formalin.

Diagnosis of abortion can be aided by examination of serum samples from the dam or body fluids collected from the pericardial, thoracic or abdominal cavities of the fetus. Serology from the dam is more valuable if baseline levels from the herd are established from samples collected at the time of pregnancy diagnosis. Negative serology has value in that it eliminates some agents from the differential list.

Noninfectious abortion may be caused by physical, nutritional, toxic and genetic factors. History and clinical signs observed in the dam may be most suggestive of a diagnosis of noninfectious abortion. Chemical testing of ocular fluid for nitrate levels is a means of diagnosing nitrate induced abortion. Fetal hepatic selenium levels can be measured in cases of abortion suspected to be caused by selenium deficiency.

The following tables detail the elements of diagnostic panels for the most common causes for abortion in livestock. The array of laboratory tests included provide the most efficient approach to determining the cause of an abortion and, once determined, control measures can be initiated.

-by Drs. Bill Wigle and Steve Lenz, ADDL Pathologists

Tissue Collection Guide for Abortion Cases

		Pathology	Virology	Bacteriology	Molecular Diagnostics	Serology
Bovine Abortion Exam (specimens from multiple fetuses should be pooled – maximum of 2 fetuses/accession)		Whole fetus and placenta, chilled OR Formalin-fixed placenta (2-3 cotyledons), lung, liver, kidney, spleen, ileum, heart, eyelid, brain (one-half), Skin if lesions	Fresh-chilled (NOT frozen) Spleen Lung Placenta	Fresh-chilled (NOT frozen) Placenta (2-3 cotyledons) Abomasal content (3-5 ml in sterile tube) Lung Liver Kidney (Skin, if lesions)	Fresh-chilled (NOT frozen) Spleen Lung Brain (one-half) Heart Kidney Placenta Abomasal content	Serum from dam
Small Ruminant Abortion Exam (specimens from multiple fetuses should be pooled – maximum of 2 fetuses/accession)		Whole fetus and placenta, chilled OR Formalin-fixed placenta (2-3 cotyledons), lung, liver, kidney, spleen, ileum, heart, eyelid, brain (one-half) Skin, if lesions	Fresh-chilled (NOT frozen) Spleen Lung Thymus Kidney Placenta	Fresh-chilled (NOT frozen) Placenta (2-3 cotyledons) Abomasal content (3-5 ml in sterile tube) Lung Liver Kidney Skin, if lesion	Fresh-chilled (NOT frozen) Placenta Lung Brain (one-half) Kidney Abomasal content Liver	Serum from dam
Porcine Abortion Exam (specimens from 3-4 fetuses should be pooled)		Whole fetus and placenta, chilled OR Formalin-fixed placenta, lung, liver, kidney, heart, umbilicus, brain (one-half)	Fresh-chilled (NOT frozen) Spleen Lung Thymus Placenta	Fresh-chilled (NOT frozen) Placenta Stomach content (3-5 ml in sterile tube) Lung Kidney	Fresh-chilled (NOT frozen) Spleen Lung Kidney Liver Placenta	Serum from dam
Equine Abortion Exam 1 fetus/accession		Whole fetus and placenta, chilled OR Formalin-fixed placenta, lung, liver, kidney, umbilicus, spleen	Fresh-chilled (NOT frozen) Spleen Lung Liver Placenta	Fresh-chilled (NOT frozen) Placenta Stomach content (3-5 ml in sterile tube) Lung Liver Kidney Skin, if lesions	Fresh-chilled (NOT frozen) Lung Kidney Liver Spleen Placenta	Serum from dam

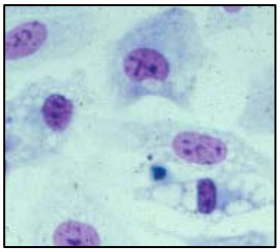
ADDL is now offering Abortion Diagnostic Panels

For each species, tissues will be examined for the following etiologic agents (associated test platforms listed), on every submitted abortion case, for the fixed fee of $145.00. Please refer to the previous table for appropriate samples to submit to ADDL if you are unable to submit the entire fetus. These "panels" incorporate several molecular diagnostic techniques (PCR tests) for agents that cannot be identified, or are difficult to isolate by traditional methods.

	Pathology	Virology	Bacteriology	Molecular Diagnostics	Serology	Fee
Porcine	Routine necropsy/ histopathology	FA (PCV2, PPV) VI (PCV2,PPV, PRV)	Aerobic culture *Salmonella* culture *Leptospira* FA 1 susceptibility	*Leptospira* PRRS	1 test (if needed) Serum from dam	$145.00
Bovine	Routine necropsy/ histopathology	FA (IBR,BVD) VI (IBR, BVD)	Aerobic culture (including *Listeria*, *Campylobacter*, *Histophilus*, others) *Salmonella* culture *Leptospira* FA 1 susceptibility	*Toxoplasma* *Neospora* *Leptospira* *Brucella spp.* *Chlamydia* *Coxiella*	1 test (if needed) Serum from dam	$145.00
Small ruminant	Routine necropsy/ histopathology	FA (BVD) VI (BVD,BD)	Aerobic culture (including *Listeria, Campylobacter)* *Leptospira* FA 1 susceptibility	*Toxoplasma* *Neospora* *Leptospira* *Brucella spp.* *Chlamydia* *Coxiella*	1 test (if needed) Serum from dam	$145.00
Equine	Routine necropsy/ histopathology	FA (EHV1) VI (EHV1, EVA)	Aerobic culture *Salmonella* culture *Leptospira* FA 1 susceptibility	EHV1 *Leptospira*	1 test (if needed) Serum from dam	$145.00

Bovine Viral Diarrhea in new World Camelids

Bovine viral diarrhea virus (BDVD) continues to plague the cattle industry, with most severe and economically devastating cases occurring in young cattle (ages 6-24 months)**.** However, cases of BVDV infection in new world camelids (NWC) have recently emerged with clinical signs similar to those in cattle. With the increasing popularity and value of NWC, practitioners and owners should be aware of their susceptibility to Bovine viral diarrhea virus.



BVDV is an enveloped, single-stranded, positive-sense RNA virus in the genus *Pestivirus* and family *flaviviridae.* Other pestiviruses include Classical Swine Fever virus and Border Disease virus. With genetic sequencing, isolates can be grouped into genotype 1 or genotype II; each genotype has at least two subgenotypes. The genotypes can be further classified as a cytopathic biotype or noncytopathic biotype, based on their effects in infected cell culture.

In cattle, clinical manifestations of BVDV infections include subclinical disease, diarrhea, respiratory infections, ill thrift, infertility, abortion, congenital defects and gastrointestinal ulcerations, mainly in the abomasum and ileal Peyer's patches (mucosal disease). Birth defects include cerebellar hypoplasia, hydrocephalus, porencephaly, ocular anomalies, and cutaneous and skeletal defects. Persistent BVDV infection can occur in a fetus infected with noncytopathic BVDV infection *in utero* at less than four months gestation. Persistently infected (PI) calves shed large amounts of viral antigen and are at increased risk to the herd. If PI calves are infected with a cytopathic BVDV biotype, or their non-cytopathic biotype mutates to a cytopathic biotype, they are susceptible to fatal mucosal disease.

BVDV infected camelids exhibit clinical signs similar to those in cattle; clinical manifestations include abortions, early pregnancy loss, stillbirths, PI crias, diarrhea, respiratory disease, and ill thrift. In one reported case, a PI alpaca cria had a thin fleece with long hair-like fibers (from primary follicles) that extended past the woolly fibers of secondary follicles. These clinical findings were similar to fleece abnormalities in PI lambs infected with Border disease. Reportedly, *in utero* BVDV infection in NWC results in PI crias; and, like PI calves, PI crias are viremic and shed large amounts of viral antigen. However, the stage of gestation during which a camelid fetus is persistently infected is unknown.

A study involving more than 12,000 alpacas in North America was conducted to determine the most prevalent subgenotype of BVDV in PI alpacas. RT-PCR identified 46 isolates which were non-cytopathic biotype, type 1b subgenotype. Only one of the 46 isolates was phylogenetically indistinct from cattle isolates; 45 of the 46 isolates had over 99% identity to cattle isolates.

A separate study of 63 alpaca herds from the US identified 25.4% herds with a seropositive cria and 6.4% with a PI cria. In some cases, seropositivity was associated with administration of bovine colostrums. Farms with PI crias reported economic losses associated with abortions, treatment of weak crias, diagnostic testing, and lost sales.

Some diagnostic tests for BVDV in cattle are valid in NWC. Viral antigens can be detected by virus isolation, PCR, or immunohistochemistry (IHC). BVDV can be isolated from lymph nodes, whole blood, fetal tissue and placenta. Serology is also available to detect antibodies. Detection of virus or viral antigen without antibody can indicate a PI animal or an animal in the acute viremic stage of infection. Detection of antibodies alone indicates previous exposure to the virus.

Because PI crias are believed to be the main source of infection, efforts should be made to identify these animals. If current diagnostic methods are used to identify PI crias, virus should be detected in serum or buffy coat cells twice (21 days apart). Virus can be isolated from buffy coat cells, genome can be detected via PCR of buffy coat cells, and antigen can be detected in tissue by IHC. IHC of cutaneous biopsies can react positively to germinal cells of the epidermis because hair follicles can retain BVDV antigen in a low percentage of non-PI animals. If IHC is positive, this test should be followed with a separate diagnostic test 30 days later. Detection of an antibody titer on an animal that is also positive for antigen does not rule out persistent infection. PI animals can have antibodies to heterologous vaccines or natural infections; however, titers are usually low. In cattle, it is recommended to test all new calves for 8-9 months after a PI animal is detected and removed. Because the gestation period of NWC is longer than in cattle, it is recommended to

test all crias born 11 months after removal of a PI animal. Seroprevalence studies show that there are few seropositive NWC; these findings indicate a large naïve population of NWC. Biosecurity is critical for keeping herds free of BVDV. Naïve animals, especially pregnant females, should not come into contact with infected animals. In addition to other NWC, cattle, goats and sheep are also primary carriers of the virus. Currently, vaccinating NWC with bovine vaccines for BVDV is not recommended. A bovine vaccine could interfere with diagnostic testing and the efficacy or safety of these products is not understood in camelids.

Bovine viral diarrhea virus (BVDV) should be considered in the differential diagnosis in NWC that have ill thrift, diarrhea, respiratory infections, abortion, and stillbirths. Introduction of BVDV into a NWC herd can lead to devastating economic losses. New additions to the herd should be screened for BVDV and biosecurity measures should be in place to prevent introduction of this disease.

-by Erica Twitchell, PUSVM Class of 2010
-edited by Dr. Tiffany Reed, ADDL Graduate Student

References:

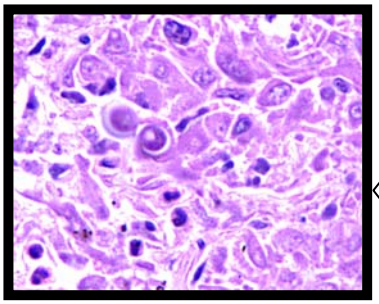
1. Belknap EB, Collins JK, Larsen RS et al: 2000. Bovine viral diarrhea in New World camelids. J Vet Diagn Invest 12: 468-570.
2. Carman S, Carr N, DeLay J et al: 2005. Bovine viral diarrhea virus in alpaca: abortion and persistent infection. J Vet Diagn Invest 17:589-593.
3. Goyal SM, Boulijihad M, Haugerud S et al: 2002. Isolation of bovine viral diarrhea virus from an alpaca. J Vet Diagn Invest 14:523-525.
4. Jones M, Boileu M: 2009. Camelid Herd Health. Vet Clin North Am Food Anim Pract 25:239-263.
5. Kapil S, Yeary T, Evermann JR: 2009. Viral Diseases of New World Camelids. Vet Clin North Am Food Anim Pract 25: 239-263.
6. Kim SG, Anderson RR, Yu JZ et al: 2009. Genotyping and phylogenetic analysis of bovine viral diarrhea virus isolates from BVDV infected alpacas in North America. Vet Microbiol 136: 209-216.
7. Mattson DE, Baiker RJ, Catania JE et al: 2006. Persistent infection with bovine viral diarrhea virus in an alpaca. J Am Vet Med Assoc 228: 1762-1765.
8. Topliff CL, Smith DR, Clowser SL et al: 2009. Prevalence of bovine viral diarrhea virus infections in alpacas in the United States. J Am Vet Med Assoc 234: 519-529.

Pathology in Practice

Dr. Tiffany Reed, ADDL Graduate student, along with co-authors Dr. Kelley Balog (PUSVM Veterinary Clinical Sciences), Drs. Katie Boes, Joanne Messick, (PUSVM Department of Comparative Pathology) and Dr. Peg Miller (the Indiana Animal Disease Diagnostic Laboratory) were featured in the "Pathology in Practice" section of the February 15, 2010 issue of JAVMA. The entire article can be found in JAVMA 236: 411-413. or http://avmajournals.avma.org/doi/pdf/10.2460/javma.236.4.411

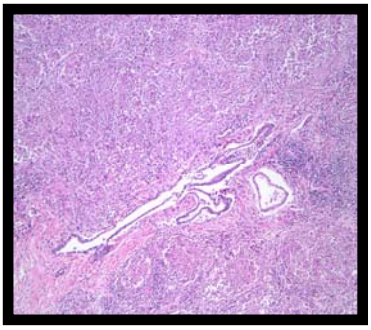
***Blastomyces* in a dog.**

Summary: A 7-year-old, intact male, German Shorthaired Pointer was submitted to ADDL following an acute history of respiratory distress, prostatomegaly, tenesmus, and anorexia. At necropsy, the lungs were dark red and non-collapsing with multifocal to coalescing, gray nodules widely disseminated throughout all lung lobes. The prostate gland was expanded by abundant pale tan soft tissue. Histologically, the lung and prostate were expanded and infiltrated by granulomatous inflammation with intralesional fungal yeasts, morphologically consistent with *Blastomyces* spp.



Numerous thick-walled, buff-colored, spherical to ovoid, broad-based budding yeasts are scattered throughout granulomatous inflammation in the prostate gland and lung. The yeasts measure approximately 5-20 µm in diameter; findings are consistent with *Blastomyces* spp.

The normal prostate architecture is replaced by aggregates of epithelioid macrophages and multinucleated giant cells (granulomas). Few serous glands are identifiable. The acinar epithelial cells are atrophied.



Histophilus somni Complex in Cattle

Histophilus somni (formerly *Haemophilus somnus*) is a Gram-negative bacterium that is a member of the Pasteurellaceae family. It appears microscopically as a cocco-bacillus, is a facultative anaerobe, non-motile, and a non-spore-forming bacteria. *H. somni* is a commensal organism of cattle that may inhabit certain mucosal surfaces, including the upper airway and reproductive tract.

H. somni was first recognized as a pathogen in cattle in 1956. *H. somni* is a pathogen of cattle worldwide, but the greatest prevalence of disease is focused in the large beef producing countries of the world, such as the United States and Canada. Infection with *H. somni* is most commonly a feedlot disease, but may be seen in dairy and grazing operations. Young growing cattle age 6-12 months are most commonly infected and show clinical signs. The actual prevalence of the bacteria is very high, and almost all cattle will be exposed at some point in their life. This can be noted in certain herds where 100% of animals have circulating antibodies to *H. somni*. Actual clinical disease, however, is uncommon in susceptible groups, with an incidence rate of 1-2% lower. Clinical disease can be devastating when it occurs. *H. somni* is capable of causing a variety of disease syndromes, including thrombotic meningoencephalitis (TME), respiratory disease (*H. somni* is a component of the Bovine Respiratory Disease Complex, BRDC), myocarditis, polysynovitis, otitis media, mastitis, and reproductive tract diseases. Historically, the most common disease manifestation was TME but, in recent years, respiratory disease and myocarditis are becoming more prevalent.

H. somni can be described as an opportunistic pathogen. The bacteria require a breakdown in mucosal immunity in order to cause disease. Many different types of events can compromise the immunity of a beef calf. These may include stress from transport, concurrent viral infection, inclement weather, weaning, etc. Certain virulence factors play a role in the disease process as well. *H. somni* exhibits a variety of different virulence factors, such as lipooligosaccharide (LOS, an endotoxin), induction of apoptosis (programmed cell death of bovine endothelial cells, the ability to inhibit destruction by phagocytic cells, histamine production, and many others.

The pathogenesis of TME involves a bacterial septicemia. Once the bacteria enter the bloodstream, they are able to evade host defenses and cause apoptosis of bovine endothelial cells. This, in turn, causes a vasculitis and thrombosis which, in the brain, leads to neutrophil infiltration and tissue necrosis (TME). In the heart this can cause myocarditis with multiple infarcts, necrosis, and abscessation.

Clinical signs of cattle with *H. somni* infection can vary greatly depending on which form of the disease the calf has. Calves with neurologic disease (TME) will often times be acutely affected and sudden death may be the only clinical sign. Other clinical signs that may be observed are fever, depression, lateral recumbency, and closed eyes ("sleeper syndrome"). If animals are still able to stand they will be ataxic, weak, and may appear blind. Regardless of the clinical signs, the course of the disease is rapid and most affected animals will die within 24 hours. The progression of disease in calves with myocarditis is also very rapid and sudden death may be the only clinical sign. A clinical diagnosis of myocarditis is rarely made, with affected cattle potentially showing signs of left heart failure (exercise intolerance, open-mouth breathing, cough, etc.). Cattle exhibiting signs of the respiratory form of the disease show nonspecific signs consistent with any pneumonic calf. Affected animals will be febrile, off-feed, show labored breathing, etc. *H. somni* is capable of causing upper airway disease as well, meaning that calves may also cough and have a foul odor emitting from their mouths.

Post mortem examination of cattle that have suffered from *H. somni* infection can reveal a variety of lesions. The most striking gross lesions will be seen in cattle with either myocarditis or respiratory disease. The left ventricular free wall is most commonly affected and will show full thickness myocardial pallor. Evidence of pulmonary congestion and edema may be noted as well. Lungs of infected cattle will exhibit a suppurative broncho-pneumonia with fibrinous pleuritis. Gross lesions of cattle with TME may be difficult to see and can be highly variable. Areas of hemorrhage and necrosis may be seen on the surface of the brain or on cut section. The brain itself may also be swollen secondary to edema.

Microscopic lesions in all affected organs include vasculitis, neutrophilic inflammation, and tissue necrosis. Colonies of Gram-negative bacteria may be seen in thrombi.

Definitive diagnosis of *H. somni* may be made in a variety of ways. These include bacterial culture, serology, and immunohistochemistry. *H. somni* can be cultured from a variety of tissues including blood, CSF, joint and pleural fluids, brain, liver, and kidney. Selective culture media is needed to ensure growth of the bacteria and samples should ideally be taken from untreated animals. Bacterial culture remains the gold standard for diagnosing *H. somni* infection. Serology may also be used to make a definitive diagnosis of *H. somni*. However, a high prevalence of seroconversion exists in many herds of cattle and may not reflect an acute infection. Therefore, acute and convalescent titers are needed to make a definitive diagnosis which is often times impossible due to the rapid course of the disease. Finally, immunohistochemistry may be used to identify *H. somni* in formalin-fixed tissues.

H. somni is susceptible to a wide variety of antibiotics, and treatment decisions are often made depending on a veterinarian's experience with a certain drug. Treatment

for all forms of the disease complex have often been with oxytetracycline. Oxytetracycline has been effective in the treatment of TME when the drug is given at the onset of clinical signs. Treatment is often unsuccessful when antibiotic therapy is delayed or if the animal is already recumbent. Treatment of bronchopneumonia associated with *H. somni* may be accomplished with a variety of antibiotics, including oxytetracycline. The bacteria are also susceptible to many of the antibiotics commonly used to treat bovine pneumonia, including some of the newer, longer-acting medications.

Prevention of the *H. somni* disease complex can be difficult due to the ubiquity of the organism. Commercial bacterins are available, but their efficacy is questionable. Field trials to test the efficacy of the vaccine are difficult to perform due to an inability to consistently recreate the disease process. Most vaccines are labeled for protection again TME only, not the other forms of disease. If calves are to be vaccinated it should be performed prior to entry into the feedlot. Metaphylaxis has also been used to prevent *H. somni* infection. Metaphylaxis involves pre-treatment with antibiotics prior to clinical illness. This is often done when calves enter the feedlot. They are given an injection of a long-acting antibiotic that is designed to protect them against infection in the early, most stressful time at the feedlot. Metaphylaxis is widely used in the prevention of BRDC, which includes *H. somni*.

-by Dr. Nathan Ahlemeyer, Class of 2009
-edited by Dr. Ryan Jennings, ADDL Graduate Student

References:

1. Fecteau G, George LW: 2004. Bacterial meningitis and encephalitis in ruminants. Veterinary Clinics of North America: Food Animal Practice 20:363-377.
2. Harris FW, Janzen ED: 1989. The *Haemophilus somnus* disease complex (Hemophilosis): A Review. Canadian Veterinary Journal 30:812-822.
3. Large Animal Internal Medicine, 4th ed. Bradford Smith. 2008.
4. Pathologic Basis of Veterinary disease, 4th ed. McGavin and Zachary, 2007.
5. Siddaramppa S, Inzana TJ: 2004. *Haemophilus somnus* virulence factors and resistance to host immunity. Animal Health Research Reviews 5(1): 79-93.
6. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses: 2007. Eds.: Radostits OM et al.
7. Wessels J, Wessels ME: 2005. *Histophilus somni* myocarditis in a beef rearing calf in the United Kingdom. Veterinary Record 157: 420-421.

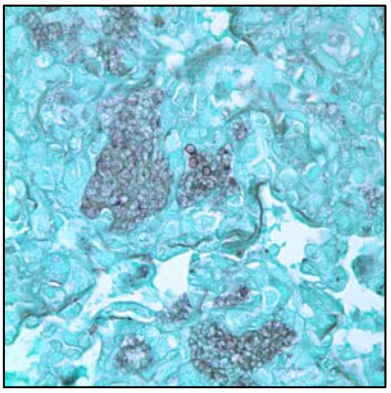
Final Diagnosis

Pulmonary Pneumocystosis in a Mouse

History: An adult female, brown and gray, B6RKO mouse was submitted dead to the ADDL for necropsy. The history indicated that the mouse had post-partum bloody vaginal discharge and respiratory distress.

Gross findings: The mouse was in poor body condition. All lung lobes were diffusely dark red to purple, firm, and oozed bloody fluid on cut section.

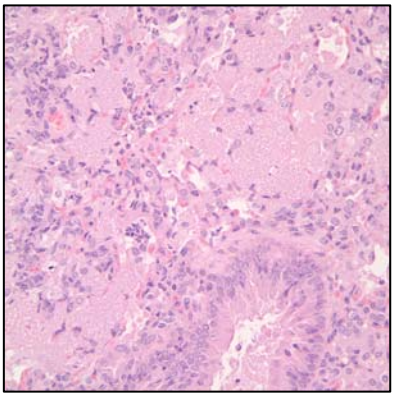
Histopathologic findings: Alveolar septa were diffusely thickened by infiltrating mononuclear cells including macrophages and lymphocytes. Alveoli were filled with eosinophilic amorphous, granular to flocculent material mixed with macrophages, neutrophils, sloughed epithelial cells and fewer lymphocytes. Bronchioles were partially filled with eosinophilic foamy material, proteinaceous debris and neutrophils. The eosinophilic material consisted of numerous indistinct, 3-5 microns in diameter, round to ovoid yeast-like organisms (fungal trophic forms or cysts) with rare pale basophilic nuclei. Gomori's methanamine silver (GMS) stain demonstrated numerous 3-5 micron in diameter, round to ovoid organisms, consistent with *Pneumocystis murina*.



Numerous round to oval cysts are demonstrated within the flocculent intra-alveolar material (GMS, x 100)

Ancillary testing: No bacteria were isolated from the lung.

Discussion: Microscopic lesions with characteristic intra-alveolar fungal organisms support a diagnosis of pulmonary pneumocystosis in this mouse. Pneumocystosis in mice is caused by *Pneumocystis murina* according to new nomenclature. At one time, all were classified as *P. carinii*; however molecular studies have revealed that the genus *Pneumocystis* contains five species that inhabit different mammalian hosts: *P. murina* infects mice, *P. jiroveci* infects human beings, *P. carinii* and *P. wakefieldiae* are found in rats, and *P. oryctolagi* is reported in rabbits. Other domestic animals are infected by *P.*



Alveoli are filled with eosinophilic amorphous material containing numerous pale eosinophils to clear, round organisms, which was accompanied by inflammatory leukocytes (H&E, x 40)

carinii. A lethal pneumonia caused by *Pneumocystis* spp. is a problem in immuno-compromised animals, including young dogs, foals, goats, pigs and laboratory animals as well as humans. Immunocompromised states due to congenital immunodeficiency, viral infection, chemotherapy, administration of corticosteroids and other underlying diseases can enhance the growth of *Pneumocystis*. The predisposed condition leading to pneumocystosis in this case was not determined.

Pneumocystis spp. resides extracellularly in the pulmonary alveoli and, as a fungal organism, a trophic form (trophozoite) and a cyst (ascus) exist. A trophic form, primarily the proliferative stage, is 1-4 microns in diameter, uninucleate, irregularly shaped and thin-walled. A cyst, the reproductive stage, is 5-8 microns, thick-walled and contains 8 round ascospores. Following inhalation of the cysts, ascospores are released in the host alveoli and develop into trophic forms. The infection is initiated by attachment of trophic forms to type 1 pneumocytes with clusters of organisms growing and filling the alveolar lumen. However, the entire life cycle has not been determined.

Clinical diagnosis of *Pneumocystis* pneumonia is difficult because specific alterations in hematological or biochemical parameters or clinical signs are usually inconclusive. Serology can provide a presumptive diagnosis. *Pneumocystis* cannot be cultured. Definitive diagnosis is based upon detection of *Pneumocystis* from respiratory fluid or biopsy samples. Histochemical stains including GMS, Grocott's, Periodic Acid Schiff and Giemsa, and immunohistochemistry are useful. Silver stains demonstrate polysaccharide moieties on cyst walls and intacystic bodies. PAS display the characteristic honeycombed material of *Pneumocystis*. Molecular diagnostic techniques such as *in situ* rRNA hybridization, DNA hybridization and polymerase chain reaction (PCR) are developed to identify the specific organisms.

-by Dr. Nozomi Shimonohara, ADDL Graduate Student

References

1. Caswell JL, Williams KJ: 2007. *Pneumocystis carinii*. In Maxie MG ed. Jubb, Kennedy and Palmer's Pathology of Domestic Animals. St. Louis, MO: Elsevier Limited. P. 593.
2. Percy DH, Barthold DW: 2007. *Pneumocystis murina* Infection: Pneumocystosis. In: Pathology of Laboratory Rodents and Rabbits. Blackwell Publishing Professional, Ames, IA. Pp83-84.
3. Keely SP, Fisher JM, Cushion MT, Stringer JR: 2004. Phylogenetic identification of *Pneumocystis murina sp. nov*, a new species in laboratory mice. Microbiology 150:1153-1165.

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

May 31……Memorial Day
July 5……Independence Day
September 6……Labor Day
November 25-26……Thanksgiving
December 23-24……Christmas
December 30-31……New Year

Congratulations to Dr. Jose Ramos-Vara, ADDL pathologist, who successfully reached the summit of Cerro Aconcagua, during a climb lasting more than three weeks in January 2010. Aconcagua is located in Argentina close to the Chilean border and, at 22,841 feet, is the highest mountain outside of the Himalayas.

⇦ Going through a Penitentes field on the way to Camp II. Penitents are unique forms of frozen snow produced by the combined effect of wind and heat and can be taller than a human being.

View of the awesome south face of Aconcagua from the north summit ⇨

⇦ View of Cerro Aconcagua (center) from Cerro El Plomo, 5,432 meters (17,795 feet) in Chile

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. 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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