

Diagnostic Forum

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

V O L 2 2 N O 2

S P R I N G 2 0 1 2

Inside the Forum

| | |
|---|------|
| White Snakeroot Toxicity | P.1 |
| Viral Hemorrhagic Septicemia in the United States | P. 2 |
| Development of DNA Vaccine against H1N1 Subtype Swine influenza viruses | P. 2 |
| Laboratory Assessment of Liver Disease in Small Animals | P. 3 |
| ADDL Schedule | P. 4 |
| Laboratory Test Result Reporting Options | P. 4 |

From the Director

Stephen B. Hooser

Bovine Spongiform Encephalopathy

On April 24th, the USDA confirmed the nation's 4th case of Bovine Spongiform Encephalopathy (BSE) in an animal that was sampled for the disease at a rendering facility in central California. This animal was never presented for slaughter for human consumption, so at no time presented a risk to the food and milk supply, or to human health in the United States. This case was determined to be caused by an atypical form BSE, meaning that it was not the same BSE agent as that which was responsible for "Mad Cow Disease"

As stated by Dr. Ron DeHaven, chief executive officer of the AVMA and graduate of the Purdue College of Veterinary Medicine, "The finding of this BSE-positive cow is not particularly surprising, and it is certainly no cause for alarm. It is not surprising because we have known for several years that there is a very low prevalence of BSE in our nation's cattle population. USDA has maintained a good, targeted surveillance program for the disease, and it is expected that we might find such cases periodically. This finding is not cause for alarm because the tissues of any infected cows that pose a food safety risk, i.e. specified risk materials or SRMs, have been kept out of the human food supply since early 2004. What this finding does confirm is that the safeguards put in place by the USDA several years ago are working as they are intended."

The ADDL West Lafayette and Heeke facilities contribute to BSE surveillance in Indiana and the U.S. through their participation in the USDA National Animal Health Laboratory Network and through collection and submission of cattle tissues for BSE surveillance testing. Since testing began, there have not been any cases of BSE detected in Indiana cattle.

White Snakeroot Toxicity

By Dr. Christina Wilson and Kimberly Meyerholtz

White snakeroot (*Ageratina altissima*) is a poisonous plant that is indigenous to the eastern part of North America. It is typically found in wooded areas, thickets or damp and shady pastures during mid-summer to late fall in Indiana. The toxicity of white snakeroot is thought to be attributed to the presence of the toxins tremetone, dehydrotremetone, and 2-oxyangeloyl-tremetone. This plant causes skeletal muscle necrosis and cardiotoxicity in livestock and grazing animals that ingest snakeroot at as little as 0.5-2% of their body weight. The plant toxins are also excreted in milk, therefore, suckling animals can also be susceptible to poisoning.

The onset of clinical signs in affected animals can occur within a few days to several weeks post-ingestion. Clinical signs of poisoning that are frequently observed can include weight loss, listlessness, constipation, dyspnea, salivation, acetone odor to the breath and urine (ketosis), muscle tremors or violent trembling, tachycardia or cardiac arrhythmias, and terminal collapse.

The ADDL Toxicology Laboratory has developed a diagnostic test for tremetone and dehydrotremetone in biological samples from animals exposed to white snakeroot. In suspect cases of poisoning, the following samples can be submitted to the diagnostic laboratory for testing: Blood (EDTA), kidney, liver, plant material, serum, or rumen/stomach contents. The clinical signs, presence of white snakeroot in the hay or pasture, increased muscle enzymes (CK, ALP, and AST), cardiac or skeletal muscle degeneration on histopathology or identification of plant fragments in rumen or stomach contents are also supportive of a diagnosis of white snakeroot poisoning. Other differential diagnoses to consider include pesticide intoxication, ionophore toxicity, vitamin E/selenium deficiency, other poisonous plant exposure (e.g. rayless goldenrod, hairy vetch), exertional myopathies, or acute infectious diseases.

Due to the high mortality of most cases, preventing exposure to white snakeroot is important. This includes limiting access to the plant and preventing consumption of raw milk from exposed animals.

References:

1. Lee ST, Davis TX, Gardner DR, Colegate SM, Cook D, Green BT, Meyerholtz KA, Wilson CR et al:2010. Tremetone and structurally related compounds in white snakeroot (*Ageratina altissima*): A plant associated with trembles and milk sickness. J Agri Food Chem 58:8560-8565.
2. Lee ST, Davis TZ, Gardner DR, Stegelmeier BL and Evans TJ: 2009. Quantitative method for the measurement of three benzofuran ketones in rayless goldenrod (*Isocoma pluriflora*) and white snakeroot (*Ageratina altissima*) by high-performance liquid chromatography (HPLC). J Agri Food Chem 57:5639-5643
3. Meyerholtz KA, Burcham GN, Miller MA, Wilson CR, Hooser SB, Lee ST: 2011. Development of a gas chromatography-mass spectrometry technique to diagnose white snakeroot (*Ageratina altissima*) poisoning in a cow. J Vet Diag Invest 23:775-779.
4. United States Department of Agriculture, Natural Resources Conservation Service, <http://plants.usda.gov>
5. Zoltani CK: 2012. In: Veterinary Toxicology: Basic and Clinical Principles. R. Gupta (editor) Elsevier, Inc., San Diego, CA, pp. 235-245.





Viral Hemorrhagic Septicemia in the United States

By Dr. Nelson Bricker,

Edited by Dr. Tsang Long Lin, ADDL Pathologist

Abstract: Viral hemorrhagic septicemia (VHS) is a disease of global concern in fish for aquaculture as well as wild species. The discovery of this disease in the Great Lakes region of the United States in 2003 has almost frozen the aquaculture industry in this area in an attempt to prevent further spread of the disease by transport of live fish. VHS affecting US freshwater fish is caused by a novel genotype known as VHS virus IVb, which affects a broad range of freshwater fish with varying levels of severity. The migration of wild

fish, movement of water within a watershed, and transmission of fomites are important means of viral spread. VHS virus may infect fish by a number of routes, including virus-laden water. Acute infections show gross hemorrhage in the eye, under the skin, in the kidney, liver, swim bladder and many other tissues. Chronic infections primarily show hepatosplenomegaly. Anemia, leucopenia, darkening of color, and behavior change may be noted as well. The new genotype shows pathogenicity in both immature and mature fish. All fish infected with

the virus carry and shed it for life. A sensitive quantitative real-time polymerase chain reaction (qRT-PCR) has been developed for detecting and differentiating VHS viruses. The broad host range and possibility of spread to other states makes testing suspicious fish populations imperative to minimize the impact on the aquaculture and ecosystem communities.

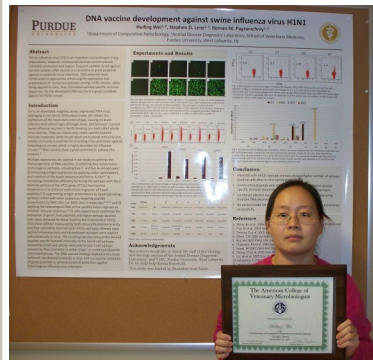
The entire article with references can be found on the ADDL website. www.addl.purdue.edu



Two sentences in the “BVD in White-Tailed Deer” article published in the Winter 2012 issue of the Diagnostic Forum were inadvertently omitted.

The following should have been included:

“Research in the Animal Disease Diagnostic Laboratory is underway with several faculty working together on this important viral disease in Indiana. Drs. Roman Pogradichniy, Leon Thacker, Duane Murphy, and Bill VanAlstine are investigating a possible link between a common cattle disease and white-tailed deer in Indiana.”



Development of DNA vaccine against H1N1 subtype swine influenza viruses

by Huiling Wei, S Lenz, D Thomson, R Pogradichniy

Abstract: Swine influenza virus (SIV) is an important viral pathogen in pig populations. However, commercial vaccines cannot provide complete protection with induced humoral immunity only and require frequent updates to fight against current isolates. DNA vaccination is an effective means of eliciting both arms of immune system, humoral and cellular immune responses. In this study, DNA vector pcDNA3.1 was inserted with a chimeric intron downstream of the CMV promoter region followed by a

Kozak sequence to enhance the expression of gene inserts. The C-terminal of VP22 gene (VP22c), encoding the tegument protein of bovine herpesvirus-1, was fused separately to the N-terminal of four quadruplicated epitopes, two B-cell epitopes (HA and M2e) and two T-cell epitopes (NP1 and NP2), which were conserved at least among H1 SIV isolates. Linkers-KK- was used to space between each copy of the two B-cell epitopes and -RVKR- was used for the two T-cell epitopes in order to enhance the presentation of epitopes to the immune system. The expression of epitopes was confirmed in *in vitro* transfection of 293FT cells and higher numbers of epitope-positive cells were achieved from those containing VP22c than those without. After the DNA plasmids were administered to mice intramuscularly in combination or separately, or boosted with recombinant proteins of quadruplicated epitopes fused to VP22c, the vaccine stimulated desired epitope-specific humoral immunity to the two B-cell epitopes and cellular immunity to the epitope NP380-393. Our results indicate that the DNA vaccines with quadruplicated epitopes fused to the VP22c may be a potential strategy in developing universal vaccines against SIV.

Huiling Wei's research project won the American College of Veterinary Microbiologists award at the Conference for Research Workers in Animal Disease annual meeting in Chicago, December, 2011.

Laboratory Assessment of Liver Disease in Small Animals

By Dr. Bill Wigle, ADDL Pathologist

Since the liver has extensive functional reserve, relatively severe injury must be present before specific clinical signs appear. In early stages of hepatobiliary disease, clinical signs are nonspecific and include anorexia, vomiting, diarrhea, weight loss, PU/PD and dehydration. In advanced disease, specific signs include jaundice, bleeding tendencies, hepatomegaly or microhepatica, hypoglycemia, hepatic encephalopathy, gastrointestinal ulceration, ascites and superficial necrolytic dermatitis.

Conditions affecting the gastrointestinal tract, endocrine, urinary, nervous, cardiovascular and hematopoietic systems, as well as neoplasia and systemic infections, can result in the same nonspecific signs seen with early hepatobiliary disease. Such non-hepatic disorders have secondary effects on the liver (reactive hepatopathies) resulting in increased serum liver enzymes which then require differentiation from primary liver disease. In fact, the majority of instances where liver enzyme levels are increased do not represent primary hepatic disease.

A complete blood count, serum chemistry panel, and urinalysis form the primary screening tests for liver disease. Hematologic tests are primarily used to determine if a systemic disease process is present and to determine if jaundice is pre-hepatic in the presence of anemia.

Alanine transferase (ALT) and aspartate transferase (AST) are "leakage" enzymes which become elevated when hepatocytes are damaged. These enzymes have both a short half-life and, if serum high levels persist with repeated measurements, ongoing disease is suggested. ALT is more specific for hepatic disease than is AST.

Alkaline phosphatase (ALKP) and gamma-glutamyl transpeptidase (GGT) are "cholestatic" or "inducible" enzymes. Bile stasis causes increased production of these enzymes which are located in the cell membranes of bile canaliculi (ALKP) and bile duct epithelium (GGT). These enzymes may also be induced by drugs or corticosteroids. Increases in ALKP alone have been associated with hepatic neoplasia and benign hepatic nodular hyperplasia. The bone isoform of ALKP may also be increased in young dogs prior to closure of growth plates or adult dogs with osteogenic tumors.

Once the presence of primary liver disease is established, the diagnostic process should proceed in a sequential manner. Elevated liver enzymes can reflect damage or the presence of inflammation in the liver, but they provide no estimate of hepatic functional capacity. Routine chemistry panel values that are indicative of liver function include bilirubin, blood glucose, cholesterol, albumen and blood urea nitrogen. Generally, advanced hepatic disease is present when these values become abnormal.

Bilirubin becomes elevated when there is excessive hemolysis, hepatocellular dysfunction and cholestasis or obstruction of the extrahepatic bile ducts. Blood glucose is reduced after loss of more than 70% of hepatic function and is often low in animals with portosystemic shunts. Cholesterol levels in serum increase in cases of extrahepatic biliary obstruction and are decreased in cases of portosystemic shunts. Reduced serum albumin is observed due to decreased synthesis in hepatic failure when about 75% of liver function is lost. Blood urea nitrogen (BUN) is reduced as a result of the liver's inability to convert ammonia to urea. This finding is most commonly associated with portosystemic shunts.

Plasma ammonia has been shown to be a sensitive and specific indicator for the presence of congenital portosystemic shunts as well as for acquired shunts. The volatility of ammonia requires that blood be collected in an EDTA-coated tube and that the specimens be placed on ice with NH₃ measurement being performed within 30 minutes. This test is essentially limited to specialty practices or those with access to a properly equipped local laboratory since samples cannot be shipped for testing.

A coagulation profile may be useful if hepatic biopsy is planned. Dogs with chronic hepatitis, with or without cirrhosis, have been shown to have reduced coagulation factors due to impaired hepatic synthesis.

Measurement of serum bile acids are more sensitive as a function test and become elevated sooner in the course of hepatic disease than the above biochemical parameters. This test is most appropriate when animals have persistent elevated hepatic enzymes and are not jaundiced.

Urinalysis demonstrating bilirubinuria in cats suggests either hepatobiliary disease or hemolysis. The presence of ammonium biurate crystals in dogs and cats occurs with portosystemic shunts.

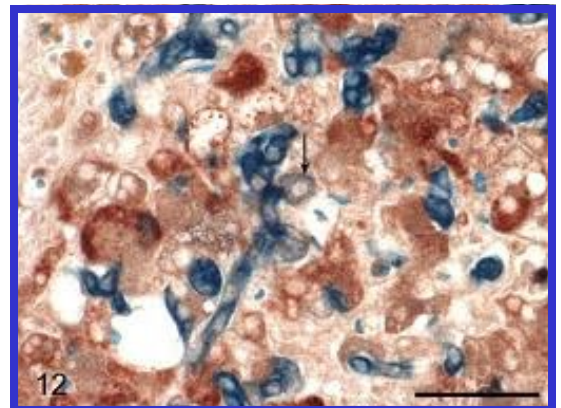
The above laboratory findings are useful in establishing the presence of hepatobiliary disease, but do not define it well. Liver biopsy provides the means to classify the type of disease, plan for specific treatment and has prognostic value. Liver biopsy specimens may be obtained via fine-needle aspiration, blind percutaneous or ultrasound-guided needle biopsy, laparoscopy and exploratory laparotomy. Diffuse liver disease may be successfully diagnosed by fine-needle aspiration or blind percutaneous biopsy. Ultrasound-guided needle biopsy, laparoscopy and laparotomy are more diagnostic when lesions are focal. Standardized histologic criteria for diagnosis of liver disease in small animals have recently been published to aid in applying biopsy findings to clinical decision making.

Hepatic biopsy is also the means of obtaining specimens for quantitative copper analysis to diagnose copper associated hepatopathy. A 1-2 gram specimen is required for chemical analysis. Levels above 1000 ppm are diagnostic. Obtaining a liver biopsy also provides a specimen for virus isolation or culture for bacteria and fungus.

A combination of diagnostic modalities is required to confirm and define hepatocellular disease in small animals. Early detection and intervention is required to maximize the effectiveness of specific therapy.

Suggested Reading:

1. Bexfield N: 2006. Diagnosis of canine liver disease. In Practice 28:444-453.
2. Rothuizen J and Twedt DC: 2009. Liver biopsy techniques. Vet Clin N Amer Sm Anim 39:469-480
3. Watson PJ: 2004. Chronic hepatitis in dogs: a current understanding of the aetiology, progression, and treatment. Vet J 167:228-241.
4. WSAVA LIVER STANDARDIZATION GROUP: 2006. Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases. Elsevier, Toronto, Canada



Purdue ADDL and Heeke ADDL will be closed on the following University holidays in 2012.

May 28.....Memorial Day
July 4.....Independence Day
September 3.....Labor Day
November 22-23.....Thanksgiving
December 24-25.....Christmas

ADDL Lab Results are available by

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

Lab results are available on the Internet. Call to set up an account or go to our web page, then

1. Click on Online Reports Tab
2. Click on Request Info and follow instructions.

Reduced UPS shipping rates for ADDL Clients

- ADDL has reached an agreement with UPS for submitters to send samples to the West Lafayette Lab at a reduced rate using its Authorized Return Service. Packages will arrive at ADDL the following morning.
- Pre-addressed labels will be provided to you by ADDL.
- Submitter will be billed \$7.00 per package (up to 15 pounds).
- Call us at 765-494-7440 or visit our website to request labels.
- If multiple cases are submitted in a single shipment, the UPS charge will be added to one case.

If you are currently using our histopathology mailers (via U.S. mail) and would prefer taking advantage of the UPS option with its guaranteed delivery time, we will provide you the formalin-filled containers without an address label at \$15./box of 12.

GlobalVetLink is available for electronically requesting and reporting Coggins tests (Equine Infectious Anemia). Both ELISA and AGID testing are available at \$8.50/test with no accession fee. In order to have access to a GlobalVetLink account, contact the company directly at www.globalvetlink.com or phone 515-296-0860.

Non-Profit Organization
U.S. Postage PAID
PURDUE UNIVERSITY

ANIMAL DISEASE DIAGNOSTIC LABORATORY
406 S. UNIVERSITY
WEST LAFAYETTE, IN 47907