Diagnostic Insights

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KANSAS STATE VETERINARY DIAGNOSTIC LABORATORY

Accredited by the American Association of Veterinary Laboratory Diagnosticians **July 2011**

Personnel Profile — Dr. Jianfa Bia

Dr. Jianfa Bai joined the Kansas State Veterinary Diagnostic Laboratory in 2007 as an assistant professor.

Dr. Bai received his PhD from K-State. He established and directed the Gene Expression Facility at K-State, and provided services and trainings in microarray and real-time PCR technologies before he joined the KSVDL.

Dr. Bai is in charge of sequencing services in the KSVDL. His laboratory is currently providing DNA sequencing services on BVDV, PCV2, PRRS and SIV.

His group is also developing new molecular diagnostic tests. Recently, a duplex real-time PCR to detect *Brucella canis*, and a multiplex PCR to detect and differentiate *E. coli* serogroups were developed. The *B. canis* test is able to detect all *Brucella* species, at the same time, *B. canis* specifically. The *E. coli* test included molecular targets of *stx*1, *stx*2, *eae*, *hly*A, and O-antigens of O26, O45, O103, O111, O121, O145 and O157 *E. coli* serogroups.

In addition to his service responsibilities, Dr. Bai is involved in few research projects. His group has cloned and characterized full genomes of 2 type I and 6 type II porcine Torque teno viruses that are becoming increasingly important to swine production. A series of potentially infectious clones have been generated, which will significantly enhance animal challenging studies as there is currently no cell culture line available to effectively propagate the virus.

Another project is to sequence VP4 and VP7 segments of about 250 group -A rotavirus strains in order to study the diversity of two segments, and to identify potentially new vaccine targets.

Dr. Bai is also working on *E. coli* super-shedding strains, and trying to identify molecular targets that can differentiate high and low shedding strains using second generation sequencing technology.

Dr. Bai can be contacted at: jbai@vet.k-state.edu or 785-532-4332.

Sorbitol Dehydrogenase in Large-Animal Serum Chemistry Profiles—Dr. Lisa Pohlman

So, What's up with the "SDH" on the Large Animal Serum Chemistry and Hepatic Profiles?

Sorbitol dehydrogenase (SDH), aka iditol dehydrogenase (ID), activity is now being included in all Large Animal **serum** chemistry and hepatic profiles.

SDH is a cytoplasmic enzyme that catalyzes the conversion of fructose to sorbitol in hepatocytes. SDH activity in the blood is usually low, but when increased is an indicator of **acute hepatocellular injury or necrosis** that may be reversible or irreversible, focal or diffuse.

The half-life of SDH is very short (~ 4 hours); therefore, after a single insult to the liver SDH activity increases rapidly and then decreases very soon after peaking. However, a continued increase in SDH activity over a period of days or weeks is indicative of ongoing hepatocellular injury or necrosis.

Measurement of SDH activity is primarily done for horses and cattle because other commonly used enzymes that increase with hepatocyte damage (eg. AST, LD) are not liver specific. Conversely, in dogs and cats ALT is relatively liver specific and more stable than SDH, thus SDH is not commonly used for these species.

If you would like to know more about SDH in large animals, Dr. Pohlman can be reached at:

lpohlman@vet.k-state.edu
or
785-532-4882.

Inside this issue:

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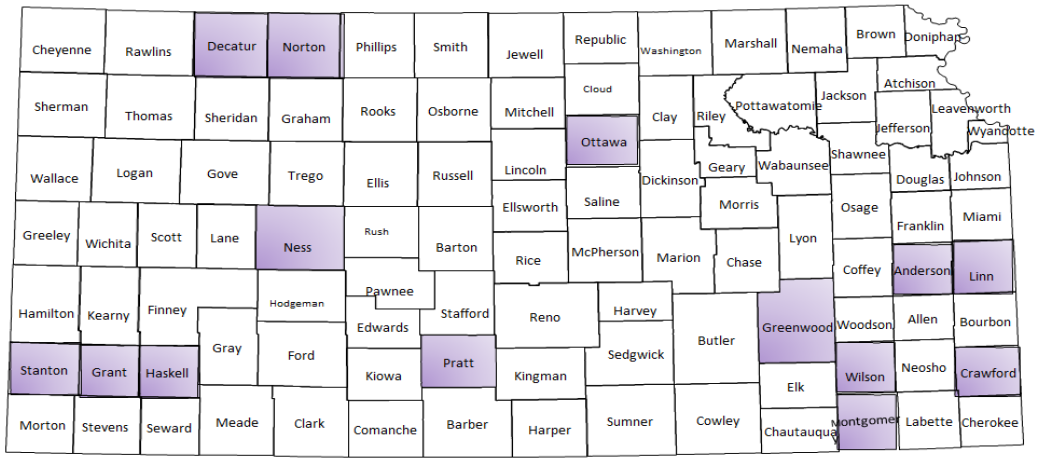
Kansas Counties With At Least One Positive *Tritrichomonas foetus* Laboratory Result in 2011

According to the Kansas Department of Agriculture Division of Animal Health & KSVDL, the Kansas counties designated below have had at least one positive *Tritrichomonas foetus* laboratory result in 2011.

In the spring of 2012, we are planning to conduct a study to determine the prevalence of Trich in Kansas cow-calf herds. **As a Kansas veterinarian, if you would like to participate in the study, please contact Dr. Hanzlicek at gahanz@vet.k-state.edu or 785-477-2001 for more information.**



Using Diagnostic Tests to Investigate Bovine Herd Health Issues—Dr. Gregg Hanzlicek

Recently, veterinarians from the KSVDL were asked by a producer and his veterinarian (Dr. Aaron Larson, KSU-CVM, 2007) to help investigate a dairy herd with health problems.

The presenting signs were sporadic episodes of cows, in multiple stages of lactation, with constipation and low production. Additionally, recently calving cows were not responding to treatments for common post-calving diseases, and some early lactation cows were diagnosed with clinical ketosis.

Two cows were necropsied by Dr. Larson and tissues sent to KSVDL for further analysis. Dr. Jerome Nietfeld found disseminated accumulation of lipids within hepatocytes in one liver and the accumulation of some lipid within hepatocytes in the second liver.

A sight visit was planned with several senior pathology-rotation students going along.

During the visit, rumenocentesis were performed to assess rumen health and serum was collected from both pre-calving and post-calving cows.

Rumenocentesis revealed reduced numbers and activity of normal rumen flora. Serum non-esterified fatty acid (NEFA) and beta-hydroxybutyric acid (BHBA) clinical pathology results revealed severe negative energy balance in the pre-calving cows and subclinical ketosis in the lactation herd.

Based on the investigation, dietary changes were instituted and the producer has reported a significant improvement in herd health.

We're on the web @ www.ksvdl.org

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Virology
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Help us help you:

- E-mail and Fax: Please make sure we have your correct email and fax information.

Rabies Prevention and Control—Dr. Mike Moore and Rolan Davis

In May we received the updated *Compendium of Animal Rabies Prevention and Control*.

This month we would like to review the compendium recommendations.

For dogs, cats and ferrets exposed to a rabid animal:

If the animal is unwanted: Euthanize

If wanted and vaccinated: booster and observe for 45 days.

If wanted but unvaccinated: Euthanize

If the owner is unwilling to agree to euthanasia, strict isolation for 6 months with a vaccine given 30 days prior to release is an option.

During this isolation you might want to consider the Texas Protocol: a vaccination series that approximates the post exposure treatment for humans. (This is not in the compendium. For more information contact Mike or RD.)

If wanted but over-due for rabies booster: Handle on a case by case basis with your local health department.

For dogs, cats and ferrets that bite humans:

If unwanted: Euthanize and test for rabies by Direct Florescent Antibody (DFA).

If wanted but has neurologic signs: Euthanize and test by DFA.

If wanted but healthy: Regardless of vaccination status these animals can be confined and observed for 10 days. If signs of rabies develop in the 10 days, the animal should be euthanized and tested. **Do not vaccinate these animals during this 10 day period as an adverse reaction might confuse the health status.**

For wild animals that bite humans:

Euthanize and test by DFA (We do not have shedding data for these animals).

We get many calls about these different time-frames, and if you do not deal with them every day they can be confusing. **So please call if you have any questions. (785-532-4503). Besides that, it gets mighty lonely here with only RD to talk to.**

New GCMS poison screening test in the toxicology laboratory—Dr. Deon van der Merwe

Gas chromatography coupled with mass spectroscopy (GCMS) is one of the most powerful "general" methods for identifying a wide range of toxicants.

GCMS is suitable for compounds that are heat stable, at least somewhat volatile at temperatures below 300^0C, and have a molecular mass between 35 and 600. These characteristics are shared by a large proportion of the pesticides in use, as well as many pharmaceuticals and natural toxins.

Mass spectroscopy can be used to identify compounds by comparison to a mass spectrum library, usually with a high level of confidence, without the need for a physical laboratory standard that contains the compound of interest. It opens up the range of potential identifications to thousands of compounds contained in searchable libraries.

GCMS libraries available at KSVDL include most pesticides, drugs and environmental toxicants in use in North America that are detectable by GCMS. It is therefore a useful screening tool when the specific toxicant involved in poisoning is uncertain, or when high confidence confirmation is needed when a compound was detected by another method such as thin layer chromatography.

There are, however, some important factors to keep in mind when considering the use of GCMS as a toxicant screening tool. The results are typically qualitative, in other words, it will indicate presence of a particular compound without providing accurate information on concentration.

When quantification is needed additional tests, requiring physical laboratory standards, are usually required. Heat labile, non-volatile, and very low or very high molecular mass compounds will not be detected, while some compounds may not be extractable into suitable solvents using general methods. Therefore, although a large proportion of potential toxicants can be identified with a general GCMS screen, the test should not be used to exclude the possibility of poisoning.

If a specific compound is suspected, it may be beneficial to let the laboratory know because extraction procedures can be optimized for certain compounds.

Suitable samples for GCMS may include stomach contents or vomitus, tissues such as liver or kidney, body fluids such as serum or urine, and environmental samples such as water and feed.

Samples should be submitted in leak-proof containers, and perishable samples should be frozen and sent on ice. Results for typical samples will be available in 2-4 days. More complex analyses may take more time.

The price is $98 per sample. To order the test, please ask for a "GCMS Toxicant Screen".

Dr. van der Merwe can be contacted at: dmerwe@vet.k-state.edu or 785-532-4333.

A Conversation About Titers—Dr. Dick Hesse and Dr. Bill Fortney

In veterinary medicine we use very specific "titers" to determine;


1. If an active infection is present; 2. If the animal had a past exposure to an infectious agent (surveillance); or 3. If an animal is "protected" against a specific infectious agent.

What is a titer?
For this discussion we will use the Wikipedia definition of titer. "A titer is a way of expressing a concentration. Titer tests employ serial dilution to obtain quantitative information from an analytical procedure that inherently only evaluates a positive or negative. The titer corresponds to the highest dilution (end point) that still yields a positive reaction for what you are looking for."

What is a titer actually measuring?
The titer or quantity/concentration of a substance is dependent upon the test and what it is designed to detect. The three most common " titers" we will discuss are infectivity titers, antigen titers, and the most common of all, antibody titers.

Infectivity titers are based on the actual level of infectivity of a virus or bacteria. These titers are determined by preparing ten-fold dilutions of the infectious stock material and looking for growth of the organism in cell cultures, bacterial medias or in some cases host animals. A lethal dose 50 (LD50) refers to the concentration of an organism that results in the death of 50% of the animals tested. An infectious dose 50 (ID 50) refers to the concentration of an organism that results in infection of 50% of the animals tested. A protective dose 50 (PD 50) refers to the concentration of a drug that protects 50% of the animals that were tested. In each of the above titration examples, serial dilutions are performed in order to determine the concentration.

How are antigen titers determined?
Antigen titers are determined by preparing a dilution series (usually two-fold) and then looking for the highest dilution that still goes a positive reaction in the test system. Viral hemagglutination of red blood cells is one method of determining "HA" concentration. Another test system that is commonly used to detect the presence of a pathogen is the enzyme-linked immunoassay or ELISA—parvovirus, influenza and rotaviruses are common "antigen ELISAs".

What are titer tests?
The most common titer tests that are conducted are the antibody assays. In these systems serum samples are serially diluted (usually twofold) and the highest dilution of the serum that gives a positive reaction is known as the antibody end point or titer. Neutralization assays, hemagglutination inhibition assays, and dilution ELISAs will provide titer information.

If the test sample is only subjected to a single dilution and not serial dilutions, and an actual endpoint is not determined then, the result is only positive or negative and no "titer" information is obtained. People try to derive titer information based on SP ratios, thinking that the higher the ratio, the more antigen or antibody is present in the sample. Sometimes this is true, but it is conditional and very misleading. Slight changes in the pathogen, antigen or antibody will greatly change the SP ratio and therefore its potential use as a titer indicator.

Our next article will deal with small animal vaccine induced "protective" titer information and how to get the most out of it.

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Dr. Fortney can be reached at: wfortney@vet.k-state.edu

Help us help you:

- **Please make sure you are using the current KSVDL forms: go to www.ksvdl.org for the latest version.**

Developing, Delivering Accurate, Innovative Diagnostic Services

The mission of the Kansas State Veterinary Diagnostic Laboratory (KSVDL) is to develop and deliver accurate, innovative, and timely diagnostic and consultative services to the veterinary and animal health community while providing support for teaching, training, and research programs.

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We're on the web!
www.ksvdl.org

Continuing Education

August 19, 2011
5th Annual Conference for Care of Llamas and Alpacas

September 24, 2011
Natural Disasters. . . . .What About the Animals?

October 7, 2011
Annual Fall Conference on Animal Diagnostics and Field Applications

October 14, 2011
Ophthalmology Conference and Wet Lab

http://www.vet.ksu.edu/CE/Conference.htm

Test Results & Schedules

Laboratory results may be accessed online 24 hours a day, 7 days a week!!

To set up an account go to:

www.ksvdl.org

KSVDL will be closed on the following days:

Labor Day: September 5, 2011
Thanksgiving: November 24 and 25, 2011
Christmas: December 26, 2011

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