Kansas State Veterinary
DIAGNOSTIC LABORATORY

JULY 2016

DIAGNOSTIC INSIGHTS

Laboratory Diagnosis Update of Canine Leptospirosis

By Dr. Kenneth Harkin, Veterinary Health Center KSU-CVM

The two tests routinely utilized for the diagnosis of canine leptospirosis are serology by the microscopic agglutination test (MAT) and polymerase chain reaction (PCR) testing of urine as these tests offer the best combination of sensitivity, specificity and ease of submission. The optimum confirmation of a diagnosis of leptospirosis with the MAT is to document a 4-fold rise or higher in the reciprocal titer over a 2-4 week period (e.g., 800 initially and then 3200 two weeks later). If the 2-week convalescent titer fails to document the expected 4-fold rise, it is recommended to check at 4-weeks. For various reasons, however, it may be inconvenient or impossible to do the convalescent titer. In those scenarios, a single reciprocal titer of 6400 or higher would yield a high confidence in the diagnosis. The sensitivity of the test declines as a single reciprocal titer cut-point for a diagnosis increases, but the specificity improves, eliminating the effect of prior vaccinations. Lower titers in the unvaccinated dog may also be considered supportive of the diagnosis when clinical signs are consistent with the disease. The MAT is intended to identify the serogroup of the infecting serovar and not the specific serovar, but even the infecting serogroup is misidentified up to 50% of the time by the MAT. This does not diminish the value of the MAT in establishing a diagnosis, however.

Some dogs, especially those < 1 year of age, may never seroconvert, but a diagnosis can still be obtained by use of the PCR. The PCR identifies the presence of the leptospiral organism in urine and is unaffected by previous vaccinations. The test is reported by the laboratory as either positive or negative for pathogenic leptospires. In the presence of supportive clinical signs, a positive PCR confirms the diagnosis of leptospirosis. The PCR can also be used to detect the carrier/shedding status of animals, something that the MAT cannot predict. A urine sample for PCR testing is best obtained prior to the initiation of antibiotic therapy, although some dogs will remain positive for several days after starting antibiotics. Urine can be collected and stored in the refrigerator for up to 5 days with no significant reduction in sensitivity of the PCR test, allowing the veterinarian time to decide whether or not to submit the test while not delaying therapy.

The PCR may be negative if the patient is not shedding leptospires in the urine or there are too few organisms to be detected, either because the patient is not in the leptospiruric phase (shedding leptospires in the urine) of the disease or due to antibiotic therapy initiated prior to diagnostics. Although it is rare that a dog with clinical disease from leptospirosis would not be in the leptospiruric phase, the PCR can be performed on other bodily

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www.ksvdl.org/accounting-and-billing/

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Blue-Green Algae: Be Aware of the Risks

By Dr. Deon van der Merwe, Kansas State Veterinary Diagnostic Laboratory

Along with long summer days and warm weather, the blue-green algae bloom season is in full swing in Kansas, and it is again necessary consider the risks when people and animals have access to lakes and ponds. Excess nutrients in ponds and lakes, in combination with warmth and sunlight, make it possible for cyanobacteria (also known as blue-green algae) to multiply exponentially, leading to discoloration of the water and/or the formation of scums, a condition known as a HAB (harmful algal bloom). HABs have the potential to generate dangerous toxins that can sicken or even be deadly to people and animals. Some of the toxins can be irritating to the skin and mucous membranes. Inhalation of water droplets, for example when swimming or water skiing, can cause irritation in the airways. Ingestion of affected water is, however, the most dangerous form of contact. It can lead to severe gastrointestinal inflammation with vomiting and diarrhea, and absorption into the rest of the body may lead to life threatening adverse effects in the liver and the nervous system. Not all types of blue-green algae or other causes of water discoloration and scums produce toxins, but if such conditions are encountered it is best to assume that the water could be dangerous until its safety can be confirmed. If water discoloration or scums are encountered at a publicly accessible lake, please contact the HAB Hotline (785-296-1664) established by the Kansas Department of Health and Environment (KDHE), or file a report on their website (http://www.kdheks.gov/algae-illness). For private lakes and ponds, samples may be sent to the Kansas State Veterinary Diagnostic Laboratory (KSVDL) for testing. KSVDL can be contacted at 866-512-5650.

Blue-green algae "bloom" or "scum." Remember to bathe pets immediately if they come into contact with Blue-green algae

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KSVDL Celebrates Dr. Brad DeBey

Dr. Brad DeBey, who has spent the past 20 years using his practical knowledge and pathology expertise with the Kansas State Veterinary Diagnostic Laboratory and Department of Diagnostic Medicine and Pathology, retired in June.

Dr. DeBey (center) oversees a necropsy procedure.

A 1983 graduate of the Kansas State University College of Veterinary Medicine, Dr. DeBey has served clientele including practicing veterinarians, animal owners/livestock producers, research scientists and regulatory officials. He's also well known for his ability to handle case material for any species. Dr. DeBey trained approximately 30 anatomic pathology residents and graduate students, and hundreds of students who have participated in the pathology rotation for fourth-year veterinary students. In addition, he has over 50 scientific publications and case reports. Dr. DeBey has been an outstanding anatomic pathologist, all-purpose diagnostician, mentor, colleague, and friend to many. We wish him the very best during his retirement. He will be greatly missed.

Equine Herpes Virus (EHV) Diagnostics

By Dr. Elizabeth Davis, Department of Clinical Sciences, KSU-CVM and Dr. Mike Moore,
Kansas State Veterinary Diagnostic Laboratory

Recent news accounts across the nation have brought much attention in the equine world to the neurological form of Equine Herpes Virus (EHV). This is a brief review of laboratory methods for establishing a definitive diagnosis of EHV 1 and 4 associated disease conditions.

EHV-1 and EHV-4 are most widely recognized as causing morbidity in horses. Both EHV-1and EHV-4 can lead to the induction of upper respiratory disease in horses, while EHV-1 is also associated with abortion, neonatal disease, chorioretinopathy and severe neurologic disease, termed equine herpes myelitis (EHM). Current estimates suggest that approximately 10% of horses that develop clinical signs of disease associated with EHV-1 infection may progress to demonstrate neurologic signs. EHV-1 viral infection results in cell-associated viremia, where infected host leukocytes effectively disseminate virus throughout the infected horse. EHV-1 has been determined to exist in two distinct strains (differ in genotype) with the abortion strain referred to as the wild type strain (EHV1w) and the more pathogenic strain that has a different (mutated) genetic sequence (EHVm). In contrast, EHV-4 is predominately restricted to the upper airways and is not generally associated with abortion or neurologic disease.

Clinical signs of respiratory disease associated with EHV-1 and 4 typically include pyrexia, marked serous nasal discharge, and occasionally cough. Secondary bacterial colonization results in nasal secretions changing from mucoid to mucopurulent in nature. Polymerase chain reaction (PCR) testing has become the diagnostic test of choice due to its high analytical sensitivity and specificity. Positive PCR test results may be obtained when culture techniques are not successful due to low level viral shedding. Samples can be collected from the respiratory tract and include nasal swab sampling or nasopharyngeal lavage collection. Additionally, due to the leukocyte associated nature of the EHV-1 virus, whole blood (anticoagulated) buffy coat sampling provides an additional sample for viral PCR testing.

In EHV-1 and 4 suspect cases, nasal swabs and whole blood samples should be tested simultaneously (in parallel) to further enhance diagnostic sensitivity. Nasal swab sampling is recommended over pharyngeal lavage sampling due to greater diagnostic sensitivity3. Samples should be collected using a synthetic swab (not cotton) to maximize PCR testing accuracy. Diagnostic testing requires careful interpretation as outlined in the 2009 ACVIM Consensus Statement on this pathogen and disease process, the interested reader is referred to this particularly valuable reference for a more detailed diagnostic outline.

In the next issue of Diagnostic Insights, we will discuss reproductive and foal manifestations of equine herpes virus.

References

1. Pusterla N, Mapes S, Akana N et al. (), Prevalence factors associated with equine herpesvirus type 1 infection in equids with upper respiratory tract infection and/or acute onset of neurological signs from 2008 to 2014, Vet Rec. 2016;178: 70
2. Lunn DP, vis-Poynter N, Flaminio MJ et al. (), Equine herpesvirus-1 consensus statement, J.Vet.Intern.Med. 2009;23: 450-461
3. Pusterla N, Mapes S, Wilson WD (), Diagnostic sensitivity of nasopharyngeal and nasal swabs for the molecular detection of EHV-1, Vet.Rec. 2008;162: 520-521

For more information about this disease or testing options, please contact KSVDL Client Care at 866-512-5650 or clientcare@vet.k-state.edu.

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DIAGNOSTIC *INSIGHTS*
www.ksvdl.org

Using the Canine Mast Cell Tumor Panel for Prognosis Guidance

By Dr. Chanran Ganta, Kansas State Veterinary Diagnostic Laboratory

Canine cutaneous mast cell tumor (MCT) is a common neoplastic disease in dogs compromising up to 20% of all cutaneous neoplasms. The diagnosis of canine mast cell tumors are usually straightforward, but unfortunately establishing an accurate prognosis was not very likely until now.

Recently, most canine mast cell tumors are histopatholgically being graded according to the 2 –tier grading system due to superior consistency in grading by the pathologists. The Grading is based on mitotic index and morphological features in MCT.

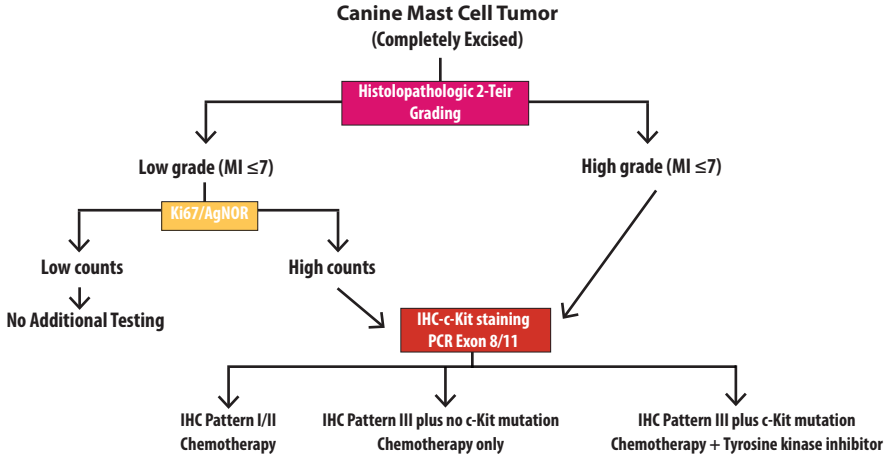
Histopathological grading provides tumor prognosis in a broad sense but prognostic panel testing assists in determining a more accurate, patient-tailored prognosis that will aid in administering specific therapeutic protocols (see figure 1).

Guidance when using the panel is shown in table 1.

Table 1. **Significance of mast cell tumor prognostic panel**

Test Type	Significance
c-Kit gene mutations on Exon 11	Poor prognosis but respond well to tyrosine kinase inhibitors like, Palladia and Kinavet-CA1
c-Kit protein expression	Staining pattern I is associated with good prognosis Staining patterns II and III are associated with decreased survival
Ki67 and AgNOR counts	Determine proliferative index and complement mitotic index for low grade tumors only

Figure 1. **Therapeutic algorithm for canine MCT**



This panel is performed on tumor biopsy specimens.

For more information please call KSVDL Client Care at 866-512-5650.

Update *(continued from page 1)*

fluids (blood, CSF, semen) or tissues (liver, kidney, lung, brain), so contact the laboratory if you would like alternative samples tested.

The PCR and the MAT can be performed on any species of animal, not just dogs.

Given that false negative test results may occur with either the MAT or PCR, performing both tests on a patient in which leptospirosis is suspected is recommended and the combination provides the greatest diagnostic accuracy.

Pricing: 6 serovar MAT: $15.50 ; PCR: $33 ; Combination: $43.50

For more information contact Dr. Harkin at harkin@vet.k-state.edu or 785-532-5690 and KSVDL Client Care at 866-512-5650 or clientcare@vet.k-state.edu

KSVDL Personnel Activities

Publications:

- Padmanabhan A, Hause BM. Detection and characterization of a novel genotype of porcine astrovirus 4 from nasal swabs from pigs with acute respiratory disease. Arch Virol. 2016 Jun 21
- Rachel M. Palinski, Zhenhai Chen, Jamie N. Henningson, Yuekun Lang, Raymond R. R. Rowland, Ying Fang, John Prickett, Phillip C. Gauger and Ben M. Hause. Widespread detection and characterization of porcine parainfluenza virus 1 in pigs in the USA. Journal of General Virology 2016 97: 281-286.
- Hause BM, Myers O, Duff J, Hesse RA. Senecavirus A in Pigs, United States, 2015 Emerg Infect Dis. 2016 Jul;22(7):1323-5. doi: 10.3201/eid2207.151591.
- Abdou Nagy, Jinhwa Lee, Ignacio Mena, Jamie Henningson, Yuhao Li, Jingjiao Ma, Michael Duff, Yonghai Li, Yuekun Lang, Jianmei Yang,Fatma Abdallah, Juergen Richt, Ahmed Ali, Adolfo García-Sastre, Wenjun Ma. Recombinant Newcastle disease virus expressing H9 HA protects chickens against heterologous avian influenza H9N2 virus challenge. Vaccine: http://dx.doi.org/10.1016/j.vaccine.2016.04.022
- Mitra N, Cernicchiaro N, Torres S, Li F, Hause BM. Metagenomic characterization of the virome associated with bovine respiratory disease in feedlot cattle identified novel viruses and suggests an etiologic role for influenza D virus. J Gen Virol. 2016 May 5. doi: 10.1099/jgv.0.000492.
- Hause BM, Palinski R, Hesse R, Anderson G. Highly diverse posaviruses in swine faeces are aquatic in origin. J Gen Virol. 2016 Jun;97(6):1362-7. doi: 10.1099/jgv.0.000461. Epub 2016 Mar 22.

Presentations:

- Drs. Jamie Henningson, Giselle Cino, Sanjeev Narayanan and Brad Njaa presented at the Davis-Thomson Foundation for the Advancement of Veterinary and Comparative Pathology 43rd Annual Pathology Course held at the Kansas State University College of Veterinary Medicine, Manhattan, KS.
- Dr. Brian Lubbers presented, BRD Diagnostics: Testing and Trends at the Texas Veterinary Medical Diagnostic Laboratory's Bovine Respiratory Disease Seminar in Amarillo, TX.
- Dr. Brian Lubbers presented, Antibiotic Regulations and Antibiotic Resistance in Cattle Production at the Noble Foundation Cattlemen's VFD Meeting – Ardmore, OK.
- Dr. Gregg Hanzlicek gave an Anaplasmosis update at the National Cattlemen's Beef Association meeting in Denver, CO.
- Dr. Gregg Hanzlicek presented Managing Neonatal Health to the attendees at the National Dexter Cattle Association meeting in Salina, KS.

Field Investigations:

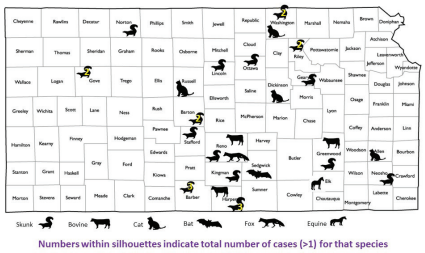
- Outbreak of summer pneumonia in unweaned beef calves on pasture
- Sudden death in multiple beef calves and cows housed on native pasture
- Multi-year reproductive failure in a cow-calf operation

Diagnostic Disease Trends Maps for Kansas

Updated weekly at www.ksvdl.org!

Disease trend maps include:

- Anaplasmosis
- Canine Brucellosis
- Canine Leptospirosis
- Johne's
- Rabies
- Rocky Mountain Spotted Fever
- Trichomoniasis
- Tularemia



Numbers within silhouettes indicate total number of cases (>1) for that species

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After-hours Sample Submissions to KSVDL

KSVDL is open for sample reception 8 am - 5 pm Monday through Friday and 8 am-noon on Saturday. Outside of those hours, samples and even deceased animals can be dropped off by going to the Emergency Desk at the Veterinary Health Center within the Vetereinary Medicine complex.

Please bring your completed submission form(s) along with your sample to the ER desk and let them know it is for KSVDL. If the sample is large or is an animal for necropsy the KSVDL intern will be paged to place the sample/animal in the necropsy cooler. Any small samples will be placed in the refrigerator where they are held until pick-up at the beginning of the next business day.

There is no need for bulky packaging of small samples (blood tubes, swabs, etc) in a cooler with ice packs. If the sample is not to be refrigerated (Trich pouch) please mark that on the sample container and relay that information to the desk personnel as well. Trich pouches will be placed in the incubator until they are delivered to the laboratory at the beginning of the next business day.

If you have any questions about after hours drop off contact Client Care during regular business hours at 866-512-5650 or clientcare@vet.k-state.edu.

Tips for Shipping Samples in the Summer

There are five things to consider when shipping samples in the summer: ice packs, absorbent padding, paperwork, insulation, and shipping. Use the following tips to ensure your samples arrive cool and collected:

- All samples except fixed tissue and InPouches or Trich tubes need to have enough ice packs to keep them cold until they arrive at the lab
- Use paper towels, newspaper or cloth towels to protect fragile contents and to help absob in case of a break or leak
- Place submission forms in a plastic bag to protect from melting ice packs
- Use an insulated cooler box to make sure ice packs last as long as possible (a recycled box that your clinic saves or new boxes that you order just for your samples. If you need a box, KSVDL offers an insulated return box for $11. Every time we receive this box we automatically ship it back to your clinic (after taking the samples out) so that it can be reused.
- Overnight shipping is highly recommended (KSVDL offers discounted UPS labels to help you out with the cost of shipping). You can order these 2 ways, online at KSVDL.org and click on Create UPS Labels or by contacting Client Care.

For questions or more information, please contact Client Care at 866-512-5650 or clientcare@vet.k-state.edu.

Developing and 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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Continuing Education

www.vet.k-state.edu/education/continuing/

August 5-9, 2016
American Veterinary Medical Association Annual Convention
San Antonio, Texas
http://www.avmaconvention.org/avma2016/public/enter.aspx
Visit the KSVDL booth at the AVMA exhibition hall!

August 26-29, 2016
Central Veterinary Conference (CVC)
Kansas City, Missouri
http://www.thecvc.com/register-now-to-attend-cvc-kc/

September 15-17, 2016
49th Annual American Association of Bovine Practitioners
Charlotte, North Carolina
http://www.aabp.org/meeting/

For more information call the Continuing Education Office at 785-532-4528.

Test Results and Schedules

Laboratory results available online, all the time!

Holiday Schedule:

Labor Day: Closed: Monday, September 5th
Thanksgiving: Closed: Thursday, November 24th and Friday, November 25th; Open Saturday, November 26th
Christmas: Open Saturday, December 24th

To receive this newsletter by email, contact: ksvdloutreach@vet.k-state.edu.

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