

Under the 'scope

Winter 2012

Working to serve you better

- Reminder: Shipping supplies and labels available from the VMDL
- General submission forms with convenient check boxes
- Small animal antimicrobial sensitivities added (see pg 3)
- Equine enteric panel added (see pg 3)

News from the Director

Diagnostic tests offer great opportunity and new challenges



Alex J. Bermudez, DVM, MS, DACPV, Director, VMDL

One of the most significant advances in the diagnosis of infectious diseases in the past 15 years has been the common use of the polymerase chain reaction (PCR) test. This testing modality detects genetic material, DNA or RNA, of the infectious disease agent. It allows veterinary diagnostic labs to rapidly identify infectious disease agents that may be difficult to culture by traditional bacteriology or virology methodologies in a timely fashion. As we have become accustomed to PCR testing methodology we have learned that there are significant strengths to the testing modality but also some new challenges in result interpretation. The strengths of the PCR technology are the sensitivity and specificity of a well developed PCR test. In addition there is a quicker turnaround time for PCR results particularly when com-

pared to viral culture techniques. Below are some issues of PCR testing that must also be considered as you interpret the results.

PCR testing detects a segment of RNA or DNA, so a positive test does not necessarily indicate that a live field strain virus (or other specific infectious agent) is actually present in the specimen. An example where this fact is important in your interpretation of results is if the animal being tested has recently been vaccinated for that same agent. For instance a dog that had recently received a parvovirus vaccine can have a positive parvovirus PCR test and yet the dog may not have had a clinical parvovirus infection. In such a case an accurate knowledge of the vaccine history, clinical history and pathology (if available) will be essential for an appropriate interpretation of the "positive parvovirus PCR result."

PCR testing is exquisitely sensitive. In some cases this has allowed us to identify the presence of disease agents in animal populations at a higher rate than previously documented. This information may be very clinically significant as is the case of the presence of trichomoniasis in Missouri cattle (see additional information in this newsletter). In other cases the mere presence of a disease agent at a low level may not be clinically

significant to the actual animal that has been presented to the veterinarian. Again this highlights the need for the holistic interpretation of PCR results considering the agent and the clinical presentation of the host. There are some agents where a positive PCR test is always significant and a positive result will require either preventive care, treatment or that regulatory action be taken. With other disease agents that are potentially less pathogenic and also common in the animal population the veterinarian will have to weigh this information with all the other information known about the case. Some viral infections can be clinically inapparent due to the host's adequate immune response yet the host will still be "PCR positive."

A final issue we have seen in the VMDL with PCR testing involves reproducibility of testing. If a sample contains a low to high number of the specific organism the PCR test will typically be highly reproducible. In the case where there is a very low titer of the infectious agent in the test material it is possible to have a positive test on a sample and retest the same sample and have a negative result. The reason for this apparently conflicting result is that the PCR test has a threshold for number of DNA or RNA segments that must be present for a positive test result. When there are very few organisms in the sample, an individual subsample may not always reach that threshold.

***Tritrichomonas Foetus* – best practices for sample submission**

Dr. Susan Schommer, Molecular Biologist

The demand for *Tritrichomonas foetus* (TF) testing is greater than ever before due to increased recognition of the disease and the rapid expansion of state import regulations. Recent changes to the MO cattle regulations for movement and sale mean that many more veterinarians are being asked to collect and submit samples for TF. Most states, including MO, accept a single PCR or 3 cultures for regulatory purposes, but be sure to check the individual state's requirements before submitting a sample. September 1, 2011 Missouri began enforcing new animal health regulations regarding testing for Trich. Missouri already requires that eligible bulls be tested before they can enter the state but the new regulations will require breeding bulls within Missouri be tested before changing possession or ownership. Furthermore, herds that have animals testing positive for Trich will now have additional regulations they are to follow. For complete details visit: <http://www.sos.mo.gov/adrules/moreg/current/v36n10/v36n10c.pdf> and refer to page 1351. Please read our guidelines below for the most accurate testing.

Accurate test results are dependent on proper sample handling. For the most sensitive PCR results, the inoculated InPouch™ TF should be received within 48 hours of collection- avoid collecting late Thursday or on Friday if you cannot drop off the sample at the VMDL. The sample must remain at room to body temperature. For shipping samples in the summer it is recommended to include a cold pack separated from the sample by newspaper, paper towels or bubble wrap in a box shipped overnight. In the coldest periods of winter the same system can be used with a handwarmer-type heat pouch (such as Grabber warmers). Once we receive the sample it will incubate overnight at body temperature and be processed the next day.

If it is not possible to get the samples to us within 48 hours or you have an appropriate incubator, samples may be incubated in-house vertically at 35-37C for 24-48 hours, frozen and then shipped frozen with cold packs. These samples can then start processing the day we receive them.

Proper handling of the sample is critical for accurate test results. Death of the *Tritrichomonas foetus* organism is believed to release DNases that can decrease the sensitivity of TF PCR. The signal of the TF organism in the sample declines after 4 days, samples received after 4 days of age that are not frozen and sent on ice will include a notation on the results indicating that a negative results must be interpreted with caution. Samples for culture must NOT be frozen.

Include the animal ID and date of collection clearly on the submission form and the InPouch™, so that we can process the sample as quickly as possible. The PCR test is \$22 and pooling is not available. InPouches are available for purchase, at cost, through the VMDL. This assay is run nearly every day and results are generally available within 48 hours of receipt of the sample. Please call 1-800-862-VMDL or email schommers@missouri.edu if you have any questions.

New Test Method for Anaplasmosis

Dr. Gayle Johnson

The VMDL has implemented a new PCR test to detect *Anaplasma marginale* that gives better concordance with suspected clinical disease than a previous test designed to detect both anaplasmosis and ehrlichiosis. During the months of August and September 2010, several cases were submitted with a history of multiple deaths of adult cattle without previous clinical illness, occurring with either multiple abortions or birth of weak, undersized calves (less than 45 pounds body weight at term). Anaplasmosis was suspected in some instances, but the PCR testing used at that time was negative in many cases. Serologic testing, which detects exposure but cannot confirm disease, was often not done.

The cELISA is a serologic test that is inexpensive and reliable for identifying carrier animals. Carrier infection can exist below the level detected by some PCR assays. Both tests have very few false positives. Like serologic testing, PCR can be used to detect both clinically affected animals and the carrier state. It is notoriously difficult to obtain a positive diagnosis of anaplasmosis in cattle unless the carcass is minimally autolyzed. The anaplasmosis PCR may be helpful in eliminating cattle with other ehrlichial infections, such as *Ehrlichia phagocytophilum* and may assist in identifying some fetuses and weak neonates lost to this disease. This new PCR test will be more successful in identifying *Anaplasma marginale* positive cattle so that early treatment measures can be taken.

News from Bacteriology– New Antimicrobial agents for small animal use

Cefovecin (Convenia) and Cefpodoxime (Simplicef) now available on sensitivity testing

News from PCR– New Equine enteric panels

1) A new Equine enteric panel is now available. Please submit both an EDTA blood tube and a fecal sample. For \$115, the following tests are included: PCR for Potomac Horse Fever, Salmonella, and Lawsonia as well as antigen ELISAs for Clostridium Difficile A&B toxin and Clostridium Perfringens Enterotoxin. Each of these tests are also available individually.

2) If you would like only the PCR portion, that is available for \$50- again please submit both EDTA blood and a fecal sample.

3) The diagnosis of Potomac Horse Fever is improved by testing both blood and feces. The pricing for PHF PCR has increased to \$35, however for that one price we will do a PCR on each of those samples.

Change in CWD Sampling.

Dr. Gayle Johnson, Anatomic Pathologist

Any cervid breeder wishing to participate in the State CWD Monitored Herd program must test all animals over 12 months of age that have died and have a result of “Not detected” for all deer. This is a voluntary program, but interstate movement to all other states requires participation for a period of at least 5 years. Also, movement within Missouri requires at least enrollment in the CWD Monitoring program but there is no established minimum time. Federal funding for the CWD sampling program is reduced, associated with APHIS budgetary reductions. Beginning January 1, 2012, APHIS will no longer pay the cost of CWD testing in captive cervids, and veterinarians must indicate the method of payment to be used. Samples may be submitted to the NVSL in Ames IA or other labs in the NAHLN network that provide testing. Testing costs will be in addition to the cost of sample procurement at the Missouri VMDL. The Ames lab is now charging \$40.00 to do the IHC test. If the VMDL takes samples from submitted deer there is the additional VMDL charge of \$23.50 + shipping for procuring the sample. Veterinarians may want to communicate directly with one of the nearby diagnostic labs that does testing and inquire about cost. We will place the prices currently listed for CWD testing by some of these labs on our website shortly. Also new is a requirement that refrigerated ear or hide with identifying tag be sent for potential genetic identification of positive animals.

Here are NAHLN approved labs that their prices as of 1/1/12. Prices may change, so submitters should call first.

NAHLN Lab	Current Price in State	Out of State fee?	Can submit heads?
Colorado State U	\$25.	no	NA
Purdue	\$35.	Yes, double test cost	\$60
Kansas State U	\$36.	no	yes
Michigan State	\$28.	no	yes
Minnesota	\$27.50	Yes, 10%	adds \$32.25
Ohio -\$12 one time producer fee	\$27.50	\$8/sample	Yes
Washington State	\$27.50	50%	no
Wisconsin	\$26.50 plus \$10 per case	no	NA
Wyoming	\$25.	no	no

Timely Topics– A Sample of Recent Publications from our VMDL

Subcutaneous Botryomycosis Due to *Bibersteinia trehalosi* in a Texas Longhorn Steer.

Spagnoli S, Reilly TJ, Calcutt MJ, Fales WH, Kim DY. Vet Pathol. 2011 Dec 21

Abstract: A 3-year-old Texas Longhorn steer had a long history of progressive swelling of the soft tissues of the jaw and neck. At necropsy, multifocal to coalescing dermal and subcutaneous pyogranulomas were surrounded by fibrous tissue. Microscopically, the pyogranulomas contained aggregates of gram-negative coccobacilli surrounded by Splendore-Hoeppli material and were separated by bands of fibrovascular tissue (botryomycosis). Phylogenetic analysis of multilocus sequence-typing data revealed that the bacteria recovered in pure culture from swabs of submandibular tissue were most closely related to *Bibersteinia* [*Pasteurella*] *trehalosi*. The bacterial colonies were immunohistochemically reactive with a rabbit polyclonal anti-*Pasteurella* class C acid phosphatase antibody. Botryomycosis is a pyogranulomatous inflammation caused by a variety of nonbranching, nonfilamentous bacteria that elicit the formation of Splendore-Hoeppli material. This case of botryomycosis is unique for its association with *Bibersteinia trehalosi*.

Pathology in Practice: *Francisella tularensis*.

Sean T. Spagnoli, DVM; Keiichi Kuroki, DVM, PhD, DACVP; Susan K. Schommer, PhD; Thomas J. Reilly, PhD; William H. Fales, PhD, DACVM

J Am Vet Med Assoc. 2011 May 15; 238(10):1271-3

An adult sexually intact male feral cat of unknown age was evaluated by a veterinarian in southern Missouri in late April because of anorexia, dehydration, and fever (rectal temperature, 39.6°C [103.3°F]) of unknown duration. Serologic testing revealed that the cat had no circulating FeLV antigen or antibodies against FIV. The cat died 4 days later despite administration of enrofloxacin SC and administration of fluid therapy SC. The body was submitted for necropsy examination. For this cat, no bacteria were cultured either splenic or lymph node specimens, and a definitive diagnosis of tularemia was made on the basis of PCR detection of *F tularensis*-specific nucleic acid in samples from the spleen and enlarged lymph nodes. The negative bacterial culture result was attributed to the enrofloxacin treatment that the cat had received; therefore, careful interpretation of negative culture results is necessary if a patient has been treated with antimicrobials.

Diminished reproductive performance and selected toxicants in forages and grains. Evans TJ.

Vet Clin North Am Food Anim Pract. 2011 Jul;27(2):345-71, viii.

This article discusses reproductive toxicants as the potential, primary causes of observed reproductive abnormalities and other variables that can affect reproductive performance in ruminants. The causes of diminished reproductive performance in ruminants are often multifactorial.

Compressive Myelopathy Associated With Ectasia (dilation) of the Vertebral and Spinal Arteries in a Dog.

Bozynski CC, Vasquez L, O'Brien DP, Johnson GC. Vet Pathol. 2011 Aug 19.

Abstract: A 4-year-old dog was presented for acute, progressive tetraparesis and cervical hyperesthesia. Symmetrical tubular structures coursing along the lateroventral aspects of the spinal cord at the fourth and fifth cervical vertebrae were identified in magnetic resonance images. At necropsy, vertebral arteries and their spinal branches were severely ectatic bilaterally, and the cervical spinal cord was compressed. Histologically, the ectatic branches of the vertebral and ventral spinal arteries were surrounded by fibrosis with scant mononuclear cell infiltrates and hemorrhage. Spinal branches of the vertebral arteries had focally severe reduction in the tunica media. A thrombus was in an arterial branch. Smaller vessels in adjacent tissue had fibrinoid degeneration. Axonal degeneration was detected in the affected spinal cord and nerve roots. The segmental degenerative radiculomyelopathy in this dog was attributed to anomalous ectasia of the vertebral and ventral spinal arteries.

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