Diagnostic Insights

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KANSAS STATE VETERINARY DIAGNOSTIC LABORATORY

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

Personnel Profile — Dr. Gregg Hanzlicek

Dr. Gregg Hanzlicek has recently been named the Director of Production Animal Field Investigations in the Kansas State Veterinary Diagnostic Laboratory.

Dr. Hanzlicek is originally from Great Bend, Kansas. After receiving a bachelors degree in animal science from Kansas State University he spent several years working in production animal agriculture.

He is a 1991 graduate of the Mississippi State University College of Veterinary Medicine after which he was a bovine veterinarian for 16 years.

In 2007, Dr. Hanzlicek came back to the Kansas State University College of Veterinary Medicine and completed a PhD in epidemiology.

He joined KSVDL in December of 2010 to provide outreach to Kansas veterinarians and participate in field investigations.

"My position offers the best of both worlds: I have the opportunity to interact with the numerous veterinary experts within the KSVDL and also private veterinary practitioners."

Dr. Hanzlicek may be contacted at: gahanz@vet.k-state.edu or 785-532-4853.

Ehrlichiosis: A PCR Test Now Available at the KSVDL

Are you seeing dogs that are febrile, anemic, depressed, and with enlarged lymph nodes? Do these dogs also demonstrate thrombocytopenia on CBC's? Are the blood protein profiles indicating a hypoalbuminemia and an accompanying hypergammaglobulinemia?

If you are thinking yes to these questions, then you might want to know that the KSVDL now offers a PCR test to detect canine ehrlichiosis due to single or mixed infections involving any of the following rickettsial agents: *Ehrlichia chaffeensis*, *E. canis* or *E. ewingii*.

These tick-borne infections are considered to be emerging diseases in dogs and other vertebrates, including humans.

The ability to rapidly detect and differentiate them can be valuable in assessing the potential for zoonotic transmission to humans and other susceptible animal species, and in handling cases where different phases of infection (acute, subclinical, or chronic) might be suspected.

The PCR test is a multiplex, real-time PCR procedure that uses the RNA recovered from whole blood for the specific detection of any or all of the three *Ehrlichia* species, simultaneously.

The lab requests that whole blood be collected in an EDTA tube (purple-cap) and be shipped on ice but not frozen.

The cost of the test is $43.00/sample.

If you have questions, please contact the KSVDL at 866-512-5650 or Dr. Richard Oberst at 785-532-4411.

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Bulktank BVDV PCR is Now Available at KSVDL

Bovine viral diarrhea virus (BVDV) in a dairy herd can be devastating.

The most common indications that this virus is circulating in the herd are one or a combination of the following:

1) Sudden abortion storms
2) Overall poor reproductive success
3) Treatments for common ailments suddenly not being effective
4) Adult cow pneumonia

Typically the virus is circulating in the herd because of a persistently infected (PI) animal. The practical first step in determining if a PI is in the herd is a bulktank milk test.

A positive test indicates that one or more PI animals are in the herd or pen.

The next step is to individually sample each animal which contributed to the bulktank milk to identify the PI.

It is extremely important that no animal that contributes to the bulktank be culled or rendered without an individual BVDV test.

We recommend bulktank samples contain 200 or less animals.

Sample: 100 ml of fresh, NOT frozen, bulktank milk

The cost of the test is $29/ sample

If you have questions about this test contact: Dr. Gregg Hanzlicek at: gahanz@vet.k-state.edu or 785-532-4853.

Laboratory Diagnosis of Canine Leptospirosis—Dr. Kenneth Harkin

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 week later).

If the 2-week convalescent titer failed 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. Lower titers, especially to non-vaccinal serovars or if the dog has not been vaccinated may also be considered supportive of the diagnosis when clinical signs are consistent with the disease. Some dogs, however, may never seroconvert, so a diagnosis of leptospirosis is still possible with negative titers.

The PCR identifies the presence of the leptospiral organism in urine and is unaffected by previous vaccinations. The test is reported 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.

The PCR may be negative if the patient is not shedding leptospires in the urine or there are too few organisms to be detected. The PCR can be performed on other bodily fluids (blood, CSF, semen) or tissues (liver, kidney, lung, brain), so contact the laboratory if you would like alternative samples tested.

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.

Dr. Harkin can be contacted at : harkin@vet.k-state.edu or 785-532-5690.

KSVDL Specializations

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Companion Animal Outreach
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Virology
Dr. Richard Hesse
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Help us help you:

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

Rabies Testing—Dr. Mike Moore

In the rabies-diagnostic-world we have three test result options: Positive, Negative and Unsuitable.

Positive and Negative are self-explanatory. Unsuitable usually means we can pick up the skull, look through the foramen magnum and read the *Kansas State University Collegian* through the eye socket.

Within a month after arrival at the Rabies Lab, I had the unfortunate experience of having to call a large animal veterinarian to discuss why we resulted the bovine brain he sent in as unsuitable. While the veterinarian had completed the hard work of removing the entire brain, it arrived ½ in formalin.

In 2003, CDC mandated that a full cross-section of fresh brain stem and cerebellum be examined by direct fluorescent antibody to be able to issue a negative result.

So if any of you are old enough to remember the recommendation of submitting a bovine or equine brain, ½ fresh and ½ formalized, please delete that from your data-base. If other tests are requested on a negative brain, we will formalize it for you.

When submitting dogs, cats and other small animals for rabies testing, please submit the whole head, preferably removed at the base of the skull. Bats and small mammals are usually submitted whole.

We also offer a press release template to the clinics that submit a positive case. Our goal is to help practitioners remind their clients that rabies is endemic in Kansas and making sure their animals are current on rabies vaccination is important. Skunks and bats are our reservoir species and they do not mind sharing the disease.

Practitioners are welcome to use the template.

Dr. Moore may be contacted at: mcmoore@vet.ksu.edu or 785-532-4503.

To Pool or Not to Pool Samples—Dr. Jerome Nietfeld

A common request is to pool samples from multiple animals to help keep costs down.

Pooling can be helpful as it allows testing of more animals without increasing costs and gives a better overall picture of the status of the group. However, pooling samples is not always a good idea.

If you want to do virus isolation, it is usually best to keep samples from individual animals separate. The reason is neutralizing antibodies, which prevent viruses from infecting cells. If one animal in the group has neutralizing antibodies to the virus in question, the antibodies will be in the tissues and likely prevent isolation of the virus, even if tissues from multiple animals contain the virus. PCR and ELISA results are normally not affected by pooling.

The only effect of pooling is possible over-dilution of the virus if most of the tissues are uninfected (virus isolation is also subject to over-dilution).

Sometimes diagnostic laboratory personnel might be reluctant to pool samples or to make large pools if they do not have data to indicate that the results will be accurate.

If a pool is positive, is it important to know which sample was infected? If so, the lab must individually test all samples in the pool. In cases where the proportion of positive animals is relatively high, you will end up spending more money by pooling.

If there is no chance that individual animals will need to be tested, it is fine for the submitter to pool samples before shipment.

If there is any chance that individual animals will be tested, send the samples separately and ask the diagnostic lab to do the pooling. They will save a portion of each sample and if a pool is positive can test the individual samples.

Dr. Nietfeld may be contacted at: nietfeld@vet.k-state.edu 785-532-4460.

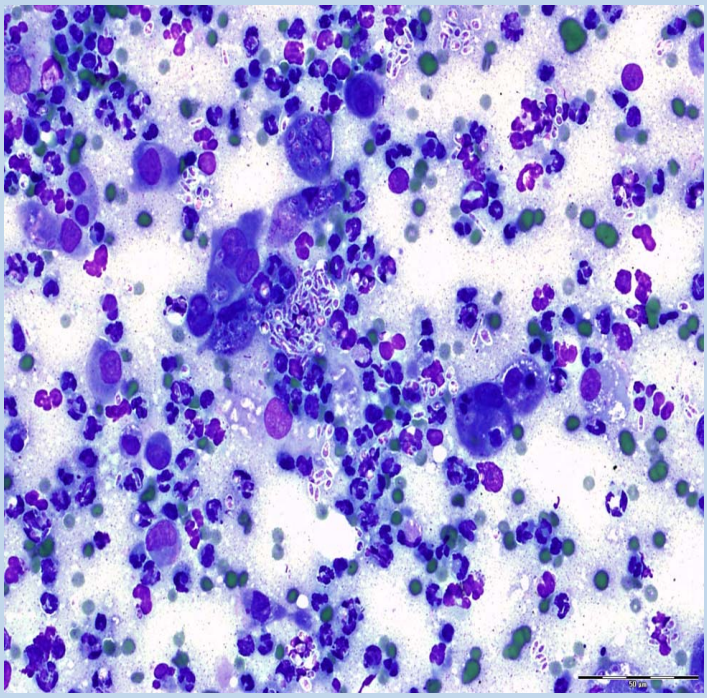
Help us help you:

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

What's Your Diagnosis?—Dr. Lisa Pohlman

Signalment & History

A 6 year-old domestic short haired cat presented with a crusted, ulcerated cutaneous lesion on the dorsal, left hind paw. Impression smears of the lesion were taken and sent for cytologic evaluation. *(See Below)*.



Cytologic Diagnosis: Pyogranulomatous inflammation due to Sporotrichosis.

Sporotrichosis is a relatively rare disease caused by *Sporothrix spp.,* usually *Sporothrix schenckii,* which is a dimorphic fungus (yeast form seen at body temperatures) found worldwide. The organism prefers soil rich in decaying and organic matter and has also been isolated on many live plants. Human infections are often associated with rose gardening since infection usually occurs via a puncture wound. More correctly, any thorny plant should be regarded as a possible source of infection. Infection via inhalation of spores has also been reported, but this is much less common. Disease has been reported in people, horses, mules, donkeys, goats, cattle, dogs, cats, rats, mice, hamsters, birds, camels, dolphins, armadillos and chimpanzees.

The mycelial form of the organism enters the tissue via traumatic inoculation and then converts to the yeast form. Local proliferation of the organism and the associated inflammatory reaction results in draining wounds that first appear similar to cat-bite abscesses or cellulitis. These woundsnare refractory to antibiotic therapy, and may progress to become ulcerated, cutaneous nodules that involve the dermal and subcutaneous tissues. Subsequently the affected area becomes ulcerated and forms large crusts. The typical inflammatory reaction is pyogranulomatous, but upon removal of the crusts there is often a purulent exudate. In immune-competent dogs, the disease is usually limited to the cutaneous form, and organisms are typically sparse or rare – hence in dogs this infection can be very difficult to diagnose. Disseminated disease is very rare in dogs unless the patient is immunosuppressed.
Conversely, most cats will develop cutaneolymphatic or disseminated disease regardless of their immune status at the time of infection. Further, in cats organisms are typically abundant and found easily within the lesions and exudates (especially just under the crusty scabs). If sporotrichosis is suspected and organisms are not found microscopically, culture of the lesion is recommended.

Identification of the organism is straight forward on a cytologic preparation if the classic round to oval to cigar-shaped yeast forms (~ 3 to 9 microns long and ~ 1 to 4 microns wide) are seen. However, if only the round-shaped yeast forms are present, these organisms can be difficult to distinguish from *Histoplasma capsulatum*. *Sporothrix schenckii* has also reportedly been misidentified as *Cryptococcus neoformans*. This error is likely due to an artifact of sample preparation in which there is contraction of the cytoplasm from the cell wall resulting in the impression of a large clear capsule.
Infection is usually caused by traumatic inoculation of the organism into tissue via soil, plant material or organic matter. Disease in dogs is most common in hunting dogs and usually associated with a small puncture wound such as a thorn or wood splinter. In cats the infection is most common in those cats that are allowed to roam; the organism is believed to be inoculated via a contaminated claw or oral cavity of another cat.

The zoonotic potential of infection from cats to people is well established. The occurrence of transmission from dogs to people is not well documented and not confirmed to occur. It is reported that the abundance of organisms in the lesions, exudates, feces and under the nails of infected cats and the relative lack of organisms in the lesions of dogs is the reason for this difference. However, transmission from cats to people has been reported even when the cat's lesions have had very few organisms, and transmission from dogs has not occurred when owners have been exposed to lesions with abundant organisms. Therefore, it is likely that there are other factors that affect zoonotic potential, and not simply the number of organisms in a lesion. Transmission from person to person does not occur.

Dr. Pohlman may be contacted at:
lpohlman@vet.k-state.edu **or 785-532-4882.**

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

June 5-8, 2011

73rd Annual Conference for Veterinarians

http://www.vet.ksu.edu/CE/2011/AnnConf11/index.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:

Memorial Day: May 30, 2011
Independence Day: July 4, 2011
Labor Day: September 5, 2011
Thanksgiving: November 24 and 25, 2011

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