University of Nebraska Lincoln

Diagnostic Center News

University of Nebraska Veterinary Diagnostic Center

Co-Editors: Dr. David Steffen & Mavis Seelmeyer

Winter 2007

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Notes From the Director

I am happy to announce that Dr. Alan Doster was awarded the Nebraska Veterinary Medical Association Outstanding Service Award at the NVMA Meeting in January. Dr. Doster has been a dedicated pathologist at the Nebraska Veterinary Diagnostic Center for 27 1/2 years and continues to work exceptionally hard to provide fast, detailed and accurate reports. Dr. Doster has always prioritized service and I expect as users of the Nebraska Laboratory, you are aware of this contribution. I would also like to note his scholarly achievements. He is widely published primarily in regard to viral infections of cattle and swine and he routinely gives seminars on diseases of food animals and wildlife. Dr. Doster is currently co-teaching a wildlife disease class and is enthusiastic about the new NU-ISU Cooperative veterinary education program. I expect our professional students will benefit greatly from his experience, expertise and enthusiasm. Thanks to the NVMA for recognizing this exceptional faculty member.

The laboratory had another good year with diagnostic volume up and numerous contracts for testing to support research and surveillance projects. Our dedicated staff works hard to meet the obligations of the contracts, and as always, expedites and prioritizes your diagnostic cases. The laboratory performed nearly ½ million tests with roughly 50% of those being cattle samples, 20% canine samples, 10% swine samples and the rest spread amongst the various other species. We have seen a rise in swine and goat submissions this past year. We are continuing to offer opportunities to veterinarians in Nebraska to get a subsidy on swine submissions if they provide surveillance samples (tonsil and nasal swab) and data (premise ID or location) for Classical Swine Fever (CSF) surveillance programs. Please contact Mavis Seelmeyer (402-472-1434), if you would like to participate and we will get the information you need. Last year, several clinics generated thousands of dollars in revenue by sampling swine herds in their areas. Several clinics missed the opportunity and left more than $50,000 unencumbered.

The laboratory accreditation site visit occurred earlier this month. New standards for accreditation have dramatically improved the "behind the scenes" operation of our laboratory. Last fall I was elected to the executive committee of AAVLD and will serve as president of the organization in two years. As an organization, we don't often seek input from users regarding issues they perceive regarding laboratories as a national system. One issue I have always been aware of is the need for consistency of assay performance across laboratories. That is a primary objective of the enhanced accreditation process. I certainly welcome any other ideas on changes/improvements labs might do nationally to improve service.

- - David J. Steffen, DVM, PhD
Director, Veterinary Diagnostic Center

Did You Know??

Cows can have regional accents.

Pigs can cover a mile in 7.5 minutes when running at top speed.

Americans spend around $3billion for cat and dog food a year.

Bulls are color blind.

Bacteriology News

Brucellosis Testing Notice: Any card test positive result for serum from swine and cattle requires that serum be forwarded for additional testing to verify negative or reactor status of the tested animal. This will require additional time (sample shipping, laboratory work, test reporting, etc.) before tests results are available. **If shipment or sale requires urgent results we recommend that samples be sent to the designated Brucella Lab for your state to avoid potential delays caused by false reactors.** The Nebraska VDC will continue to perform brucellosis serological testing associated with diagnostic cases and upon request for the convenience of our clients. Positive test samples forwarded for confirmatory testing may require an additional two to five business days for reporting.

PCR Testing for *Mycobacterium avium subsp. Paratuberculosis* (Johne's)

We are now certified to test for *Mycobacterium paratuberculosis* (Johne's disease) using the following three methods:

Solid culture – turn-around-time is 10-12 weeks ($22.50/sample)

Liquid culture – turn-around-time is 8-10 weeks ($25.00/sample)

Direct fecal PCR – turn-around-time is 1 week ($20/sample) (*Direct fecal PCR will be the default assay if no specific test is requested.*)

If you order either type of culture, you will receive the same report as you have in the past. PCR is reported as positive or negative only. ***Please specify which test method you want on your submission form.***

The VDC Bacteriology Laboratory with support from Virology and has recently been evaluating the use of a real-time PCR test for the detection of *Mycobacterium avium* subsp. *paratuberculosis.* Newer methods to extract DNA from feces has resulted in a tremendous improvement in sensitivity of rt-PCR. This assay has now proven to be comparable to the sensitivity achieved by liquid or solid culture. For animals that are shedding high to moderate numbers of bacteria in feces, the detection sensitivity is approximately equal (> 90%). For animals shedding low numbers of organisms, the rt-PCR test is more sensitive (culture ~ 60%, rt-PCR >70%). Some reports suggest that the real sensitivity may be close to 90%. This reflects an estimated threshold of detection of DNA from 5 - 10 organisms per 2 gram sample of feces. The r2 for quantitative correlation is approximately 92.5%. The specificity and relative precision are very good (> 95%) for both the rt-PCR assay and cultures with PCR confirmation of species. The manufacturer of the detection kit reports a false positive rate of 5.3% and a false negative rate of 6.3%. The predictive value of a negative test is > 95% and the predictive power of a positive test is >72%. The rt-PCR also works very well with fresh feces and frozen feces. The rt-PCR has the obvious advantage of faster turn around time (3-5 working days) compared to culture (3-10 weeks). There is also some cost advantage to the rt-PCR assay. The Nebraska VDC is certified by USDA-NVSL to test by all three methods but NVSL check test performance of the rt-PCR was superior (virtually 100% accurate). Evaluation of the assays will continue. But in the interest of cost and time efficiencies, **the default method of testing will be by rt-PCR unless other testing is specified by the client.**

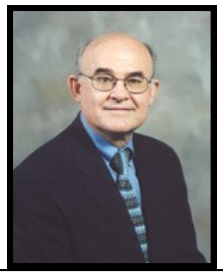
***Trictrichomonas foetus* Testing:**

Just a reminder for those of you testing for *Tritrichomonas foetus*: if you are not inoculating the samples into the In-pouches, you may be compromising the integrity of the test. *Tritrichomonas foetus* does not live outside the body for more than a few hours without the special media that is in the In-pouches. For best results, the sample should be inoculated into the In-pouch as soon after collection as possible, kept at room temperature or incubated at body temperature (37°C), and shipped to the lab as soon as possible. Do not refrigerate the inoculated In-pouches and if you are shipping in the winter use a warming pack to keep them from freezing in transit. Don't send your specimens on a Friday or the day before a holiday, because the specimens may be not be delivered in a timely manner and the specimens could be exposed to extreme temperatures.

In-pouches can be ordered from the Bacteriology Lab or directly from the manufacturer Biomed Diagnostics (1-800-964-6466).

- - submitted by Dr. Scott McVey, DVM, PhD, Faculty Supervisor, Microbiology
and by Deb Royal, Supervisor, Microbiology

Department Employees



Dr. David K. Hardin

Dr. Hardin grew up in Mountain Grove, Missouri on a dairy and swine farm. He attended Cabool High School, graduated in 1970 and then enrolled at the University of Missouri-Columbia where he graduated from the College of Veterinary Medicine in 1977.

After graduation, Dr. Hardin established and operated a veterinary practice in Hartville, Missouri from 1977-81. In 1981, Dr. Hardin returned to the University of Missouri to receive advanced training in theriogenology. Upon completion of the Theriogenology residency he accepted a teaching position at Texas A&M University.

In 1986, Dr. Hardin moved to Mississippi State University and continued teaching theriogenology and dairy production medicine.

In 1991 he returned to the University of Missouri as the Director of Veterinary Medical Extension and Continuing Education where he served as director from 1991-2003. During this time he developed the Missouri Dairy Plan. This plan served as a production and economic model for dairy producers who wished to expand their dairy operations. He developed the first international workshop for pet food regulations and the first pet breeder workshop for veterinarians and pet producers.

In 2003, Dr. Hardin accepted a position as department head for the Department of Pathobiology & Population Medicine at Mississippi State University where he served in this role from 2003-2006. While department chair, Dr. Hardin was instrumental in developing and implementing a major curricular change at CVM-MSU.

In May 2006, Dr. Hardin joined the faculty at UNL as Associate Dean, Cooperative Program in Veterinary Medicine and Head, Department of Veterinary & Biomedical Sciences.

About The Diagnostic Center

The Veterinary Diagnostic Center consists of the following areas: Front Office/Administration and Computing, Bacteriology Lab, Histology Lab, Pathology, Laboratory Compliance, Toxicology Lab and Virology Lab which includes the IHC Lab. There are five pathologists and one pathology resident. At the current time, there are 31 employees.

Virology Lab Updates

Two new PCRs are available: canine distemper virus and equine herpes virus type 1

Canine distemper virus:

Antemortem specimens--- conjunctival scrapings, pharyngeal washings, urine, whole unclotted blood, serum and cerebrospinal fluid.

Postmortem specimens: lung, tonsil, bronchial lymph node, bladder and brain.

EHV-1 rhinopneumonitis:

Antemortem specimen--- nasopharyngeal swabs which should be placed immediately in viral transport medium (**available from the lab at no charge**) and transported to the lab with cold packs.

EHV-1 abortion:

Postmortem specimens----lung, liver, adrenal and lymphoreticular tissues.

EHV-1 neurologic disease:

Antemortem specimens--- nasopharygeal swabs and buffy coat

Postmortem specimens--- CSF, spinal cord, spleen, liver, kidney and pharynx.

Both are realtime PCRs which means that results will be available by early afternoon on the day of testing.

- - submitted by Judi Galeota, Supervisor, Virology

University of Nebraska Veterinary Diagnostic

Nebr. Veterinary Diagnostic Center
Univ. of Nebraska
Fair St. & E. Campus Loop
1900 N. 42nd St.
P. O. Box 82646
Lincoln, NE 68501-2646

Phone: 402-472-1434
Fax: 402-472-3094
E-mail: vdc2@unl.edu

We're On the Web:
http://vbms.unl.edu/

The Nebraska Veterinary Diagnostic Center is accredited by the American Association of Veterinary Laboratory Diagnosticians

All regulatory testing for export is done in compliance with the code of federal regulations and technicians performing the test have been tested annually by the USDA through the National Veterinary Services Laboratories check-testing program. Additional assays within the lab regarding toxicology, microbiology and parasitology are assessed annually by check testing through the Veterinary Laboratory Association. Positive and negative control samples are included in all serologic and toxicologic testing protocols that require them.

Ancillary testing is reviewed by a single case coordinator who assures that test results are in agreement and any unusual test results are investigated to ensure that standard operating procedures are followed and that results can be replicated. Standard operating procedures are on file in each of the laboratories and available for inspection. A copy of our Quality Manual is available upon request.

Nebr. Veterinary Diag. Ctr.
Univ. of Nebr.
Fair St. & E. Campus Loop
1900 N. 42nd St.
P. O. Box 82646
Lincoln, NE 68501-2646