University of Nebraska Lincoln

University of Nebraska Veterinary Diagnostic Center

Co-Editors: Dr. Alan R. Doster & Mavis Seelmeyer

Diagnostic Center News

Spring/Summer 2012

Notes From the Diagnostic Center

In This Issue:

Did You Know??

- - The tongue of a pig has six thousand more taste buds than a human's.

- - Scientists believe that the closest living relative to the Tyrannosaurs Rex is the chicken.

New Diagnostic Laboratory Now Reality

Governor Dave Heineman signed the appropriations bill on April 2nd that will provide the funding for the construction of a new Veterinary Diagnostic Lab. The new facility will be located west of the Life Science Annex in the area of the recreation fields. The building will be approximately 47,000 gross square feet at a total project cost of approximately $55 million. The Nebraska Legislature appropriated $50 million for planning and construction. The remaining $5 million is to be raised from private funds.

- - taken from http://vbms.unl.edu/ Website; submitted by Dr. David Hardin, Dept. Head, School of Veterinary Medicine and Biomedical Sciences

Congratulations—- to Dr. Alan Doster, Director, Veterinary Diagnostic Center. Dr Doster was the recipient of the "Faculty of the Year" award from the class of 2014.

If you would prefer to receive your Newsletter by E-mail, please provide us with the E-mail address you would like to have us use and E-mail that request to us at: vdc2@unl.edu Thank you!

FDA Press Release: Ban on Extra-label Cephalosporin Use

By Dale A. Moore, DVM, PhD, Director, Veterinary Medicine Extension, WSU

On January 4, 2012, the US Food and Drug Administration issued an order that will prohibit most extra label use of cephalosporin drugs in food animals (*For the full press release, see webpage*: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm285704.htm?source=govdelivery).

The order is to take effect April 5, 2012. From now until March 6, 2012, the FDA will accept comments about their ruling. What can you do to make sure you are either in compliance or can make meaningful comment?

►Take a look at all the antibiotics you are using.

►Are you using drugs that contain cefiofur? Drugs with cephaparin are NOT included because the only approved use in food animals is for intra-mammary treatment in cows and there is no use of this drug in people.

►If you are using a cephalosporin drug in food animals, how are you using it?

►How does your use correspond to the label with regards to:

Dose – mg or ml per pound or kg

Route of administration

Frequency – how often you give it

Duration of treatment with the drug

Species or class or age of animal

►If you are using the drug in a manner that is NOT on the label, find out why, start using and training treaters to use it according to label instructions.

Are there exceptions to this order? Yes, there are exceptions. These drugs can be used to treat an extra label disease condition on the order of the veterinarian but ONLY using the labeled dose, route, frequency and duration. There are some exemptions for Minor Species like ducks, etc. There are **NO allowable *prevention* uses** of these drugs – that means you cannot give every newborn calf a "dose" to try to "prevent" scours.

What about the other drugs we use? There are still a number of drugs we cannot use in food animals or that we cannot use off label (like the sulfa drugs in dairy cattle). It might be a good idea to go through all the drugs you are using and make sure everyone is using them on label too.

(taken from the Washington State University Veterinary Medicine Extension Newsletter)

DIAGNOSIS OF LEPTOSPIROSIS

LABORATORY TESTING

Laboratory testing for Leptospirosis falls into 2 categories; tests for the demonstration of leptospires or its DNA and tests for antibody detection. Each assay has a number of advantages and disadvantages. Some suffer from lack of sensitivity and others lack of specificity. Therefore, no single technique can be recommended for diagnosis. Most tests for the demonstration of leptospires or its DNA are not serovar specific. The only definitive test that is serovar specific is culture which is expensive, quite laborious, and time consuming. The current testing protocol recommends serological testing combined with one or more techniques to identify the organism in the tissue or body fluids. Current tests include serology (microagglutination testing), darkfield microscopy, immunoflorescence, culture, histopathology with special stains and polymerase chain reaction (PCR) assay. Serology and PCR are the most widely used.

The current popular protocol for the collection of urine in cattle involves collection from cattle after injection of furosemide. Furosemide is a diuretic which increases glomerular filtration rate flushing more leptospires into the urine and produces dilute urine which enhances survival of the organism. There is the possibility of a urine specimen being contaminated with water that contains a non-pathogenic leptospira; therefore, attention to detail in taking the urine sample and laboratory handling are important. The stability of the DNA can be affected by the pH of the urine, so it is recommended that urine be refrigerated and transported to the laboratory as quickly as possible (within 24-48 hours). If testing is delayed, pH of the urine can negatively affect the quality of leptospiral DNA, resulting in a false negative.

The current popular protocol for microagglutination testing (an antibody detection technique) is collection of paired serum samples collected 2-4 weeks apart. Serum should be spun in a serum separator tube or removed from the cells and placed in a sterile tightly sealed container.

Recommended tissues from an aborted fetus for leptospira testing are fresh and fixed placenta (with minimum autolysis), kidney, liver, and thoracic fluid sent on ice.

INTREPRETATION OF MAT RESULTS IN BOVINE ABORTIONS

In the investigation of abortions caused by incidental host infection with leptospirosis. Detection of high antibody titers (≥800-1600) may sufficient to establish diagnosis. However, in maintenance host infections, particularly serovar Hardjo infection, infected animals often have a poor antibody response to infection. Often at the time of abortion, antibody titers may be quite low or negative against serovar Hardjo. In these cases, the herd serologic response to infection or detection of the organism in tissue or fluids are often more helpful than is the individual's antibody titer in establishing the diagnosis.

It is important that paired samples be tested since antibody response to an infection is shown by an increase in the antibodies which are indicative of acute infection.

Interpretation of MAT results is complicated by; cross-reactivity of antibodies between serovars, antibody titers induced by vaccination, and a lack of consensus about which antibody titers are considered to be significant for the diagnosis of infection.

Antibodies produced in an animal often cross-react with other serovars. So, in general, the infecting serovar is assumed to be the one with the highest titer.

Vaccinated animals will also develop titers. So, in general, a low titer (100-400) is indicative of a vaccine-induced response. Titers usually persist 1 to 3 months after vaccination. However, some animals develop high titers after vaccination (particularly those vaccinated several times a year) which may persist for 6 months or more.

Many consider a titer of >200-400 significant with the exception of infection with serovar Hardjo. A titer of 100 may be considered significant with Hardjo. However, this cut-off level may be exceeded in vaccinated animals.

INTREPRETATION OF MAT RESULTS IN DOGS (Lepto, contd.)

Typically, a titer of >800 in an unvaccinated dog is regarded as supportive of a clinical case of leptospirosis. During the first 6 weeks of infection, there can be cross reactivity between different serovars resulting in multiple positive serovar titers which make it difficult to determine which serovar is actually causing clinical disease. If a dog has been vaccinated, titers of <400 are often seen for several months. In cases that are clinically suspicious but do not have a corroborating titer, then either collection of an additional serum sample in 2-4 weeks to see if the titer is changing or submission of urine or kidney for PCR would be useful for a more definitive diagnosis.

TESTING PERFORMED AT THE VDC

PCR	$28/sample
Microagglutination Testing (Serology)	$10/sample
Fluorescent Antibody Testing	$17/sample
IHC	$18/sample

References

J Vet Intern Med. 2011 May-Jun; 25(3):426-32; Proceedings of the 6th Western Dairy Management Conference March 12-14 , 2003, Reno, NV-158

SUSCEPTIBILITY TREND FOR E. COLI FROM BOVINE ENTERIC SAMPLES FROM MARCH OF 2011 THROUGH MARCH OF 2012

	Total	Sensitive		Intermediate		Resistant	
Ampicillin	50	28	56.0%	0	0.0%	22	44.0%
Ceftiofur	50	35	70.8%	2	4.2%	13	25.0%
Chlortetracycline	50	16	32.0%	2	4.0%	32	64.0%
Clindamycin	50	0	0.0%	0	0.0%	50	100.0%
Enrofloxacin	50	43	86.0%	0	0.0%	7	14.0%
Florfenicol	50	10	20.0%	19	38.0%	21	42.0%
Gentamicin	50	41	82.0%	2	4.0%	7	14.0%
Neomycin	50	31	62.0%	0	0.0%	19	38.0%
Oxytetracycline	50	15	30.0%	0	0.0%	35	70.0%
Penicillin	50	0	0.0%	0	0.0%	50	100.0%
Spectinomycin	50	1	2.0%	32	64.0%	17	34.0%
Sulphadimethoxime	50	22	44.0%	0	0.0%	28	56.0%
Tiamulin	50	0	0.0%	6	12.0%	44	88.0%
Tilmicosin	50	0	0.0%	0	0.0%	50	100.0%
Trimethoprim/ Sulphamethoxazole	50	41	82.0%	0	0.0%	9	18.0%

The Veterinary Diagnostic Center's Quality Program

The UNL Veterinary Diagnostic Center (VDC) is committed to providing accurate and prompt responses to your request for assistance. To assure accuracy in testing we aspire to maintain accreditation through the American Association of Veterinary Laboratory Diagnosticians (AAVLD). The standards set by the AAVLD are outlined as minimum essential requirements and these meet or exceed those required by the international community. The VDC has an active quality control program managed by a full-time Quality Assurance Specialist who oversees the quality program.

Unlike many commercial laboratories, all testing in our laboratory is performed following verifiable standard operating procedures utilizing validated procedures. Each accession processed is supervised by a licensed veterinarian with specialized training in diagnostic medicine. Several of our faculty are board-certified in specialty areas such as Veterinary Pathology. Our laboratory managers are experienced in clinical testing and research methods and work closely with competent well trained staff in the performance of assays.

The quality system ensures that tests performed by the veterinary diagnostic laboratory sections are appropriately controlled. The VDC is active in ongoing participation in a variety of proficiency testing programs to confirm the accuracy of our test results. Our laboratory participates in programs administered by the National Veterinary Services Laboratories (Ames, IA) and the National Animal Health Laboratory Network (Plum Island Animal Disease Center). Additionally, quality is evaluated and maintained by the use of known positive and negative controls when applicable.

We understand timely, relevant test results are important. Our commitment to a well-managed quality system helps us provide exceptional service to our clients. We ask your assistance with our program by requesting complete information on accession forms and specific tests requests or detailed clinical and gross pathology data be provided with each case so we can serve you most efficiently.

- - submitted by Sharon Clowser, Laboratory Compliance Specialist

Bacteriology and Parasitology Lab Update

Many of the supplies and media used in the laboratory are limited in their days of use. Some have antibiotics or cells in them that degrade causing them to not work properly after the expiration date. In this era of budget cuts, it is not prudent to throw away costly media because it expired. We have worked diligently to assess how much media we need on a routine basis to meet our clients needs without being wasteful. The end result is; we don't store extra media or supplies. So we are asking all our clients to please notify the lab if they are planning to send in cases with a large number of samples. For bacteriology, mycology, and parasitology it would be more than 10 samples, for brucellosis testing more than 100 samples and molecular diagnostics more than 20 samples.

We often receive submissions requesting anaerobic culture from samples where, in most cases, anaerobic bacteria would not be considered a pathogen such as a urine, lung or skin scrapings. It seems likely that clients are just marking all possible culture types on the submission form and as a result are paying for cultures that are not necessary. Types of samples that require anaerobic culture include deep wounds, punch biopsies, or bone cultures. When anaerobic bacteria should be considered a possible pathogen, it is essential that samples be collected and anaerobically transported properly for the survival of the bacteria. Examples of acceptable samples for anaerobic culture are blocks of tissue placed in a sterile, sealed container, samples collected using a commercial anaerobic specimen collector, or liquid exudates placed in a sterile, air-tight container.

The direct phone number to the bacteriology lab is 402-472-8470.

- contributed by Deb Royal, Supervisor, Microbiology Lab

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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.

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