

Diagnostic Center News

Spring 2013

News From the Diagnostic Center

Notes from the Director

In This Issue:

Bacterial Culture Results from Bovine Lungs	2
Bacteriology Lab Proper Sample Submission	3
UNL Bovine <i>E. coli</i> Interpretation	4
Submission of Expired Trichomonas Transport Media	4
Sample Collection for Infectious Bovine Keratoconjunctivitis	5
Meet Our New Employee	5
Fact Sheets Added to Website	6

Numerous reports of epizootic hemorrhagic disease (EHD) in deer and cattle were reported in Nebraska and the Midwestern United States late last summer and fall due to the drought conditions. This spring, frequently asked questions of the Veterinary Diagnostic Center have inquired about EHD and its ability to persist in the cattle population and cause problems including reproductive disease and congenital fetal defects.

EHD is closely related to Blue Tongue Virus. Both are Orbiviruses and are transmitted by biological vectors, primarily biting flies in the Culicoides family. Common names for the EHD vectors include biting midges, sand gnats, and no-see-ums. Mosquitoes and other species of gnats have also been incriminated as carriers of EHD. Last year's drought is thought to have produced favorable habitat which included moist creek beds from receding waters, damp soil, and manure piles which were ideal for reproduction of the insect vectors. The drought is also believed to have concentrated the deer and livestock populations around water sources which in turn increased exposure to vectors. Most outbreaks develop in late summer and early autumn as replication of the virus in midges is temperature dependent. A marked decrease in the appearance of new cases occurs with the onset of freezing temperatures which kill the vectors that transmit the virus.

EHD is a disease primarily of white-tailed deer but on occasion can affect other ruminants including cattle, mule deer, pronghorn antelope, elk, bighorn sheep, and bison. Nearly 100% of the deer population can be seropositive in some regions of the country. Anecdotal evidence from field cases indicates that life-long neutralizing antibodies levels can develop in deer against the homologous serotype. Sheep can be experimentally infected but rarely develop clinical signs. Goats do not appear to be susceptible to the disease and do not become viremic after experimental infection. Viremic, non-symptomatic deer can occur. The incubation period in deer is 5-10 days. Infected deer can be viremic for up to 2 months and serve as reservoirs of infection. EHD typically recurs each year and varies from a few scattered cases to severe epizootics with high mortality. Variability is thought to be related to the abundance and distribution of insect vectors, EHD serotype, frequency of exposure, herd immunity especially maternal antibody levels, and genetic variability in the susceptibility of the host.

(continued on page 6)

Did You Know??

Shark corneas are used in eye transplants and shark bone marrow can be used to graft human bones.

- - - taken from
www.funfacts.com



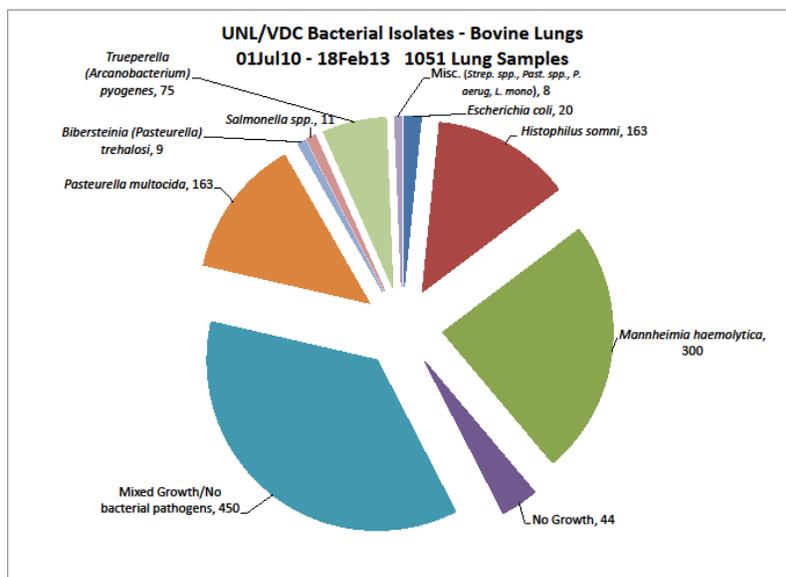
PLEASE NOTE: The Diagnostic Center is now able to accept payments with either Visa or MasterCard. Please call the main office at 402-472-1434 for further information.

Bacterial Culture Results from Bovine Lungs at the UNL Veterinary Diagnostic Center

J. Dustin Loy, DVM, PhD, ACVM, Bruce W. Brodersen, DVM, PhD

We commonly receive requests about the incidence of bacteria which are isolated from bovine lungs. This chart summarizes the culture results from July 1, 2010 to February 18, 2013. This report does not include age. There were 1,051 individual lung specimens during that time with 1,243 isolates, specimens with mixed contaminating growth, or no bacterial pathogens. One hundred eighty-seven individual lung specimens had more than one bacterial pathogen isolated. Twenty-four percent of the isolates were *Mannheimia haemolytica*, 13% were *Pasteurella multocida*, and 13% were *Histophilus somni*. This chart emphasizes that 45% of the lungs were heavily contaminated or overgrown with *Proteus* or *Bacillus spp.*, or a mixture of contaminants. *Bibersteinia trehalosi* constituted only 0.74% of the isolates.

Bibersteinia is an important pathogen of small ruminants, primarily causing pneumonia in sheep and severe septicemia in lambs. Improved diagnostic techniques have helped to increase the ability of diagnosticians to differentiate *Bibersteinia* from other members of the *Pasteurellaceae*. However, the role of *Bibersteinia* in bovine respiratory disease remains nebulous. Currently, there is little direct evidence for it being a primary pathogen. Recent work has shown that *Bibersteinia* can inhibit the *in vitro* growth of *Mannheimia haemolytica*, and thus confound culture results.



Sample Collection for Infectious Bovine Keratoconjunctivitis (Pinkeye)

This year the VDC has had a large number of submissions for culture of eyes for infectious bovine keratoconjunctivitis (IBK). This disease is caused mainly by the bacterium *Moraxella bovis*. Reports have also shown IBK to be associated with other organisms such as *Moraxella bovoculi* and *Moraxella ovis*. Other bacterial species, such as *Mycoplasma bovoculi*, have been shown to exacerbate or predispose animals to infections with *Moraxella* sp. When collecting specimens during suspected cases or breaks of IBK, sample collection is critical to isolating the organisms responsible. In order to maximize the probability of isolating pathogens, at least 5-10 eyes should be sampled, ideally from animals in the acute stages of disease. The acute stages of IBK are often only indicated by increased tear production and one or both eyes may appear to be slightly closed, and these should be sampled in lieu of animals with more advanced disease. Samples should be collected as aseptically as possible in the conjunctival sac using a moist swab, with two to three passes from medial to lateral canthus. This maximizes the amount of lacrimal secretions in the swab. Care should be taken to only swab the conjunctiva, as soil microbes and environmental organisms can contaminate the sample and make it unusable. Samples should immediately be placed in a transport media (such as Amies) and refrigerated to prevent overgrowth of contaminating organisms. These should be shipped overnight if possible for culture the following day. The diagnosticians and staff at the VDC can then help determine what pathogens might be present in the eye and their implications. Diagnostic evaluation often includes conducting molecular speciation and can include *in vitro* susceptibility panels on bacterial isolates from swabs if necessary.

-- submitted by J. Dustin Loy, DVM, PhD, DACVM,
Veterinary Microbiologist



Meet Our New Employee



Kristen Reynolds

Kristen Reynolds is new to the Diagnostic Center. She is a Histology Technician in the Histology Lab and began her employment on March 4. Kristen is from Lincoln and obtained her BS in Biology from the University of Iowa. She is also a HTL (ASCP). Kristen's hobbies are playing with her new Great Pyrenees puppy and reading with her six-year-old son.

We welcome Kristen to our staff!

Bacteriology Lab Proper Sample Submission

Our mission in the bacteriology lab is to help our clients make the best diagnosis based on the testing performed and that begins with the proper collection and shipping of samples. Below is a guide to the proper handling of samples for submission.

Test	Container	Temperature
Culture (tissue/fluid)	Any sterile container	Refrigerated (ice packs)
Culture (swab)	Culturette swab or sterile swab in a sterile container with sterile saline or sterile water	Refrigerated (ice packs)
Anaerobic culture (tissue/fluid)	Any sterile container or anaerobic transport media	Refrigerated (ice packs)
Anaerobic culture (swab)	Anaerobic culturette swab or sterile swab in anaerobic transport media	Refrigerated (ice packs)
Campylobacter culture (reproductive)	Campy transport media with antibiotics added	Refrigerated (ice packs)
Campylobacter culture (fecal)	Any sterile container	Refrigerated (ice packs)
Fungal culture	Any sterile container	Refrigerated or room temperature
Johne's culture/PCR	Any sterile container	Refrigerated or frozen
Tritrichomonas foetus PCR	Inpouch or transport tube	Media should be incubated at 37°C then frozen and shipped on ice packs
Tritrichomonas foetus culture	Inpouch	Room temperature
Other PCR	Any sterile container or swab	Refrigerated (ice packs)
Parasitology	Any sterile container	Refrigerated (ice packs)
Serology	Any serum collection tube separated or removed from the cells	Refrigerated (ice packs)

Please submit tissues or exudates if possible. A swab should be used as a last resort; however, if it is necessary to use a swab, ensure that it is in date. Remember to consider the temperature outside and the shipping time. In the winter, samples can freeze during shipment and in the summer they can overheat. We have been seeing about a one-day delay on delivery of samples shipped via the US Postal Service since January 28th, when packages were re-routed through Omaha before delivery in Lincoln, so it may be necessary to add extra ice packs or heat packs to ensure the quality of the sample during shipping, especially if shipping via USPS.

An alternative in some areas of eastern Nebraska is to set up an account with Nebraska LabLinc for courier service. Account managers for veterinary clients are Sharon Pfifer at 402-465-1997 and Amy Thomas at 402-465-1990. They can be contacted for more information.

- - - submitted by Debra K. Royal, Bacteriology Lab Mgr.

University of Nebraska Veterinary Diagnostic Center
Bovine E. Coli Intrepretation

Organism: Escherichia coli

Source: Faeces, Large Intestine, Large/small Intestine, Small Intestine

Species: Bovine

Date Range: 3/12/2012 to 3/12/2013

Antibiotic	Total	S		I		R	
Ampicillin	54	30	55.6%	0	0.0%	24	44.4%
Ceftiofur	54	48	88.9%	1	1.9%	5	9.3%
Chlortetracycline	54	17	31.5%	3	5.6%	34	63.0%
Clindamycin	54	0	0.0%	0	0.0%	54	100.0%
Enrofloxacin	54	47	87.0%	0	0.0%	7	13.0%
Florfenicol	54	13	24.1%	23	42.6%	18	33.3%
Neomycin	54	41	75.9%	0	0.0%	13	24.1%
Oxytetracycline	54	14	25.9%	0	0.0%	40	74.1%
Penicillin	54	0	0.0%	0	0.0%	54	100.0%
Spectinomycin	54	1	1.9%	42	77.8%	11	20.4%
Sulphadimethoxime	54	19	35.2%	0	0.0%	35	64.8%
Tiamulin	54	1	1.9%	8	14.8%	45	83.3%
Tilmicosin	54	0	0.0%	0	0.0%	54	100.0%
Trimethoprim/ Sulphamethoxazole	54	46	85.2%	0	0.0%	8	14.8%

Submission of Expired Trichomonas Transport Media

We often receive Trichomonas In-pouches or Transport Tubes that expired prior to collection of the samples. We have contacted the company that makes the media (Biomed Diagnostics) to determine the usefulness of the media after the expiration date. We were told that the viability of the organism greatly decreases when pouches or tubes are used after the expiration date. Our recommendation is that our clients do not submit samples in expired pouches; however, if we receive expired pouches, we will run the test and add a comment to the report stating the expiration date along with the following comment:

“The media used in the collection and transport of this test were past the expiration date set by the manufacturer. The validity of the results of this test cannot be guaranteed by the University of Nebraska Veterinary Diagnostic Lab.”

Pouches/tubes should not be used if they are expired, if the liquid appears to be cloudy, leaking, dark brown or dried, or if the integrity of the pouch or tube appears to have in any way been compromised. Pouches/tubes can be ordered directly from the lab at 402-472-8470 and if shipped via USPS the pouches usually arrive in 1-3 days. They can also be shipped via FedEx (at an additional charge) to arrive the next day.

The VDC will no longer exchange or compensate individuals for pouches that have either expired or where the veterinarian wishes to return excess unexpired pouches. Pouches can be delivered to veterinarians by overnight FedEx shipments (at an extra cost) if needed. Pouches must be ordered by 3 :00 p.m. to assure next day delivery.

- - submitted by Debra K. Royal, Bacteriology Laboratory Manager

EHD (cont'd.) - - - -

Most EHD infections in cattle appear to be subclinical. Disease is often sporadic. Seroprevalence in cattle is estimated to be as high as 15% in some areas of the country. EHD has not been reproduced experimentally in cattle. Although experimentally infected cattle may become viremic, they remain asymptomatic. Typical lesions, when apparent in field cases, include fever, oral ulceration, salivation, lameness associated with coronitis, and weight loss. In pregnant cows, fetal resorption or hydranencephaly may develop if infection occurs between 70-120 days gestation. Death is uncommon in cattle but sloughing of the hooves may occur months after initial infection.

Lesions can be confused with those produced BVDV, photosensitization, and other vesicular diseases such as Foot and Mouth Disease. Hence, the requirement for vigilance and the need to contact State and Federal Veterinarians for diagnostic assistance when a vesicular disease is either suspected or encountered in a herd.

Reproductive or concurrent disease problems have not been encountered in cattle herds known to be infected with EHD. Studies of cow herds that were confirmed to be infected last year with EHD and had clinical signs of disease are underway, but as of this date, no correlation between infection, reproductive losses and other disease syndromes have been confirmed.

- - - submitted by Alan R. Doster, DVM, PhD, ACVP, Director, Veterinary Diagnostic Center

Fact Sheets Added to the Website

The following fact sheets have been added to the UNL-VDC website at <http://vbms.unl.edu/nvdl>

Tritrichomonas foetus PCR Testing and Shipping Information
Blood Culture Collection and Shipping Instructions
CEM Collection and Transport Information

A reminder that the Federal Brucellosis Test Record (VS Form 4-33) can be found on the website in the submission forms area.

Nebr. Veterinary Diag. Ctr.
Univ. of Nebr.
151 VDC
Fair St. & E. Campus Loop
P. O. Box 82646
Lincoln, NE 68501-2646