

In this issue:

Message from the Director 1

Bovine Respiratory Disease Complex 2

Freezing of Formalin Fixed Samples 3

From the Wyoming Department of Health 4

Apologies 4

Message from the Director

This past June WSVL had a site visit by a team from the American Association of Veterinary Laboratory Diagnosticians (AAVLD), an organization with a mission of “continuous improvement and public awareness of veterinary diagnostic laboratories by ... education, communication, peer-reviewed publication, collaboration, outreach, and laboratory accreditation.” As such, AAVLD sets the standards for operation of veterinary diagnostic laboratories and evaluates whether laboratories are operating up to those standards. Thus the goal of the site visit team was to look at the operations of the WSVL from top to bottom. All aspects of the laboratory were open to examination, from how samples are received and routed within the lab to how results are reported to our clients. Particular attention was paid to features of the laboratory process directly affecting the reliability of diagnostic results: ongoing staff training, adherence to standard operating procedures, equipment maintenance and calibration, standardization of reagents and so on. Other aspects of the WSVL operations such as data and facility security, results of internal audits, budgets, staffing and contracting are also scrutinized. It is important to note that AAVLD accreditation is not an end in itself but a process requiring continual input by our staff, something that is not often recognized outside the WSVL. Renewal of accredi-

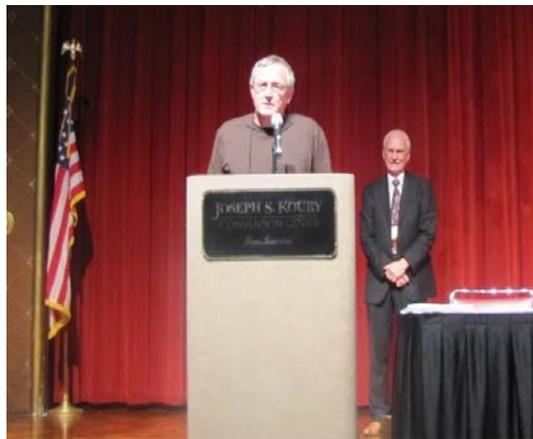
tation status is required periodically so staff pay constant attention to maintaining AAVLD standards. I am pleased to report that following the site visit and a review by the full AAVLD Accreditation Committee, the WSVL was granted full accreditation status, the direct result of

Health Association for his contributions to the advancement of national animal health programs. Congratulations Dr. O’Toole and Dr. Logan, the awards are very well deserved.

We had another site visit this summer by a team from the Centers for Disease Control to begin the certification process for the new BSL-3 laboratory at the WSVL. Staff and faculty from the WSVL, Veterinary Sciences Department and UW Environmental Health and Safety met with the CDC team, toured the facility and reviewed construction documents and proposed operating procedures. More work remains to be completed but we have received positive and productive comments from CDC. The certification process is moving forward and we anticipate timely approval to begin research and diagnostic activities in the BSL-3 facility.

There have been some changes at the WSVL over the summer. Carol Hearne retired this spring after 27 years operating the electron microscopy laboratory. Other employees who have moved on include Mercedes Thelen from Histology and Katy Fambrough from Trimming/Necropsy. New faces in the lab are Megan Dillon who has taken over duties in EM and John Henningsen in Trimming/Necropsy. Welcome Megan and John.

Will Laegreid, Director WSVL



Dr. Donal O’Toole receiving his Distinguished Career Service Award at the 55th Annual Meeting of the AAVLD in North Carolina, Oct 2012.

hard work by WSVL staff and faculty.

In a related development, Dr. Donal O’Toole, veterinary pathologist and professor at WSVL, was this year’s recipient of the Distinguished Career Service Award at the 55th Annual Meeting of the AAVLD. The award was presented during the President’s Banquet jointly hosted by the AAVLD and US Animal Health Association (USAHA) in Greensboro, NC. Dr. O’Toole received the award for “his years of service and commitment.” The State of Wyoming was well represented in the awards given at the recent AAVLD meeting. In addition to Dr. O’ Toole, Dr. Jim Logan, Wyoming State Veterinarian received the National Assembly Award from the U.S. Animal

“...a sad fact is that respiratory disease still remains the #1 cause of morbidity and mortality in cattle from about 6 weeks to 2 years of age.”

Bovine respiratory disease complex: where have all the viruses gone?

We are all in agreement that bovine respiratory disease complex is multifactorial. Stress, the environment, nutrition, management factors, and/or predisposing viral infections all contribute to what we recognize as BRD. Severe morbidity and fatalities typically are the result of bacterial pneumonia caused by the well-known respiratory pathogens that include *Mannheimia hemolytica* and *Pasteurella multocida*.

Despite development of new vaccines, improved antibiotics and a dramatic increase in our fundamental knowledge of BRD, a sad fact is that respiratory disease still remains the #1 cause of morbidity and mortality in cattle from about 6 weeks to 2 years of age.

It is a fact that pathologists seldom see microscopic evidence incriminating viral infection (Fig 1).

A common defense is that lesions such as necrotizing bronchiolitis can be overshadowed by the severe changes typical of bacterial pneumonia (Fig 2) but this is not entirely honest. Another fact is that virology sections in veterinary diagnostic laboratories seldom isolate or otherwise incriminate and confirm viruses in fatal cases of BRD.

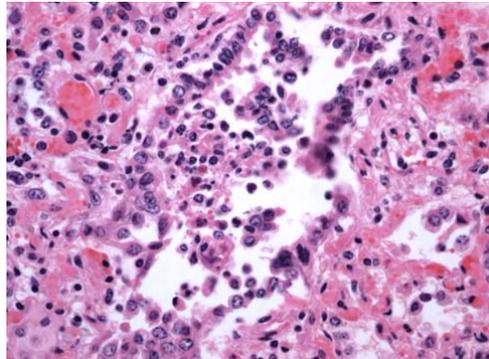


Fig 1. Lesions such as this, a necrotizing bronchiolitis with syncytial cells suggesting BRSV or possible PI-3, are seldom observed in fatal cases of BRD.

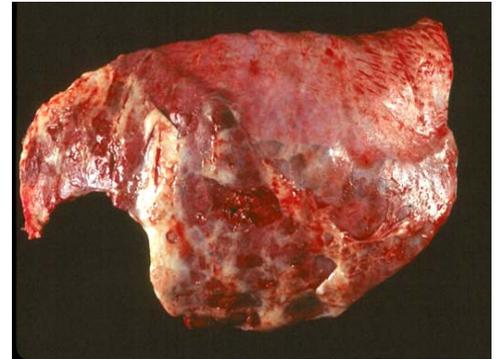


Fig 2. Lobar pneumonia and pleuritis typical of *Mannheimia hemolytica* infection

What then, can we conclude from these negative results? A possible inference is that viruses play only a minor predisposing role in BRD. Another is that all BRD vaccines provide excellent protection whether used correctly or inappropriately. Still another conclusion is that diagnostic techniques are inadequate. Few will believe any of these to be true. The most likely conclusion is that, by the time fatalities occur, the viruses are no longer present. Most authorities will agree that the key to recovering or identifying viruses

in BRD lies with sampling at the earliest time clinical signs are evident.

Deep nasal swabs saturated with nasal secretions are a sample of choice for identifying viruses during the earliest stages of infection. Do not, however, submit swabs in bacterial transport media. With this sample, we can perform virus isolations and PCR testing. PCR tests may also be pursued in fatal cases of BRD as these procedures are very sensitive and can detect only traces of persisting virus. Due

to the costs of PCR tests, we DO NOT run these routinely; you must request these procedures at the time samples are submitted. If you have questions, please do not hesitate to contact the laboratory.

Don Montgomery, Pathologist

Myrna Miller, Virologist

Jackie Cavender, Virology Section

Marce Vasquez, Virology Section

Find and Like us on Facebook!

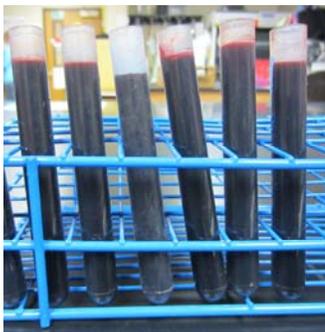
Facebook/WyomingStateVetLab

Notes from the Lab



Freezing of Formalin Fixed Samples

It is no secret that it gets cold in Wyoming during the winter. However, this can affect diagnostic samples as well as people. Freezing of small biopsy specimens in 10% neutral buffered formalin occasionally occurs during transit to the WSVL. This can preclude meaningful



histological evaluation. Addition of isopropyl alcohol (10% by volume) to the fixative lowers the freezing point of the fixative and may help prevent freezing of these specimens. Shipping the sample in an insulated container also reduces the risk of freezing. Isopropyl alcohol can interfere with immunohistochemical staining procedures. It is therefore NOT recommended for samples that are intended for immunohistochemistry, such as BVDV ear notches.

Blood samples may also freeze in the winter. This can make them extremely hemolyzed or even necrotic, often rendering them unsuitable for testing. To help prevent this we recommend that you ship your samples in a cooler box with a few ice packs to help regulate the temperature. Centrifuging the samples and taking the serum off will also solve this issue. Serum can freeze with no damage.

Testing for Reportable Diseases

When the WSVL receives an accession form with “rabies suspect” or “please check for anthrax,” all samples associated with that accession are placed on hold until a negative result is obtained for those diseases. Please be aware that requests for testing for diseases such as plague, tularemia, brucella, Q fever, anthrax or rabies can slow down your case. This is done to prevent unnecessary exposure of WSVL staff and the lab environment to potentially deadly pathogens but also results in delays in testing for other infectious agents, toxins, etc. We do not want to discourage submission of samples for any of these important diseases but would like to remind our clients this may cause some delay in reporting of other test results.

“...all samples associated with that accession are placed on hold until a negative result is obtained”

Bovine Leukosis ELISA Test Will Be Offered

The Regulatory Serology Section of the WSVL will soon be offering the Bovine Leukosis (BLV) ELISA test. This test had been discontinued due to a low number of samples being received. We have been referring the tests to RMRAHL in Denver for the past several years.

We are pleased to say we will begin offering it again on January 15, 2013! The cost will be \$7/animal and 1 mL of serum is needed. The serum can either

be pulled off the clot and put in a separate tube, or a red or tiger top tube can be sent. We will run this test on Thursdays. The test kit we will be using is highly sensitive and specific. It showed 98% Sensitivity and 100% Specificity when tested by the manufacturer. It is the same test kit we previously used.

BLV is an infectious, non-zoonotic viral disease of cattle. It may lead to persistent lym-

phocytosis and may cause some adult cattle to develop tumors with associated symptoms. Transmission between animals occurs by the transfer of lymphocytes, generally by trauma and the use of common bleeding needles and surgical procedures. Only about 5% of infected animals develop clinical signs. The ELISA test is used to identify carrier cattle.

Bovine Leukosis testing available starting January 15, 2013!

Wyoming State Veterinary Laboratory

Department of Veterinary Sciences
University of Wyoming
1174 Snowy Range Road
Laramie, WY 82070
<http://wvovet.uwyo.edu/>

Find and Like us on Facebook:

www.facebook.com/WyomingStateVetLab

Phone: (307) 766-9925
800 442 8331 (Toll-free Wyoming only)
Fax: (307) 721-2051

Dr. Will Laegreid, Director
wlaegrei@uwyo.edu
(307) 766-9929

Administration 9925, 9926,
9927, 9928

Laboratories ((307) 766 + # below)

Sample Receiving	9921
Virology	9933
Bacteriology	9931
Parasitology	9958
Toxicology	9961, 9962
Clinical Pathology	9958
Electron Microscopy	9948
Regulatory Serology	9924
Diagnostic Serology	9950
Necropsy Office	9920

Faculty ((307) 766 + # below)

Dr. Jeff Adamovicz	9905
Dr. Gerry Andrews	3139
Dr. Todd Cornish	9967
Dr. Jonathan Fox	9953
Dr. Frank Galey, Dean	4133
Dr. Myrna Miller	9934
Dr. Ken Mills	9932
Dr. Don Montgomery	9977
Dr. Donal O'Toole	9976
Dr. Merl Raisbeck	9963
Dr. Hermann Schätzl	6605
Dr. Brant Shumaker	9970
Dr. Charles Stith	9939
Dr. Chaoqun Yao	9959

WSVL Advisory Board:

Dr. Mike Driscoll	Mr. Ed Weppner
Dr. Fred Emerich	Mr. John Morris
Dr. J.D. Fox	Mr. Bill Lambert



COLLEGE OF AGRICULTURE AND
NATURAL RESOURCES

FROM THE WYOMING DEPARTMENT OF HEALTH: Human Exposure to the RB51 Brucellosis Vaccine Used in Livestock

The Wyoming Department of Health is interested in collecting information from Wyoming veterinarians regarding adverse health events after accidental exposure to RB51 brucellosis vaccine. The RB51 vaccine was conditionally licensed for use in cattle in 1996 and fully licensed in 2003. RB51 was developed to replace Strain 19; in part because cattle vaccinated with Strain 19 would give false positive tests results on brucellosis surveillance tests while RB51 does not result in the development of cross reacting antibodies¹. Although Strain 19 has been reported to cause brucellosis after accidental human exposure², the safety of the RB51 vaccine in humans following exposure remains largely undefined.

One study³ published in 2004 describes the accidental exposure of 26 individuals to RB51 either by needle stick injury (21 people) or spray exposure (5 people). At least one systemic symptom was reported in 19 of the individuals. There was no evidence that any of the individuals developed brucellosis. However the authors caution against concluding too much from such a small number of cases.

In Wyoming approximately 195,000 RB51 vaccinations are given to cattle each year⁴. One survey⁵ of needle stick exposures among veterinarians found a needle stick frequency rate of 1 needle stick per 1000 injections. If Wyoming veterinarians have a similar needle stick frequency, one would expect as many as 195 accidental inoculations of RB51 each year.

An on-line, anonymous survey has been developed for Wyoming veterinarians to provide information to the Wyoming Department of Health regarding their accidental exposures to RB51. There are also plans in place to administer this survey to veterinarians in Idaho and Montana. The survey can be accessed at <http://www.surveymonkey.com/s/RB51Survey>. A paper copy of the survey can also be mailed to you by contacting Kerry Pride at 307-777-8258 or Kerry.pride@wyo.gov.

1. "Brucellosis; Approved Brucella Vaccines." Code of Federal Regulations Title 9, Part 78, 1996.

2. Spink WW and H Thompson. *Human brucellosis caused by Brucella abortus, strain 19*. JAMA 1953 Nov 28;153(13):1162-5.

3. Ashford DA, di Pietra J, Lingappa J, Woods C, Noll H, Neville B, Weyant R, Bragg SL, Spiegel RA, Tappero J, Perkins BA. Adverse events in humans associated with accidental exposure to the livestock brucellosis vaccine RB51. Vaccine 2004 Sep 3;22(25-26):3435-9.

4. Estimate from the Wyoming Livestock Board. 5. Patterson CJ, La Venture M, Hurley SS, Davis JP: Accidental self-inoculation with Mycobacterium paratuberculosis bacterin (Johnes's bacterin) by veterinarians in Wisconsin. JAVMA 1988 May 1;192(9):1197-9

Apologies

The diagnostic staff and faculty at the WSVL take pride in the accuracy and timeliness of their work. Unfortunately, the current budget situation in the state has precipitated changes in University personnel practices resulting in delays in hiring or the potential elimination of some staff positions. Our staff is only one deep in many key positions so delays in hiring may significantly affect turnaround time for some accessions. The WSVL wishes to apologize for any delays you may have experienced in receiving results and for any future delays that may occur. In the meantime, if you have a case that is critical in nature, be sure to let us know and we will try to expedite to the extent possible.