# Wyoming State Veterinary Laboratory Newsletter

## December 2003


**University of Wyoming**
**Department of Veterinary Sciences**
**1174 Snowy Range Road**
**Laramie**
**WY 82070**
**http://wyovet.uwyo.edu/**

**Main office/Director**
**Phone: 307 742 6638**
**800 442 8331 (Toll-free Wyoming only)**
**Fax: 307 721 2051**


**WEB case access:**
**http://wsvl-web1.uwyo.edu:8083/Login.asp**

**To phone laboratories directly**
**307 742 6681 + EXT. BELOW**

| | |
|---|---|
| Mail Room | 122 |
| Virology Lab | 162 |
| Bacteriology Lab | 132 |
| Parasitology Lab | 182 |
| Toxicology Lab | 233 |
| Clinical Path Lab | 182 |
| EM Lab | 151 |
| Regulatory Serology | 142 |
| Diagnostic Serology | 163 |
| Dr. A. van Olphen | 161 |
| Dr. Merl Raisbeck | 231 |
| Dr. Ken Mills | 131 |
| Dr. Don Montgomery | 204 |
| Dr. Todd Cornish | 191 |
| Dr. Beth Williams | 211 |
| Dr. Donal O'Toole | 104 |
| Dr. Bill Jolley | 181 |
| Dr. Lee Belden | 766 2134 |
| Dean Frank Galey | 766 4133 |

**WNV Coordinator for WY:**
**Terry Creekmore: 307-742-6681, Ext. 105**
(If Terry unavailable, call Dr. Todd Cornish
**307-742- 6681 Ext. 191**)

**WSVL Advisory Board:**

| | |
|---|---|
| Dr Mike Driscoll | Mr. Ed Weppner |
| Dr. Jim Briddle | Mr. John Morris |
| Dr. J.D. Fox | Mr. Bill Lambert |

**MESSAGE FROM THE DIRECTOR**

***FINALLY, A THIRD PATHOLOGIST***

November 10, 2003 was a red-letter day for the Department of Veterinary Sciences. That was the day Dr. Don Montgomery came to work in the department. It was with pleasure and no small sense of relief that we hired Don as a pathologist, which gets us back to full strength in this discipline. Turn-around on pathology reports is back to where it should be, now that we have adequate cover. For one year there was a heavy burden on Dr. Williams and Dr. Cornish to cover a caseload requiring three people. Thank you for enduring this situation until we got the right person hired.

Dr. Don Montgomery comes to us from the Texas A&M laboratory in Amarillo, where he worked for 23 years as a pathologist. Don is a Texas native. He got his DVM at Texas A&M (1976) and obtained a PhD (1981) in pathology, studying a neurodegenerative disease of Kerry blue terriers. He became ACVP board certified in anatomical pathology in 1982. His path crossed briefly with that of Dr. Williams and mine when we were pathology trainees at CSU and he was there studying for the boards. He impressed everyone with his meticulous technique when studying the lesions of epilepsy in a large colony of beagles. Don's longstanding interests are diagnostic pathology of companion animals and livestock, neuropathology, and diseases of feedlot cattle. We are extraordinarily fortunate to have been able to winkle Don out of his native Texas. He was a regular visitor to Wyoming because of personal connections. We are delighted to welcome him to the department. Please make use of Dr. Montgomery's knowledge as a veterinarian, pathologist and researcher.

Donal O'Toole

## WEST NILE VIRUS

### SUMMARY IN DOMESTIC ANIMAL AND WILDLIFE IN WYOMING - 2003 SEASON

West Nile Virus (WNV) was identified in Wyoming in Goshen County in August 2002. It has since spread to 20 counties in Wyoming. In 2003, there were 394 human cases of WNV, and 9 fatalities reported from Wyoming. The state ranks fifth among the 45 states reporting WNV human illness.

During 2003, the WSVL tested samples from 446 horses for WNV. Of these, 230 were positive (51.5% tested positive) and located in 20 of Wyoming's 23 counties. The first WNV positive horse in 2003 was identified 22 May in Goshen Co. The last positive horse was diagnosed on 8th October in Park Co. In addition, 555 dead birds from 74 species were tested for WNV. Results indicate that 182 of those birds comprising 36 species were WNV positive (32.8% tested positive). West Nile virus-positive birds were identified in 16 of Wyoming's 23 counties. The first WNV positive bird was identified in Cheyenne on April 28. The last positive bird was collected October 9 in Albany Co. Evaluation of onset dates indicates that equine and avian cases peaked in the week ending August 8.

The ecological factors that supported increased WNV transmission in portions of Wyoming this year will continue to make those parts of the state higher risk areas for WNV transmission in the future. Precipitation levels will likely be the biggest influence on mosquito populations and WNV activity in Wyoming in 2004. If this year's precipitation levels are at or above normal, the population of *Culex sp*. mosquitoes that transmit WNV could equal or exceed levels encountered in 2003. The threat of human and equine WNV infection in 2004 may approach levels seen in 2003.

Terry Creekmore
WNV coordinator
January 2004

## RECENT CASES

### DISEASE-INFESTED RODENTS MAKE POOR PETS

We recently had a number of wild caught prairie dogs that tested positive for *Francisella tularensis* type A, which is the most pathogenic strain responsible for tularemia ("rabbit fever"). The animals were captured to provide a natural food for black-footed ferrets as part of ferret recovery effort. Prairie dogs are also captured for the pet trade. Tularemia is an infectious disease caused by a hardy bacterium, and it occurs in animals, especially rodents, rabbits, and hares. The bacterium is transmitted by direct or indirect methods, including tick and horse fly bites, contact with infected animal carcasses, consumption of contaminated food or water, or inhalation of aerosols. Symptoms include high fever, chills, head and muscle aches, weakness, chest discomfort, and a dry cough. The disease, which can be treated with antimicrobials, cannot be spread person to person. Tularemia is not uncommon. Some 200 cases of tularemia in humans are reported annually in the United States, mostly in persons living in the south-central and western states. Considering this and the fact that prairie dogs are known carriers of plague, they make a poor choice as a pet. On a personal note I don't think they are cute. On another front of the prairie dog as pet issue, there is no evidence of any link between "monkey pox" and wild prairie dogs. The prairie dogs reported in the news were probably infected by confinement near a Gambian giant rat or other African rodent, thought to be the original carriers of monkey pox to the United States. The Gambian rat is believed to be the source of infection to prairie dogs at a Chicago pet distribution center. One should not be concerned about exposure to monkey pox through being around or handling wild prairie dogs.

Ken Mills
Bacteriology section
January 2004

*"Rinderpest has probably had more impact on humans and domestic livestock than any other animal disease….The only positive aspect of rinderpest is that its control was a major stimulus for the establishment of veterinary schools in Europe in the 18th century.*

Paul Rossiter (2001): Rinderpest In: Infectious Diseases of Wild Mammals, 3rd Edition, Eds. ES Williams and I PK Barker, p. 37. Iowa State University Press.

### SALMONELLA IN A WYOMING DAIRY

Wyoming does not have many dairies but we do have some. As with dairies across the country, *Salmonella* can be a problem. We recently isolated two different serotypes from different animals in one dairy that had lost a number of animals with diverse clinical presentations. The first animal (adult) had severe diarrhea and Dr. Cornish found necrotizing enteritis on necropsy. From this animal we isolated *Salmonella* from lung and ileocecal lymph node.

All *Salmonella* isolates are sent to the Wyoming Public Health laboratory for serotyping and Pulsed Field Gel Electrophoresis (PFGE), which provides the laboratory and practitioner with information that could be useful in tracking the source of the organism. In this case, the *Salmonella* was identified as *S. mbandaka* and the PFGE did not match other isolates from the Wyoming database. The PFGE is a technique that determines the organism's fingerprint, which can then be compared to computer-stored fingerprints of previously isolated *Salmonella*. We have had a couple animal isolates that matched human isolates and through efforts of the state epidemiologist transmission possibilities have been identified.

The second *Salmonella* was isolated from another adult but in this case results of the necropsy were abomasal bloat and tympany. *Salmonella* was isolated from lung, bile, and ileocecal lymph node and serotyped as a *S. cerro*. PFGE did not give us a match in the Wyoming database.

A consistent finding on necropsy of these two animals was the presence of lots of crushed gravel in the digestive system, which may have caused mechanical damage that led to some of the problems. The bottom line in these cases, considering different clinical disease, rocks in the guts and two different Salmonella isolates was a suggestion of management change.

Ken Mills

## BRUCELLA REACTORS IN A SUBLETTE COUNTY HERD

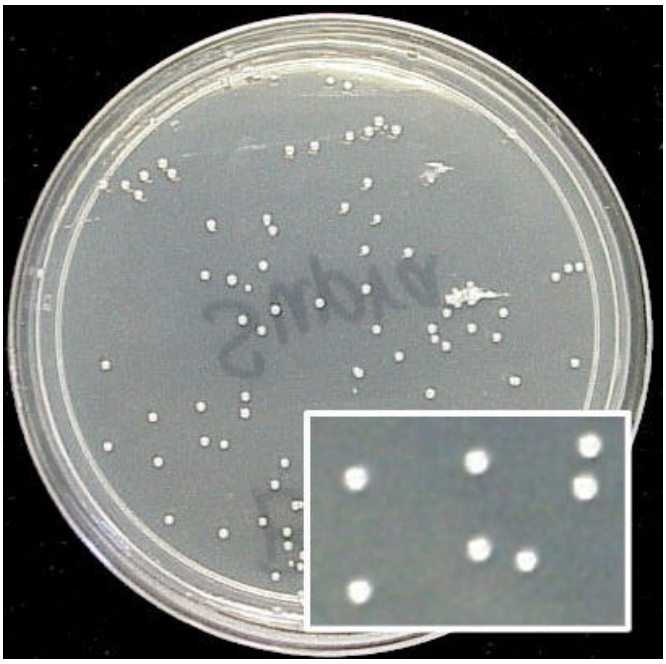
In December 2003, a beef herd containing *Brucella* reactors was identified in Sublette. Testing at WSVL and the National Veterinary Services Laboratory identified 31 reactors, many strongly positive on the rivanol test, which is an excellent index of infection. The owner agreed to depopulate the herd.

A team of WSVL, federal and state diagnosticians examined the 31 reactor cattle at the WSVL on 6 – 8 January 2004. The purpose of heavy sampling at necropsy was to isolate the *Brucella abortus* organism from as many animals as possible and to establish which biovar was responsible for abortion. The USDA's NVSL laboratory, which is responsible for the official culture results, anticipates reporting culture results to the AVIC, Dr. Bret Combs and to state veterinarian Dr. Jim Logan in 2 - 3 weeks. Additional samples were collected by Dr. Steve Olsen from the National Animal Disease Center (NADC) in Ames, Iowa so that current molecular assays can be applied to these isolates.

*Necropsy of Brucella reactor cattle, 6 January 2004. All personnel wore respirators to minimize the risk of contracting brucellosis. From left: Dr. Don Montgomery (WSVL), Dr. Steve Olsen (NADC), Brian Parrie (WSVL), Dr. Donal O'Toole (WSVL).*

Dr. Don Montgomery, who recently joined the WSVL as a pathologist, harvested an extensive set of lymph nodes and placental and fetal samples to compare three laboratory methods for diagnosing *B. abortus* infection in serologically positive cattle. Although there is a good body of information about the lesions of brucellosis in cattle, there is surprisingly little published information about lesions and location of bacteria (by culture, PCR, or immunohistochemistry) in "hot" reactors. Dr. Montgomery and Dr. Mills will have the studies completed and available for presentation at the summer meeting of the WVMA.

The Wyoming Game and Fish Department's diagnostic unit has a *B. abortus* isolate from an elk feed ground adjacent to the affected ranch. Additional testing of elk, including attempted isolation of *B. abortus*, may allow conclusions to be made about where infection originated in the herd. A technique developed by Dr. Betsy Bricker at NADC involving multi-locus analysis of variable number tandem repeats in DNA of *B. abortus* may help define the role of elk in transmission to this herd.



*Brucella abortus* in culture

The identification of a *Brucella*-infected herd in Wyoming is obviously serious. Dr. Logan, as state veterinarian, proposed additional testing of female cattle of Wyoming cattle so that adjacent states are reassured that the state has an effective surveillance system in place. At this time we are aware of just one affected herd. The Wyoming Livestock Board has current information on its web page about changes in the state rules regarding cattle and brucellosis:

http://wlsb.state.wy.us/

The USDA also has a section devoted to brucellosis on its web page:

http://www.aphis.usda.gov/vs/nahps/brucellosis/

On that web page, titled "UMR" (Uniform Methods and Rules"), there is a PDF document describing the USDA's policies for testing, controlling and eradicating brucellosis.

The WSVL posted a question-and-answer piece about brucellosis aimed at producers on its web site (reprinted below):

http://wsvl-web1.uwyo.edu/WSVL/updates2003.htm

*"Germany alone lost an estimated 28 million head of cattle in the 18th century, and the whole of Europe about 200 million [due to rinderpest]"*
C. A. Spinage: Cattle Plague – A History (2003). P. 203
Kluwer Academic/Plenum Publishers

## Brucellosis (Bang's disease) in cattle and wildlife

*This question and answer piece was developed in response to questions from producers in Wyoming after the recognition of a positive herd of cattle in Sublette County, Wyoming in November 2003.*

Q. What is brucellosis?
A. In cattle, brucellosis is a chronic bacterial disease caused by *Brucella abortus*. Its main effect is abortion. It can cause decreased milk production, weight loss, loss of young, infertility, and lameness.

Q. What are the signs of brucellosis in cattle?
A. The most obvious sign in pregnant animals is abortion, retained placentas, and birth of weak calves. Not all infected cows abort, but most do so between the 5th - 7th months of pregnancy. Most infected cows abort once, but some may abort during additional pregnancies. Calves born from later pregnancies may be weak and unhealthy. Other signs of brucellosis include apparent lowering of fertility with poor conception rates, retained afterbirths and metritis.

Q. Why is brucellosis a big deal?
A. The disease is highly contagious and historically was a major source of production loss to livestock owners in the United States. As recently as 1957, there were 124,000 infected herds in the United States. By the 1970s some 20% of American veterinarians had serological evidence of brucellosis. Today the country is nearly free of the disease. Recognition of brucellosis in a brucellosis-free state has serious economic impacts on domestic livestock markets and threatens export markets. *Brucella abortus* can infect people and cause disease. It is a high-category (Biosafety Level-3) pathogen and listed as a potential bioterrorism agent (i.e., it is a Select Agent). Clinical signs in people resemble influenza, and signs include fever, sweats, malaise, anorexia, headache, muscle and back pain. Less common signs are undulant fever, arthritis, and inflammation of the testicles. Neurological symptoms occur acutely in up to 5% of cases. In the chronic form (>1 year from onset), symptoms include chronic fatigue, depression, and arthritis. Treatment usually consists of doxycycline and rifampin used in combination for 6 weeks to prevent recurring infection. Depending on the timing of treatment and severity of illness, recovery takes several weeks to several months. Mortality is low (<2%), and generally associated with endocarditis. Additional information on brucellosis in people is available at the CDC web site:
http://www.cdc.gov/ncidod/dbmd/diseaseinfo/brucellosis_g.htm

Q. Who calls the shots when brucellosis is identified in a herd?
A. Control of brucellosis in a state is regulated jointly by the USDA's Area-Veterinarian-in-Charge and the state veterinarian under the Cooperative State–Federal Brucellosis Eradication Program,

Q. What is the strategy for testing a cattle herd suspected to have brucellosis?
A. This is a two-step process. Initial laboratory screening uses inexpensive, easy to perform, rapid, *highly sensitive* and *fairly specific* tests. Sensitivity is crucial for screening, so the occurrence of some false positive samples is tolerated in order to detect all infected animals. Then, to identify the false positive reactions, a second set of tests is then performed. Secondary tests are more expensive and complicated, and designed to *maximize specificity*. Generally, screening tests for Wyoming cattle are done at the WSVL and confirmatory tests are done at the USDA's National Veterinary Services Laboratory in Ames, IA.

Q. Why is the WSVL involved in testing for brucellosis?
A. It is important that Wyoming has independent testing capability for the disease in cattle. The WSVL signed an agreement with the USDA to perform front line screening tests such as the Rivanol test. The WSVL bacteriology laboratory has the ability to culture *B. abortus* from tissues. Personnel who perform the serological testing are monitored on a continuing basis and are formally certified by the USDA on their knowledge of accepted testing procedures. Considerable brucellosis research has been done in the laboratory in collaboration with other agencies.

Q. What do blood tests detect?
A. Blood tests detect antibodies to *Brucella abortus*. The most useful antibody to measure is IgG1. Some antibodies produced in response to vaccination cause false positives, particularly IgM. Many tests were developed so that IgM is precipitated or otherwise eliminated so it does not cause false positive reactions.

Q. Why are there so many blood tests to *Brucella abortus*?
A. Various tests were developed so that they maximize ease of use, sensitivity, specificity, cost-effectiveness and ability to distinguish vaccinated from naturally infected cattle. No one test combines all these attributes. For that reason, the number of tests run on individual samples is determined by the Area-Veterinarian-in-Charge and his/her superiors and the state veterinarian, based on circumstances of the herd.

Q. What tests does the WSVL run for *Brucella abortus*?

A. We run 4 tests: the standard agglutination test ("SPT"), the buffered acidified plate antigen ("BAPA") test, the card test ("CARD") and the rivanol test ("RIVANOL").

Q. How are these tests interpreted?
A. Interpretation is based on the test reaction ("negative," "suspect," or "reactor") AND vaccination status of the animal being tested. Details about interpretation of specific tests are provided in a USDA document dated Feb 1, 1998, *Brucellosis Eradication: Uniform Methods and Rules*, http://www.aphis.usda.gov/oa/pubs/bruumr.pdf

Q. What is the gold standard for diagnosis of brucellosis in cattle?
A. The confirmatory serological tests done at the USDA's National Veterinary Services Laboratory are helpful in establishing the status of a herd. But the definitive test is isolation of *Brucella abortus* from tissues of infected animals, either at slaughter or at necropsy. Culture allows the agent to be subtyped as to biovar. Culture takes ~2 weeks since the organism is easily overgrown by bacterial contaminants and slow to grow. Newer tests, such as polymerase chain reaction (PCR), can supplement bacterial culture as a "gold standard" method.

Q. How do I stay abreast of the current brucellosis situation in Wyoming?
A. The office of the state veterinarian for Wyoming is posting updates on brucellosis on the Wyoming Livestock Board's web site at http://wlsb.state.wy.us/. This is the most current and accurate source of information. The WSVL does not pass out confidential information about test status of individual herds to third parties, including the press. As tests are completed, results are relayed to the state veterinarian and the USDA's area veterinarian in charge. Tests are NOT reported via the Web, so there is no way that third parties can see raw test results from individual herds.

Q. Where can I find reliable generic information on the Web about brucellosis in cattle?
A. There is a good clearinghouse on the VetGate site in the United Kingdom that carries information from various countries (USA, UK, Israel and South Africa). The site is at http://vetgate.ac.uk/browse/cabi/detail/ac86a3d1a3b4ca5fcb5b57a54da15103.html

Q. Where can I or my veterinarian find current scientific information on brucellosis in cattle?
A. A good collection of current articles on brucellosis is in the journal *Veterinary Microbiology* issues 1 – 4, pp. 1 – 603, dated 20 Dec 2002. Articles are written by international specialists and represent the state of the art in brucellosis research and testing as of last year. Copies of the articles are available from medical libraries or – for a fee – from the publisher Elsevier via its Science Direct web site: http://www.sciencedirect.com/

Q. How does USDA APHIS decide whether to depopulate a herd?
A. Once infection is found, the herds are tested and positive animals are removed. Additional tests are conducted every 30 – 180 days until a herd is negative. Depopulation of affected herds was adopted in the mid-1970s as a management option for intractable, heavily affected herds. The decision about depopulation is made at a state and federal level, based on the risk that a reactor herd presents.

Q. Is it possible to prove cattle acquired infection from a wildlife source, such as elk or bison?
A. There are techniques that can help in fingerprinting strains, but scientists have limited experience in locking in firm conclusions that infection originated from wildlife, particularly elk and bison, since such events are relatively rare. Promising techniques have been developed, such as multi-locus analysis of variable number tandem repeats (VNTRs) in DNA of *Brucella abortus* isolates. The VNTR method requires that researchers have bacterial isolates from the suspect wildlife source *and* cattle in order to undertake a comparison. Epidemiological studies, such as proximity of infected elk to cattle, and incidence of infection in elk, are helpful for establishing where infection originated.

Q. Is it possible to prove cattle acquired infection from vaccination?
A. Provided the organism is cultured from the reactor cattle, yes. Growth characteristics in the laboratory help to distinguish vaccine strains (Strain 19 and RB51) from wild-type strains. Polymerase chain reaction (PCR) methods can distinguish wild biovars from vaccinal strains (*J. Clin. Microbiol* 38: 3085-3086).

Q. Can bacteriophages (viruses that infect bacteria) be used to successfully treat brucellosis in cattle?
A. We are not aware of any published, peer-reviewed studies on the use of bacteriophages to treat cattle with brucellosis. Indeed there are no recognized successful medical treatments for cattle chronically infected with *Brucella abortus*. Testing and culling reactor cattle is the standard method to control and eradicate brucellosis in the United States and other countries.

Q. Why won't state and federal authorities allow treatment with bacteriophages for brucellosis in cattle?
A. There are practical, regulatory and theoretical reasons for rejecting bacteriophage treatment for brucellosis at this time. The method has never been shown to work for this disease whereas testing and culling of positive cattle does. Environmental impacts of bacteriophages are unknown. The safety of using biological agents of undefined concentration, purity and potency is a major consideration, which is why the USDA and FDA tightly regulate the medical use of experimental treatments such as bacteriophage therapy in animals and people, respectively. The organism that causes brucellosis lives inside cells, not free in the body, so it is not clear how (or whether) bacteriophages could enter infected cells to attack *B. abortus*. Phage genetic material, under some conditions, can integrate into the bacterial genetic code, conferring resistance to further infection as well as endowing bacteria such as *B. abortus* with new antigenic and/or toxigenic properties. Unless established to be safe and effective in controlled experimental studies, the use of

bacteriophages must be considered an unproved technology to treat cattle with brucellosis.

Q. Why did the USDA switch from vaccination with Strain 19 to RB51?
A. Strain RB51 is associated with fewer adverse post-vaccination reactions in cattle, such as abortions and localized inflammation at vaccine injection sites, compared to Strain 19. More importantly, and unlike Strain 19, RB51 does not stimulate the production of antibodies that can be confused on standard diagnostic tests with antibodies produced in natural infection.

Q. Is RB51 as good as Strain 19 in providing protection against brucellosis in adult cattle?
A. Yes. Studies indicate that both vaccines protect 70 – 80% of animals in herds against challenge with wild-type *B. abortus*. Like all vaccines, protection is not perfect. The probability of infection is determined in large part by the size of the challenge dose of wild-type *Brucella abortus*.

Q. Why hasn't vaccination of wildlife controlled the problem in elk and bison?
A. Only a limited number of vaccines have been studied in wildlife, mostly S19 and RB51, both of which were developed for domestic livestock. Neither is as effective in wildlife as they are in cattle. RB51 provides lower levels of protection to vaccinated wildlife.

Q. How does the USDA classify states based on their brucellosis status?
A. There are 3 classes. Class Free status for a state or area means it is essentially brucellosis free. Class A and B states or areas have 0.25% and 1.5% Brucella-positive herds, respectively. If Wyoming loses its Class Free status, this imposes additional costs due to testing and import restrictions on Wyoming producers.

Dr. Donal O'Toole
Director, WSVL

Dr. Ken Mills
Bacteriology section

December 14, 2003

## PATHOLOGY FOREIGN ANIMAL DISEASE COURSE TRAINING AT PLUM ISLAND

Four of the diagnosticians at the WSVL have completed training at the USDA's foreign animal disease school at Plum Island in New York. They are Dr. Beth Williams, Todd Cornish, Donal O'Toole and Alberto van Olphen. The two most recently certified attended the course on November 3 – 7, 2003.

The purpose of this training is to expose personnel at state veterinary diagnostic laboratories to the clinical signs and lesions of high impact foreign animal diseases of cattle, sheep, horses and poultry. Trainees examined live affected animals in BL-3 facilities with various diseases such as rinderpest, foot and mouth disease, exotic Newcastle disease, avian influenza, sheep pox, contagious bovine pleuropneumonia, African horse sickness, classical swine fever and African swine fever. Animals are examined post-mortem and USDA personnel demonstrate typical gross lesions. This is supplemented by lectures each morning discussing the biology, location and politics of each disease. As part of the course trainees are provided with CDs illustrating the clinical signs and lesions of each of the major OIE list A disease. These include some excellent videos of the various diseases.

The course was excellent, as were the take home course materials. If any of you are asked to give extension-type talks on foreign animal diseases to your clients and would like access to the USDA CD set, please let me know.

*"At first, merchants contravening the bans on transport and sale of sick animals were to be imprisoned, some in chains to await later punishment. But when attempts to cure the disease had no effect, edicts were issued [by the Pope] forbidding bringing cattle into the city district of Rome under penalty of death (for a layman) or the galleys for life (for an ecclesiastic)"*

The Great 1709 European Panzootic in: C. A. Spinage: Cattle Plague – A History (2003). P. 109
Kluwer Academic/Plenum Publishers

## SELECT AGENT LABORATORY ESTABLISHED AT WSVL

As a result of the anthrax-in-the-mail cases in 2001, the federal government tightened up regulations governing high impact infectious agents of people, domestic animals and zoonotic agents ("Select Agents"). A new law, the Public Health Security and Bioterrorism Preparedness and Response Act of 2002, required that human and veterinary diagnostic laboratories be registered to isolate and hold select agents. These include *Yersinia pestis*, *Francisella tularensis*, *Brucella abortus*, *Brucella melitensis*, and *Coxiella burnetii* (Q fever agent).

At your end as a veterinarian this should have minimal impact since you can continue to submit tissues from suspect cases of plague, tularemia, Q fever and brucellosis to the laboratory. At our end it will require major changes in laboratory practice. Once a select agent is isolated and definitively identified from a sample submitted by you, we have to either destroy the isolate within a defined period of time, or maintain detailed records and inventory of the isolate. In either case, the USDA and/or CDC must be informed of each isolate. The WSVL must keep any retained isolates in a secure laboratory, with limited access.

With funding from the Wyoming Department of Health and the Wyoming Game and Fish Department, the WSVL established a Select Agent laboratory. The funding originated from Wyoming's CDC Bioterrorism grant. We are currently going through the final stages of commissioning for the SA laboratory. All personnel who handle confirmed select agents successfully passed an FBI background check.

In coming years it is likely that security in the WSVL will have to be tightened and there will be increased exchange of information and training between the WSVL and the state public health laboratory in Cheyenne.

## BOVINE TRICHOMONIASIS CONTROL—AT WORK

Since the required testing of bulls for *T. foetus* infection began in March 2000, more than 21,000 samples were analyzed in the parasitology laboratory at WSVL. Of those tested, >250 bulls (1.2%) were infected. Many of the samples analyzed have been 2nd, 3rd or more samples from individual animals. Most however were from single exams of animals either before turnout or after roundup.

Until 2003, more positive bulls from the beginning of the control program until 2003 were in Fremont County (49 of 1875 in 2001 (2.6%); 34 of 1666 in 2002 (2.0%)). A significant drop-off was seen in 2003, with only 6 of 1000 animals found infected. Numbers of infected bulls in other counties fluctuated as seen in Sheridan with 1 of 201 in 2001, 19 of 440 in 2002 and 2 of 547 in 2003; Carbon county with 10 of 1106 in 2001, 1 of 863 in 2002 and 20 of ~950 bulls in 2003. Sublette county bulls tested prior to 2003 were all negative. But in 2003, 7 of 300 bulls were positive.

Infected bulls have sporadically been identified in other counties in our State, but those listed above consistently harbored most of the bulls identified as carriers of the protozoan. Overall, it appears that the numbers of infected bulls have diminished in the counties where producers and veterinarians enthusiastically participated in the control program.

Bovine trichomoniasis is a problem that will probably never disappear. But with persistent cooperation between all involved parties, including producers, large animal veterinarians, enforcement and the diagnostic laboratory, it can be minimized to allow for increased profitability for cattle producers.

Bill Jolley
Parasitology Laboratory
January 2004

## TWEAKING TRICH TESTING

The side of a bull's penile/prepucial mucosa from which a smegma sample is taken may affect the confidence level of the analysis for *T. foetus*. A recent article in the Canadian Journal of Veterinary Research (2003; 67:138-141) found that bulls sampled from the right side ON the right side (by a right-handed sampler) was 96.1% sensitive for detection of a *T. foetus* infection, whereas bulls sampled from the left side ON the left side were only 88.8% sensitive. The results noted in the article may answer some of the questions we encounter when bulls test negative on the 1st and/or 2nd analysis and positive on the 2nd and/or 3rd.

Fecal coliform contamination of smegma is another problem that lowers diagnostic confidence in evaluation of trich cultures. In a laboratory project conducted by students Tanya Madden and Melissa Moore at WSVL, we found that culture media contaminated with *Escherichia coli* killed trichomonads in 6 - 8 hours. In a follow-up study, the students discovered that synergistic antibiotics cefixime and gentamycin could be added to the Diamond's culture medium in place of or in addition to the normal penicillin-streptomycin combination. This successfully suppresses the buildup of *E. coli* and three other contaminants (*Pseudomonas aeruginosa* and two *Bacillus* spp.). The antibiotics did not kill or inhibit the reproduction of the trichomonads.

If the antibiotics were added to transport media in sample tubes obviously contaminated with feces, prior to shipping to the Vet Lab for culture and examination, survival of *T. foetus* trophozoites will be enhanced. At 6ug/ml cefixime and 16ug/ml gentamycin, the addition of the drugs would cost about $0.048/L and $1.28/L, respectively. If you decide to do this, please put a notation on the form to indicate that you have added antibiotic to Diamond's medium.

Bill Jolley
Parasitology Laboratory
January 2004

## LEGAL AND FORENSIC CASES

This week we received canine carcasses for evaluation for animal cruelty. They were submitted by a sheriff's department with a history that the dogs might have been the victims of a spousal dispute. We get two or three such cases a year.

If you are confronted with a forensic case and need to submit it to the WSVL, please remember the following:

- These cases are handled differently. We follow chain-of-custody procedures.
- Mark the LITIGATION WORKUP box on top right of WSVL form
- Such cases are a time-sink, with phone calls, documentation, conferring with legal brethren, and a need to cover all major bases.
- We often end up in court as expert or subpoenaed witnesses. This is a major, costly, but important service of the laboratory.
- In such cases, we charge by the hour ($150/hour for the faculty member) including any phone calls. This is in addition to cost recovery for tests performed. If you have a litigious client who is demanding a litigation workup, make sure they understand that the bill will be $1,000 or more. The high cost may preempt frivolous submissions.
- A history is critical. If you don't tell us what you or your local police department specifically want to have checked, it may not be done. Some of these requests or suspicions are odd, and we need to know them up front.

From: The Wyoming State Veterinary Laboratory
Department of Veterinary Sciences
University of Wyoming
1174 Snowy Range Road
Laramie, WY 82070
http://wyovet.uwyo.edu/

**To:**