

Wyoming State Veterinary Laboratory

Newsletter

December 2002

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MESSAGE FROM THE DIRECTOR

Bovine viral diarrhea virus (BVDV) is the most important, laboratory-diagnosed cause of reproductive wastage in Wyoming cattle. It is a problem for three reasons:

1. It is impractical to keep truly closed beef herds under Wyoming conditions.
2. Commercial vaccines are limited in their ability to stop transplacental infection. Reproductive loss due to BVDV occurs regularly in vaccinated herds. Losses as high as one third of the calf crop occurred in 2002 in well-vaccinated herds in Wyoming. This happens when calves are exposed to a biotype of BVDV that is antigenically distinct from the strain(s) of virus in the vaccine.
3. In the past it has been expensive and difficult to detect all persistently infected animals, particularly calves, using traditional methods such as virus isolation from blood.

Drs. Todd Cornish and Alberto van Olphen from the Department of Veterinary Sciences at UW presented a large-scale study at the 45th USAHA-AAVLD meeting in St Louis in which they compared the sensitivity and specificity of two ear notch methods to detect calves persistently infected with BVDV. Virus isolation was used as the "gold standard" to assess the two ear notch tests. The study was done in cooperation with veterinarians and large producers in Wyoming. This is the first time a rigorous comparison like this has been done, with results presented before a veterinary audience. Both tests detect >99% of BVDV-infected calves.

You can get an updated version of the abstract given by Cornish *et al.* from the laboratory (307 742-6638 or TCornish@uwyo.edu or alvo@uwyo.edu). Dr. Lynn Woodard, extension veterinarian for the Wyoming State Veterinary Laboratory, has written a more detailed account of how persistently infected animals can be eliminated from a herd. Go to the Wyovet website <http://wyovet.uwyo.edu/>, then click on Disease Updates and Alerts.

The most cost and time efficient testing regimen to eliminate BVDV in an infected herd is as follows:

1. FOR CALVES < 4 MONTHS:

Calves < 4 mo. of age should be tested with the PBS/Serum ELISA. All calves in a BVDV infected herd must be tested.

Collect a sample of skin from the edge of the ear OR collect serum. The skin sample must be 1 x 1 cm. or larger. Place the skin in a 10 ml red top tube containing 2 ml of 0.1M phosphate buffered saline (PBS) pH 7.4. Be certain all tubes are individually labeled with animal I.D. numbers. The

samples must remain cool (in a cooler with ice packs is fine) while sampling is taking place. The samples should be immediately sent to the laboratory (same day if possible) or held in the refrigerator and shipped the next morning on ice packs. Ear notchers work fine for sampling. It is imperative that the sampling device be flamed between animals. Flaming prevents BVDV contamination occurring from one sample to the next. We can supply PBS-filled tubes at \$1.00 per tube if you need them.

Positive animals must be isolated from all herdmates. Since there is no one-time diagnostic test presently available to distinguish between persistently and acutely infected animals, it is necessary to retest positive animals in 3 to 4 weeks from the original sampling date. For the 2nd test, a purple or green top tube of blood should be submitted for virus isolation (VI) from each positive animal. If the VI is negative, the animal was **acutely** infected and cleared virus during the 3-4 week isolation period. Acutely infected animals may be returned to the herd. Animals that are VI positive are not able to clear the virus and are **persistently** infected. These animals should be isolated until they can be disposed of. Alternatively, all animals testing positive on the first sampling can be disposed of. This means that some acutely infected animals may be culled. **DAMS OF ALL POSITIVE CALVES MUST BE TESTED FOR BVDV** (see below).

The PBS/Serum ELISA has an average turn around time of 4 days. Turnaround may be shorter or longer depending on day of arrival and the condition of samples. Cost is \$3.25/sample. Samples are run twice a week.

2. FOR CALVES > 4 MONTHS, DAMS OF POSITIVE CALVES, COWS WITHOUT CALVES, BULLS AND ALL REPLACEMENT ANIMALS:

These animals may be tested with either the PBS/Serum ELISA test (see sampling protocol above) or with the BVDV Rapid ELISA test. For the Rapid ELISA test, collect plasma or serum from each animal in a sterile purple or green, or a red top tube. Samples should be handled, labeled and shipped as described above. Animals testing positive should be isolated and retested as described above.

The Rapid ELISA has an average turnaround time of 7 days. It may be shorter or longer depending on day of arrival and condition of sample. Cost is \$5/sample. This test is run once a week at the WSVL.

3. Other BVDV TESTS

BVDV immunohistochemistry (IHC)

BVDV immunohistochemistry is available for use on animals of any age. A 1 x 1 cm. or larger piece of skin from the edge of the ear should be collected in a red top tube containing 3-4 ml formalin. The BVDV IHC test has an average turnaround time of 1-4 weeks. Cost is \$3.50/sample. The BVDV IHC method is technically more demanding and more prone to error since a larger number of people are involved in processing the samples.

Virus isolation (VI)

Virus isolation testing is available for animals of any age and is the preferred method for aborted bovine fetuses. Tissue samples (lung, kidney, spleen, thymus, brain and/or mesenteric lymph nodes) should be sent postmortem. Blood (purple or green top tubes) should be submitted from animals that are still alive. Samples should be kept cool and sent to the laboratory on ice packs as soon as possible. Virus isolation is the gold standard to confirm an animal is infected but is too expensive for routine screening of herds. Virus isolations have an average turnaround of 2 weeks. Cost is \$16.00/sample. Virus isolations are set up once a week.

If you have questions regarding BVDV or BVDV testing procedures please call Dr. Alberto vanOlphen at 307-742-6681 ext.161 (alvo@uwyo.edu).

These tests (PBS ELISA, RAPID ELISA, IHC and virus isolation) are offered by the WSVL.

Citation:

T. Cornish, D. O'Toole, A. van Olphen, L. Woodard, D. Miller, and J. Cavender: 2002, A comparison of ear notch immunohistochemistry, ear notch antigen capture ELISA test, and virus isolation for the detection of calves persistently infected with bovine viral diarrhea virus. 45th AAVLD national meeting, St. Louis, Missouri. November 2002.

BVDV ear notch testing to detect persistently infected calves

We strongly recommend that, in screening large herds for persistently infected calves, you request the PBS ELISA. This test is faster and easier to standardize than the IHC test.

To encourage you to use the PBS test, there is now a price differential between these two tests:

**PBS ELISA ON EAR NOTCH
\$3.25 per sample
- submit in PBS solution**

**FORMALIN FIXED EAR NOTCH
\$3.50 per sample
- submit in formalin**

CAUTIONARY VETERINARY TALES

Below are two cases of iatrogenic disease in veterinarians: tularemia in an experienced diagnostic pathologist, and brucellosis in a food animal veterinarian in Wyoming.

A diagnostic pathologist in a western veterinary laboratory nicked himself with a necropsy blade while collecting specimens for CWD testing from the head of a deer. After washing the cut for five minutes, he thought

nothing of it and finished what he was doing. Three days later, the site of injury was swollen and red. By the evening of post-injury day (PID) four, a fever of 101°F developed. By next morning, it had risen to 103°F. Not connecting the fever with swelling and erythema in the finger, he treated himself for "flu". By PID 6, antebrachial and axillary lymph nodes were enlarged and painful with fever ranging from 101– 103° F. He saw a physician on PID 7, the injury site was cultured, and he began taking levofloxacin. Severe night sweats and headaches with reduced fever after four days led to a switch to IV gentamicin delivered via indwelling catheter. He developed tinnitus after four days and switched to doxycycline. *Francisella tularensis* was cultured from the wound in his finger. At the time of writing, the veterinarian was on the mend after his episode of rabbit fever.

A Wyoming veterinarian recently received a needle stick to the hand while using RB-51 vaccine. He sought treatment immediately but developed an infection at the stick site and consequent infection of the tendon sheath. Bacterial isolation found a mixed culture, including a heavy growth of *Brucella*.

RB-51 vaccine replaced strain 19 in the brucellosis eradication program in 1996. Since that time, several unintentional inoculations of this vaccine or conjunctival exposures have been reported to CDC. A few individuals reported inflammation at the inoculation site, and others have had intermittent fever, chills, headache, and myalgia. CDC maintains a registry of human exposures to RB-51. RB-51 is a genetically stable, rough morphology mutant of *B. abortus* strain 2308 that lacks the polysaccharide O-side chain on the bacterial surface. It was developed by passage in a selective media, resulting in a strain that maintains its immunogenicity but is less virulent than strain 19. Standard diagnostic tests do not detect measurable antibody titers to RB-51, eliminating the cost of trace-backs and retests on false positive animals. Studies in mice, sheep, and cattle showed RB-51 is protective against experimental challenge with *B. abortus* but is less likely than strain 19 to cause abortion.

One of the characteristics used in the development of the RB-51 vaccine complicates chemoprophylaxis. Treatment recommendations for strain 19 exposure include the use of doxycycline and rifampin but, since rifampin was used in the selective media for RB-51 development, it is ineffective.

Doxycycline treatment for 21 days is the preferred regime for post-exposure prophylaxis in adults exposed to RB-51.

Ken Mills/Donal O'Toole

PATHOLOGY SERVICE

We are currently operating with two pathologists, instead of our normal complement of three. The Department will interview three candidates for the vacant position in January-February. The new faculty member will be in place by July 1, 2003 if the Gods are with us.

To give Drs. Williams and Cornish relief other than Roloids® some cases are going to Dr. Quist, a PhD board-

certified, contract pathologist in Montana. This introduces a delay, since it takes a day to get slides to her, and reports returned to the WSVL electronically must be cut and pasted before they can be finalized

When you have a case requiring the fastest turnaround for your client, please write: "**EXPEDITE REPORT**" in the clinical history section of the accession form. Dr. Williams or Cornish will read such cases in-house.

I ask you to exercise discretion in doing this, since not all cases are emergencies and we still need to be able to send "overflow" cases to Dr. Quist until the new pathologist is on board.

We retain tissue blocks in the laboratory. If there is a hold up – for example, if Dr. Quist is not available that day to read a case – please contact the laboratory. We will cut additional slides at no extra cost to be read in house pronto.

WY DISEASE ROUND-UP

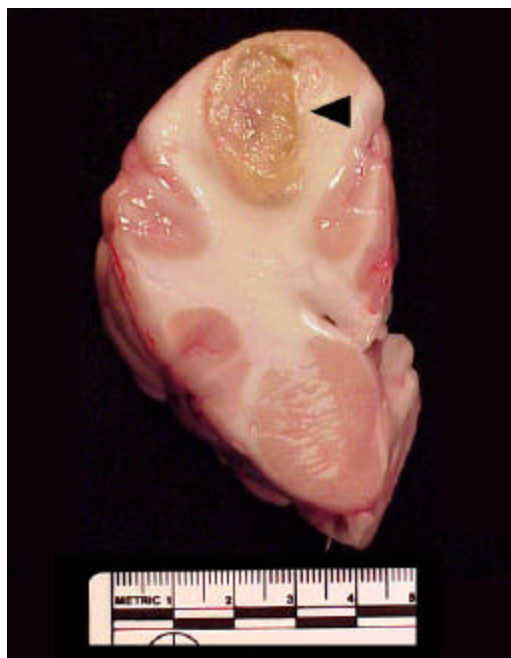
The laboratory had an increased number of outbreaks of **thromboembolic meningoencephalitis (TEME)** in October and November. Typical presenting signs were pneumonia, lameness and encephalopathy. We had cases from Gillette, Torrington and Alliance. Diagnoses on several outbreaks were based on histopathology alone, since the causative organism, *Haemophilus somnus*, is fastidious. We therefore need brain for both histology and bacteriology.

We continued to see cases of **Pigeon Fever in horses** due to *Corynebacterium pseudotuberculosis* myositis of the pectoral area into November. Other states that are normally free of PF, such as Kentucky, also saw this disease. Dr. J. D. Fox in Cheyenne estimates he saw more than 110 cases this Fall. Although horses generally have lesions in the muscles of the sternum, atypical locations have also been seen, such as the pinna.

Seven of 50 cows in north-central Wyoming suffered from **aldicarb (Temik) poisoning**. The cattle were grazing a barley field over-sown with radishes for approximately a week when the first four animals were found dead. Several more became recumbent while the referring veterinarian investigated the problem. Clinical signs were paddling, hypersalivation, dyspnea and diarrhea. Surviving cows and calves from affected cows were clinically normal. A tentative diagnosis of organophosphate/carbamate intoxication was supported by finding a partly eaten Temik package in the field. The diagnosis was made by finding depressed (0 μmole/ml/min) cholinesterase activity in blood. Treatment with atropine was successful in most surviving affected animals. Aldicarb is an insecticide marketed under the trademark Temik. It is for use against nematode parasites of alfalfa, beets and potatoes. Unlike many carbamate insecticides, it has a relatively high mammalian toxicity (LD₅₀ of approximately 2 mg/kg). Under approved conditions of use (soil incorporation), human or animal exposure is unlikely but we continue to see cases due to improper disposal. Cattle do not seem put off by its faint

chemical odor and will readily eat it if available. Although supportive treatment and atropine can be successful if started early enough, poisoning is rapid and animals often die before anything can be done. As with all restricted-use pesticides, the Temik label includes instructions for safe disposal. Producers should be encouraged to comply with these instructions.

A 6-month old paint filly was presented to a veterinarian in poor condition, recumbent, paddling, with a high white cell count. At necropsy the filly had a large abscess in the medial aspect of the thigh and two abscesses in the brain. We cultured *Strep. equi* from brain. The foal had **streptococcal encephalitis** due to hematogenous spread of infection from the abscess in the thigh.

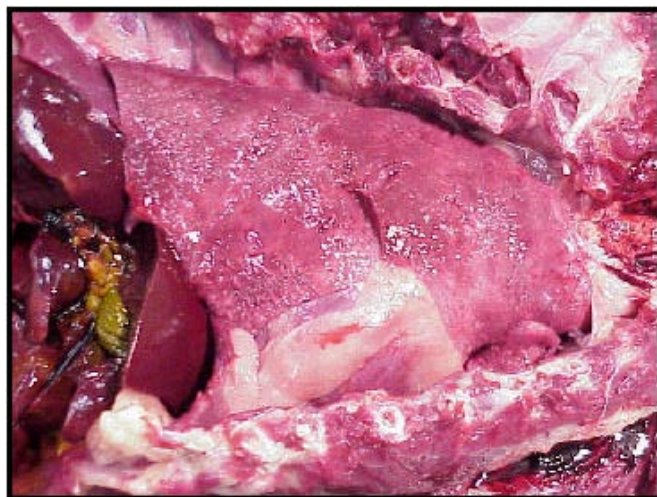


Abscess in brain of 6-month filly due to Strep. equi.

New **West Nile Virus** cases stopped for the winter with the end of the 2002 mosquito season. The last WY case in a horse was in October 29 in Crook County. Elsewhere in the newsletter is a map of cumulative cases of WNV. Dr. Todd Cornish and Terry Creekmore, WNV project coordinator for Wyoming, anticipate new cases will start again in May 03, since infected mosquitoes emerge late April/early May. Both strongly recommend that you encourage clients to get their horses vaccinated before that time. Horses should be vaccinated (twice, 3 weeks apart) or boosted (once) by March. Approximately 15 clinical cases occurred in horses receiving only the first dose of vaccine or right around the time of the second dose; as far as we know they all survived. All known fatalities in WY horses were in non-vaccinated horses, indicating the vaccine is effective. We have more follow up to do on the outcome of some of the serologically positive horse. The only vaccine on the market at present for horses is made by Fort Dodge. There is

currently no human vaccine for WNV, although two commercial companies are working on a human product. One (Acambis Inc) has a \$3M development grant from NIH. Phase I trials begin in Spring 2003. This live attenuated chimeric vaccine is based on a successful yellow fever virus, and has inserted genes for envelope proteins of WNV. It has been tested on hamsters, mice, monkeys and horses. If you have questions about WNV, or need a speaker to talk to a local audience, please contact Terry Creekmore at **307-742-6681, ext. 105**. The USDA recently declared WNV an enzootic disease, recognizing that it is here to stay in the US. It also means that the National Veterinary Services Laboratory will no longer run tests for free. Wild bird surveillance at WSVL will be free in 2003 (paid for by public health money), but contact Dr. Todd Cornish or Terry Creekmore before submitting carcasses. Equine serology (IgM ELISA) will be \$6.00/sample in-state and \$10.00/sample out-of-state. Diagnostic PCR is \$30/sample, but again, please contact the laboratory (Todd Cornish or Terry Creekmore) prior to submitting samples.

An adult dog that presented at a veterinary clinic in Cheyenne with dyspnea and depression was diagnosed with **pulmonary candidiasis** at necropsy.



Diffuse granulomatous pneumonia in lungs of a dog with pulmonary candidiasis.

The owners obtained the animal four years earlier in Nevada and the dog subsequently lived with its military owners in Korea and returned to the US in March 2002. It had "always" had a sporadic cough or episodes of gagging. He was depressed for one month prior to presentation. The examining clinician noted atrophy of muscles of the head. Radiographically, diffuse lung disease was identified. A fungal organism was identified by cytological examination of the dog's sputum. The dog was euthanized and submitted with a request that we examine the dog for an exotic fungus, since there was history of illness in the family. Gross changes in the dog consisted of diffuse consolidation of all lobes of the lungs, and moderate bilateral atrophy of temporal muscles. At necropsy the dog had chronic diffuse interstitial

pneumonia with extensive fibrosis, and modest numbers of intralesional, intra-alveolar *Candida*-like yeast, and identification is still pending. The Wyoming Department of Health was informed of the case, since there was a human health concern.

Five rams from a group of 114 were submitted for necropsy with a history of being off food and weight loss. The rams had **urolithiasis** of varying severity with hemorrhagic cystitis, hydronephrosis, ruptured bladders and uroperitoneum. One ram had a ruptured ureter. The pelleted ration was formulated of approximately equal parts oats, alfalfa and soy hulls. The producer was advised to get the rams off the ration, put them on a diet with more roughage, add salt (NaCl), and provide free access to clean water at all times. Uroliths are still being analyzed at the time of writing. We assume soy hulls were the source of the problem.

Contributed by Terry Creekmore and Drs. Ken Mills, Beth Williams, Todd Cornish and Merl Raisbeck

INCREASED RISK OF NITRATE TOXICOSIS IN WY CATTLE

Given the continuing drought, we are likely to see an increased number of nitrate poisoning cases in cattle over the next few months. Drought stress exacerbates the tendency of many plants to accumulate nitrate. The scarcity of usable forage forces producers to use feedstuffs of dubious provenance. While it always a good idea to test hay before feeding, it is especially important during the current drought.

Some things to consider:

- **Sampling:** Hay should be sampled after it is cut and cured, not before. Use a bale corer, which you can get from a county agent, to collect 10–15 sub-samples from each lot (stack, truck load etc.) of hay. Samples collected by grabbing a handful here and a handful there are not reliable.
- **Testing:** Use a reputable laboratory! Many environmental testing laboratories don't understand this kind of sample. While I'm sure there are many others, I have had good results with the WY Department of Agriculture's Analytical Services Laboratory in Laramie ((307)-742-2984; accession forms are available on line at <http://wyagric.state.wy.us/aslab/aslab.htm>) and Livestock Nutrition Laboratory Services at the University of Missouri.
- **Results:** Nitrate results can be expressed several different ways. The commonest are KNO_3 , NO_3 ion, and nitrate-nitrogen ($NO_3 - N$). Make sure you know how the results are being reported. In fact, one measure of a laboratory's competence in this area is whether its personnel make concrete recommendations based upon results. If they just send you a number, think about switching laboratories.
- **Interpretation:** The older literature (e.g., 1960s) contains many references to an entity known as lowland

abortion, which was supposedly caused by 200 – 300 ppm dietary NO_3 . Since that time, many people have fed a lot of NO_3 without reproducing the syndrome. Some investigators have fed as much as 2.5% dietary NO_3 without clinical effects. To the best of my knowledge there are no creditable reports of NO_3 poisoning at dietary concentrations of less than 1.0%. At the WVSL we use 0.5% as a "safe" cutoff. Water is a different critter because cattle may drink quite a bit before they eat. Many authorities suggest concentrations around 500 ppm as the cutoff. If both feed and water contain appreciable concentrations of nitrate, you have to consider the contributions from the two sources. Please give me a call if you need help.

Solutions:

- Horses are quite resistant to nitrate intoxication. Apparently the NO_3 ion is absorbed by the upper gastrointestinal tract and eliminated in the urine before it can be reduced to the toxic NO_2 ion. Assuming the hay is in other respects good, consider feeding moderately high NO_3 hay to horses.
- If the NO_3 concentration is not too high (<1.5%), it can be diluted to acceptable concentrations with clean feed. The feed must be thoroughly mixed before feeding (e.g., using a grinder) otherwise some cattle may get a toxic dose. Merely throwing out one bale of "bad" and two bales of "good" hay does not constitute dilution and is asking for trouble.
- Fermentation, i.e., haylage, may decrease NO_3 somewhat if there is sufficient soluble carbohydrate present. Most Wyoming forages lack the necessary energy to fuel the reaction.
- A probiotic feed additive (Bova Pro®, FarMor Biochem, Milwaukee) is based upon a patented *Propionibacterium* sp. developed in Oklahoma State University and is advertised to decrease rumen NO_3 and blood methemoglobin concentrations by 40 – 50%. Preliminary data looked promising when it was introduced several years ago, but I don't have first-hand experience with the product in Wyoming.

*Dr. Merl Raisbeck
December 2002*

Supporting pre-veterinary and veterinary students

The Department of Veterinary Sciences operates a scholarship program for pre-veterinary students. The Jensen scholarship supports talented pre-veterinary students who have set their sights on becoming veterinarians. It was the brainchild of Dr. Lynn Woodard, who wanted an endowment with annual cash scholarship to recognize the best pre-veterinary student in the Pre-Veterinary Program at the University of Wyoming. By best I don't just mean the smartest, although grades are a big consideration. Lynn wanted to give a leg up to students with "the right stuff":

communication and leadership skills, a good work ethic, familiarity with the realities of veterinary practice, and plain horse sense. Thanks to donations from veterinarians, faculty and staff in the department, Montfort Corporation and the WVMA, we have made annual awards over the past six years. This year's award was \$1,000. It went to Jessica Zebroski, who is a Lander native



Jessica Zebroski – 2002 recipient of the UW Rue Jensen Pre-Veterinary Scholarship

Past recipients were Mary Hayes (1997; California native at UW), Kelly Hehn (1998; Casper), Laura Hammonds (1999; Lusk), Katie Leonard (2000; Pennsylvania native at UW) and Mary Spivey (2001; Laramie). To date, every Jensen recipient has gone on to veterinary school.

So when the College of Agriculture or the UW Foundation approaches you for gifts, please consider earmarking some of your donation for the Rue Jensen Scholarship. By giving a leg up now to promising pre-veterinary UW students, we load the dice: they are more likely stay the course, get to veterinary school, and more likely to come back to our state to practice.

The ability to increase the award to two pre-veterinary scholarships annually, at the level of \$1,000 or more per student, will help aspiring veterinarians at UW on their long, expensive and competitive journey to a DVM.

Dr. Ron Foland, President-elect of the Wyoming Veterinary Medical Association, is working on a complementary program. The WVMA is establishing an annual cash award scholarship for Wyoming veterinary students. The proposal is timely. There is growing recognition of the difficulty of attracting veterinarians to mixed rural practices in states like Wyoming. A proposal dealing with the problem of too few rural veterinarians was discussed at the December Mega-Ag convention in Casper.

I encourage you to contact Dr. Foland and donate to the WVMA scholarship for veterinary students.

Donal O'Toole

If you are interested in supporting Wyoming pre-veterinary or veterinary students:

Rue Jensen Scholarship for Pre-Veterinary Students at UW – contact:

- Dr. Lynn Woodard 307 742 6638
Woodard@uwyo.edu
- Ann Leonard, UW Ag development office
Aleonard@uwyo.edu

WVMA Scholarship for WY Veterinary Students – Contact:

- Dr. Ron Foland, Frontier Veterinary Clinic, 307-322-5533 rondvm@yahoo.com
- WVMA office c/o Vicki Smith, Executive Director, (800) 272-1813
msvickil@mindspring.com

Abortion sample kits

Abortion season looms. The Wyoming State Veterinary Laboratory is providing abortion kits for customers who are interested. Kits have been sent to most of our clients and are available for \$3.00 a kit. Kits include a box for mailing, labeled Whirl-Paks, syringes, needles and fetal tissue collection lists for histology, bacteriology, virology and toxicology. Having veterinarians (or their technicians) collect samples for testing by following a check list decreases result turn around time for abortion cases and will standardize the samples you take. Don't forget to collect placenta, when it is available – this is the sample we most commonly don't receive, and it is arguably the most important sample, particularly in cattle, sheep, and horses.

*Brian Parrie,
Necropsy manager*

WSVL Web Site

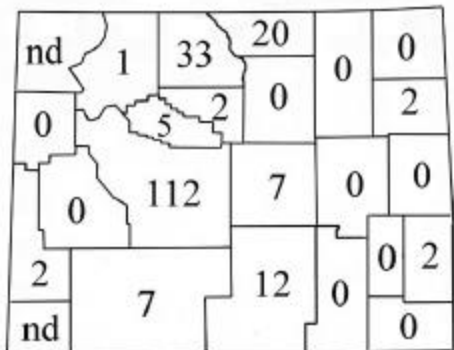
We revamped our web site to standardize the format and improve ease of updating. If you have suggestions of useful links, including WY clinic web sites, please contact us. Back issues of the newsletter are poster there

<http://wyovet.uwyo.edu/>

If you would like to get the newsletter electronically in PDF format, please send us your email address. We will put you on the list

Trichomonas in bulls

1 January 2000 – 30 November 2002
218 positive of 16,914 tested



WNV in horses

August – November 2002
96 positive horses

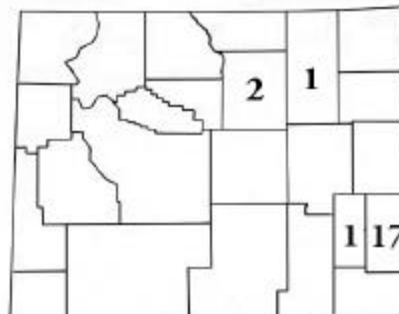


Comment: the hot spot remains Goshen county. We anticipate cases will be statewide in 2003.

Numbers by county (numbers positive/numbers tested):
AL: 0/495; BH: 33/1427; CA: 12/2332; CO: 0/208; CP: 0/25;
CR: 0/12; FR: 112/4854; GO: 2/236; HS: 5/684; JO: 0/84;
LA: 0/216; LI: 2/313; NA: 7/472; NI: 0/243; PA: 1/441; PL:
0/233; SH: 20/879; SU: 0/620; SW: 7/400; TE: 0/83; UI: no
data; WA: 2/1077; WE: 2/126; YNP: no data.

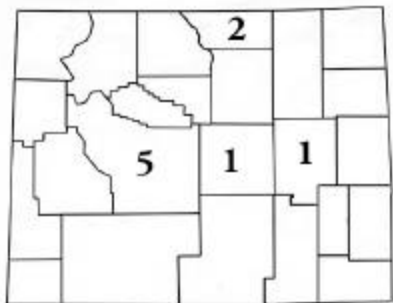
WNV in birds

August – November 2002
21 positive birds



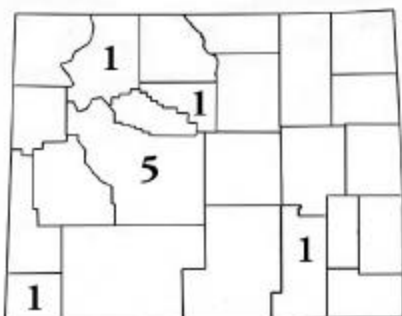
Skunk rabies

1 January 2002 – 30 November 2002
9 positive skunks



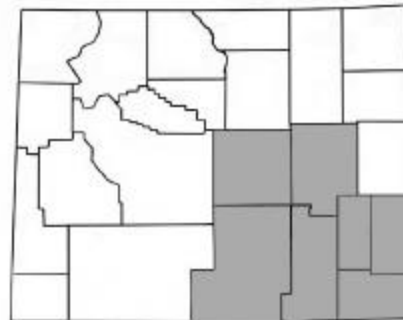
Bat rabies

1 January 2002 – 30 November 2002
9 positive bats



CWD in free-ranging cervids

Fall 2002



The recognized endemic CWD area in WY has expanded in 2002 due to surveillance by Dr. Williams and her colleagues in WGF. Cases have been recognized for the first time on the western slope of the Snowy Range and the Sierra Madres.

From: The Wyoming State Veterinary Laboratory
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