MESSAGE FROM THE DIRECTOR

SHIPPING NEWS

As a result of an incorrect message out of Denver, some local postal services in Wyoming are confused about the difference between diagnostic and infectious samples. They have been telling veterinarians they must put biohazard stickers on the box. This is not necessary. It is causing problems in shipping, since Biohazard stickers cause alarm among postal workers. Please DO NOT use biohazard stickers for routine diagnostic accessions.

If you have problems with local postal service, tell them that the correct information is in 49 CFR (Code of Federal Regulations) 173.134 Class 6, Division 6.2 – Definitions and Exceptions. What this says is as follows:

What veterinarians send to our laboratory are diagnostic specimens, NOT infectious samples. Samples become officially “infectious” only after the agent has been isolated in a laboratory. Diagnostic samples include animal-derived materials such as excreta, secreta, blood and blood components, tissue, and tissue fluids that are transported for diagnostic/investigational purposes. To meet code, clearly write “DIAGNOSTIC SPECIMEN” on the outside of the box.

Diagnostic specimens must be packaged in a triple package, consisting of a primary receptacle, a secondary packaging, and an outer package. The primary receptacles must be packed in such a way that, under normal transport conditions, they cannot break, be punctured, or leak.

The secondary package must be secured and cushioned so that a leak will not compromise the cushioning material or the outer package.

If you are sending a liquid such as blood, make sure the primary receptacle is leak-proof and there is absorbent material between the primary receptacle and secondary packaging. If several blood tubes are sent, they must be individually wrapped or separated so as to prevent contact. Absorbent material must be of sufficient quantity to absorb the entire contents of the primary receptacles.

This is the most recently available regulation on shipping diagnostic specimens. If and when these regulations change, the Wyoming State Vet Lab will let you know.

Our shippers and blood tube mailers meet the standards required by 49 CFR. These are available by request on accessions forms, via e-mail or by phone. If you have questions or concerns about proper mailing procedure or if your local postal service is trying to determine how your package should be mailed, please contact Brian Parrie, who takes care of our shipping and receiving (307 742 6681 ext. 121 and email Parrieb@uwyo.edu).

We rely heavily on the service provided by the post office. Please make sure that your packages do not leak in the mail. Avoid using “Biohazard” stickers for routine accessions!
West Nile Virus Update: Don’t let the vaccine freeze!

West Nile virus was first identified in Wyoming on August 16, 2002. It subsequently spread to 15 of Wyoming’s 23 counties. Transmission of the virus will begin with the emergence of mosquitoes this spring. We anticipate that West Nile virus will be established in the remaining eight Wyoming counties by the end of the summer. Historically, the second year of virus transmission has been markedly worse than the initial year. Wyoming residents can expect an increase in the number of horses infected with West Nile virus this summer. After this year, the disease should settle down and become part of the normal infectious flora of the state.

The WNV vaccine made by Fort Dodge, which is currently the only product on the market, cannot be allowed to freeze. According to the company, if it freezes before use the pH changes and the vaccine is considered no longer effective. You should advise clients to make sure the vaccine is not allowed to freeze before use. Your clients should be vaccinating their horses now. If horses have not been vaccinated before, they need two injections 3 - 6 weeks apart. If they were vaccinated last year, they need a single booster before insects emerge. Foals from previously vaccinated mares can be given a 2 dose series (3 - 6 weeks apart) starting at age 3 - 4 months. Foals from mares not previously vaccinated should be given a 2 dose series (3 - 6 weeks apart) starting at age 6-8 weeks.

If you have an animal (e.g., dog, sheep, camelid) that you suspect is WNV-infected, please contact Dr. Todd Cornish at the laboratory prior to submitting samples.

Useful and regularly updated web sites for information on WNV developments are:

Overview of WNV disease at Cornell’s web site:
http://www.cfe.cornell.edu/erap/WNV/

CDC site on human disease:
http://www.cdc.gov/ncidod/dvbid/westnile/

USDA site on animal disease:

Maps of WNV distribution:
http://cindi.usgs.gov/hazard/event/west_nile/west_nile.html

NEMATODE PARASITES OF PRIMARY IMPORTANCE FOR WYOMING CATTLE

- Trichostrongylid roundworms infect virtually all cattle and other ruminants, every month of the year; mature, tolerant animals are the infection reservoir for young stock.
- Ostertagia ostertagi and other Ostertagia species are the most prevalent and most detrimental nematodes for young cattle (<3 years old).
- Detrimental effects are most pronounced in late summer/early fall (in calves), and during mid-winter to early spring (yearlings and 2-year-olds).
- Late summer/early fall disease in calves is due to adult, egg-laying worms in the lumen of the abomasum: diagnosis by fecal flotation + egg counts, done at a veterinary laboratory.
- Winter disease in yearlings and 2-year-olds is due to inhibited L4 larvae embedded in the abomasal mucosa, near the gastric glands; HCl production is inhibited, abomasal pH rises, digestion of protein ceases, animal development and condition declines to the degree dependent on number of hypobiotic/inhibited larvae present: diagnosis is by direct observation of abomasal mucosa (count the white bumps). Adult, egg-laying worms are scarce or non-existent during winter months and so egg counts are useless to detect this form of the disease.
- Late winter/early spring “wrecks” are due to high numbers of inhibited larvae simultaneously breaking out of hypobiosis, becoming adults in the lumen of the abomasum; this usually occurs within two weeks of a warm weather episode in late February, March or April: diagnosis is by necropsy examination of abomasal mucosa for ulceration/inflammation OR by fecal egg counts 2-3 weeks after clinical onset.
- Treatment strategies:
  ➔ Use an effective enteric dewormer (target adult worms) on calves coming off range/at weaning.

WNV testing fees at WSVL

Equine serology (IgM capture ELISA)
- In state: $6.00
- Out of state: $10.00

Virus detection in unfixed equine tissues (PCR test)
- In state: $28
- Out of state: $28

Virus detection in formalin-fixed equine tissues (IHC)
- In state: $15
- Out of state: $15

Testing tissues from birds (PCR and IHC)
- No charge (subsidized)

Testing non-equine and non-avian species:
- Please contact Dr. Cornish prior to submitting samples for testing
Apply effective systemic anthelmintic (target inhibited larvae) to animals during late fall/early winter, after grazing is minimal.

If possible, apply enteric dewormer to adult animals prior to spring/summer turnout to minimize and/or delay buildup of worms in pasture during grazing season.

Complete elimination of trichostrongylids is effectively impossible, for several reasons: (1) anthelmintic treatments seldom eliminate 100% of inhibited worms, (2) worms are present on virtually all pasturage inhabited by wild and domestic ruminants, (3) many infective, ensheathed L3 larvae on vegetation can over-winter, especially when covered by an insulating layer of snow, (4) the worms have terrific reproductive potential, quickly building up in number on forage fed on by animals in pasture/on range.

Dr. W. R. Jolley, Parasitologist, WSVL
Willjo@uwyo.edu

“People who write obscurely are either unskilled or up to mischief”
Peter Medawar
Science and Literature in Plato’s Republic

Noteworthy cases

ACUTE HEPATOTOXICITY AND DIC IN A YOUNG BITCH ON CARPROFEN (RIMADYL)

A 5-year old GSD bitch was brought to a Wyoming veterinarian with lameness in her left pelvic limb. She was put on carprofen (87.5 mg BID) for 14 days. She came back in and a drawer sign was detected in the left stifle. She was put on a COX-2 inhibitor. She was off carprofen for 4 days. The veterinarian did surgery for a ruptured cruciate on 6th Feb. She went home on carprofen for 5 days BID. When she came back the bitch was unwell, constipated, icteric, and vomiting. On the 15th Feb there was a seroma at surgery site and alkaline phosphatase and ALT were up (837 and 862 IU respectively). The veterinarian stopped all medications and put her on amoxycillin, prednisolone and Tagamet. On 19th the dog seemed better and the veterinarian planned to send her home. At that time she was still vomiting but less icteric. On the 20th icterus was marked and DIC was present (petechiae present in mucous membranes). The veterinarian gave her half a unit of blood, she arrested and was revived, but she never regained consciousness. At necropsy she had a nutmeg liver, and blood in the peritoneal cavity, omental bursa, mesentery and the bowel. Internal hemorrhage was the proximal cause of death.

The time line fits an idiosyncratic reaction to carprofen, although use of the COX-2 inhibitor may have played a role, given the timeline. Most dogs manifest a hepatic reaction to carprofen 5 - 30 days after initiation of treatment, but this can occur as late as 180 days later.

SUSPECT SODIUM ION TOXICOSIS KILLS 132 CATTLE

This episode is still being investigated. Preliminary toxicological and histological data point to possible salt poisoning.

A Wyoming rancher found several beef cows dead on a feed ground in early March 2003. Another 20 cows had neurological signs (ataxia, circling and convulsions). Time from clinical onset to death was 2 - 3 hours. The owner’s veterinarian submitted fixed and fresh tissues to the laboratory. A total of 132 cows died over 24 hours.

The veterinarian and the owner brought the carcasses of 4 freshly dead cattle to the WSVL for necropsy. Gross lesions were non-diagnostic. The only noteworthy histological change, present in one of four brains, was chronic quiescent polioencephalomalacia lesions. Dr. Raisbeck visited the ranch to collect more samples and conduct a field investigation. Two cows with neurological signs were shot and the brains collected. One had chronic PEM lesions. Toxicological tests for other causes of mass mortality (nitrate, sulfide, metals and organophosphates) have been negative to date.

SUSPECT SODIUM ION TOXICOSIS KILLS 132 CATTLE

Site of loss of 132 cattle in Wyoming. March 2003. Three dead cattle (arrowheads) are shown along a creek.

Sodium-ion intoxication (“salt poisoning”) in cattle results from either high sodium diets or - more commonly - water deprivation. Clinical signs include ataxia (hypermetria, knuckling, incoordination), visual impairment, profuse tearing, convulsions and death. The condition is usually characterized by a relatively short course from onset of signs until death, variable morbidity and high mortality. Although monogastric species such as horses, poultry and swine are more sensitive than ruminants, it occurs in cattle and sheep as a result of well failures, frozen water supplies, industrial contamination, etc. Diagnosis is based upon demonstrating elevated brain concentrations of sodium in serum (good), CSF (better) or brain (best). Survivors may develop lesions of polioencephalomalacia. Therapy has traditionally been considered futile, but Dr. Lisle George of the UC Davis has reported success with feeding calves water of gradually decreasing salinity over a period of weeks.
ABORTION FOLLOWING USE OF LIVE ATTENUATED IBR VACCINE

Live attenuated IBR vaccines may cause abortion when given to pregnant cattle. The warning on vaccines such as Pfizer’s PregGuard 9 specifically says: “Do not use in pregnant cattle (abortion can result).” Some producers are however confused by advertising that states that some products can be used on pregnant animals. They are unaware of the small print: the cattle should have been previously vaccinated with the same product prior to breeding.

In a case reported several years ago, diagnosticians at Kansas investigated abortion or birth of stillborn or weak calves in 75 of 125 cows that were given an attenuated IBR vaccine 30 days earlier. Dr. Nietfeld and his colleagues concluded that the virus isolated from dead calves was probably from the vaccine, based on restriction fragment endonuclease testing. Recently our laboratory was involved in a similar episode.

An owner vaccinated 20 pregnant heifers with a commercial vaccine containing live attenuated BHV-1. None was vaccinated previously for IBR. The label warning notwithstanding, the owner was under the mistaken impression that vaccination of pregnant animals with the product was safe because “you can do that now.” Approximately one month later heifers began to deliver stillborn calves. Samples from two dead calves were submitted. Hepatic lesions were strongly suggestive of herpesviral infection and were positive on immunohistochemistry for herpesviral antigen. At the time of writing we are attempting to confirm a presumptive diagnosis of iatrogenic herpesvirus abortion.

Some owners - indeed, some veterinarians! - are taking risks with live attenuated vaccines. This is particularly the case when the vaccinated animals are naïve and pregnant. Many attenuated virus can cross the placenta and cause disease and death in the fetus.


Todd Cornish/Donal O'Toole 28 March 2003

FATAL ZINC PHOSPHIDE TOXICOSIS IN A GROUP OF HORSES

In early January 2003, three of 11 horses were found dead within 40 yards of a stock tank after being turned into a dormant alfalfa field. One horse died within 24 hours of turning in, and the other two died one day later. Remaining horses were removed from the field and there were no more deaths or illness.

Necropsy and histopathological examination were unremarkable with the exception of mild pulmonary congestion. The endocardial lining of the heart, particularly the right side, was congested (“almost black”). The alert practitioner noticed a handful of gray-black oats in the stomach of the dead horses. He submitted them for analysis to the WSVL.

Differential diagnoses focused on ionophore intoxication because a new “mineral barrel” was introduced at the same time that the horses were moved. The history was atypical of acute ionophore poisoning. Acid hydrolysis of stomach contents produced phosphine gas (PH₃), suggesting recent exposure to zinc phosphide (ZnP₃) or aluminum phosphide. The owner of the field was asked about recent pesticide use. It transpired that a third party had been asked to “take care of” a small corner of the field in question while he was poisoning prairie dogs in an adjacent field. The ZnP₃-based bait was scattered on the ground around prairie dog burrows, following the traditional paradigm “if a little is good...”

Zinc phosphide is an approved rodenticide for agricultural pests. Directions instruct that a small amount of bait be placed in individual burrows, not broadcast on the surface. Used properly, ZnP₃ poses little hazard to non-target species, since compound disintegrates quickly in carcasses and the environment. That said, ZnP₃ is toxic in virtually all mammalian species. Accidental poisoning most often occurs in dogs, which develop acute signs of depression, tremors, weakness and seizures.

ZnP₃ is rapidly hydrolyzed to phosphate gas in acidic solutions. If you suspect it as a cause of death, it is essential that gastric samples be analyzed promptly. In cases of ZnP₃ exposure where there was a delay in taking gastric samples, a negative laboratory result should be regarded skeptically. Lesions due to ZnP₃ are non-specific (pulmonary congestion with mild changes in liver, heart and kidneys). The stomach contents of poisoned animals may have a characteristic acetylene or dead-fish odor. Confirmation requires chemical analysis.

Dr. Merl Raisbeck
March 29, 2003

DEATH OF NEBRASKA RANCHER FOLLOWING ACCIDENTAL SELF-INJECTION WITH MICOTIL

Tilmicosin phosphate (Micotil® (Elanco)) is a popular drug for the control of bovine respiratory disease. It is particularly effective against Pasteurella, Mannheimia and Actinobacillus spp. A single injection results in therapeutic concentrations in the respiratory tract for 3 – 4 days.

Repeated unprotected exposures of people to tilmicosin can cause acute anaphylactic reactions. More importantly, tilmicosin can be fatal due to cardiotoxic effects in people, dogs and pigs. In horses it causes gastrointestinal signs, including colic. There is a warning on the label about its use in horses.

Accidental exposure of humans to tilmicosin is not rare. Over one 30-month period, a regional poison information center in Canada collected 36 cases of accidental human exposure. Most patients injected <1 ml by needle punctures, and had local effects and no longer term effects.
In a recent unfortunate case, a young rancher in Saronville, Nebraska died following accidental self-injection with tilmicosin. He had a loaded syringe in the pockets of his coverall when a cow kicked him. The syringe contents were injected into his groin. He complained of a rapid heart rate and dizziness and was taken to hospital by ambulance. He died one hour later. The amount he self-injected was not reported in the press story.

When using this drug to treat cattle, please respect its potential cardiac toxicity. Needle sticks are common in veterinarians.

Omaha World-Herald. Dead rancher’s family stunned by freak accident. March 12, 2003

“For science is the long history of learning how not to fool ourselves.”
Richard P. Feynmann
American physicist

NEW TESTS

ELISA TEST FOR NEOspora ANTIBodies

Neospora caninum is an important cause of abortion in many states, particularly those with a large dairy industry such as California. Neosporosis is generally rare in Wyoming, although it has been seen in adjacent states, including Colorado and Nebraska. We are offering an in-house serological test for Neospora. This is an ELISA kit made by VMRD. This competitive ELISA detects antibodies in serum. The basis of the test is inhibition by positive serum of binding of horse radish-peroxidase-labeled Neospora caninum specific antibodies to N caninum tachyzoite p65 antigen. The test takes approximately 2 hours to run. The cost is $9.50/sample for 1-10 samples, and $6.50/sample for 11+ samples.

THYROID HORMONE TESTING FOR DOGS AND CATS

The laboratory recently purchased a chemistry analyzer that increases our testing capabilities. One is for T3 and T4. We need a minimum of 0.5 ml of serum to run both tests. Please supply a clinical history. If it is a cat in which you suspect hyperthyroidism, let us know whether there are signs suggestive of adenomatous hyperplasia: weight loss in presence of polyphagia, palpable thyroid gland(s), hyperactivity, tachycardia, polyuria/polydipsia, bulky stools, vomition. If it is a dog and hypothyroidism is your rule out, please let us know if typical signs are present.

Our current cuts offs are:

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<th>Hypothyroidism</th>
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<td>Very unlikely</td>
</tr>
<tr>
<td>1.5 - 2.0</td>
<td>Unlikely</td>
</tr>
<tr>
<td>1.0 - 1.5</td>
<td>Unknown</td>
</tr>
<tr>
<td>0.5 - 1.0</td>
<td>Possible</td>
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<tr>
<td>&lt;0.5</td>
<td>Very likely</td>
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Cost:

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<tbody>
<tr>
<td>T3</td>
<td>$12</td>
</tr>
<tr>
<td>T4</td>
<td>$12</td>
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<tr>
<td>Both T3 and T4</td>
<td>$22</td>
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</tbody>
</table>

POSITIVE EIA HORSE IN CARBON COUNTY

Regulatory serology identified a positive EIA (Coggins-positive) in Carbon County in February. The horse tested negative in 1996 when purchased out of state and moved to Carbon County. It is unknown when or where the horse was exposed. Fortunately, it does not appear to have transmitted the disease to other horses in the herd. It remains an isolated diagnosis.

NEW ANAPLASMOsis TEST

WSVL is now performing CELISA for anaplasmosis. This test replaces the long tedious complement fixation test previously used for regulatory testing. The test costs $5.00/sample. We prefer to run this test once a week to make the testing more cost effective. But, if you are in a jam and needs results pronto, please call Becky Wills to let her know your situation.

“The theory of legal procedure is that if you set two liars to expose one another, the truth will emerge.”
George Bernard Shaw
Too True to be Good

VISITING VIROLOGIST IN DR. VAN OLPHEN’S LABORATORY

Dr. Lidia Gogorza is a visiting scientist from Argentina who will spend four months in the virology laboratory to work on the development of a test for the detection of BVDV in seropositive animals. In addition she will share her knowledge and different perspectives on the study and teaching of viral diseases.
Dr. Lidia Gogorza, visiting virologist from Argentina

Dr. Gogorza received her DVM and doctorate degrees from the Universidad del Centro de la Provincia de Buenos Aires, Argentina, where she holds a research and teaching faculty position in the Department of Animal Health and Preventive Medicine at the School of Veterinary Medicine. Her research focus is the bovine immune response to viral infections, particularly bovine leukemia virus and BVDV.

TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHY (TSE) DIAGNOSTIC TESTING AT WSVL

In 2001, the WSVL was one of the first state veterinary diagnostic laboratories to become a designated USDA TSE testing laboratory because of our long experience with scrapie and chronic wasting disease (CWD).

This program is designed to expand regulatory and research TSE testing beyond the National Veterinary Services Laboratory (NVSL) in Ames, Iowa and is a unique partnership between state and federal animal disease diagnostic laboratories. Because of the huge need for testing TSE capacity, skilled state laboratories including ours (now numbering 15) were tapped to complement centralized testing. The USDA provided specialized equipment for immunohistochemistry and some reagents. In return, we supply technical and diagnostic expertise. Thus the WSVL is able to provide needed TSE testing for Wyoming in-state and also participate in federal programs to control scrapie and CWD; both state and national programs win with these arrangements. We believe that this state-federal cooperative animal disease diagnostic program could serve as a template for other diseases with regulatory implications and where local, timely testing is desirable.

“A committee is a cul-de-sac down which ideas are lured and quietly strangled.”

Barnett Cocks
New Scientist

Dr. Lisa Wolfe of the CDOW, assisted by Dr. Jean Jewell of the Dept Vet Sci, performing tonsillar biopsy on an anaesthetized mule deer at WGFD facilities at Sybille as part of Dr. Beth Williams’ ongoing research on CWD.

Over the past year, personnel at the WSVL, in collaboration with folks at the Wyoming Game and Fish Department, have carried out several testing projects on new laboratory assays for CWD of deer and elk. Currently the most reliable CWD diagnosis is by immunohistochemical identification of the disease agent (pathologic isoform of the prion protein) in fixed tissue sections from either brain stem or lymphoid tissue, depending on the species of animal being tested. WSVL performs thousands of such tests on hunter harvested animals each year, both in conjunction with the WGFD hunter survey, for contracts with other states, and in conjunction with NVSL.

Heightened concern over the past year about CWD outside the historically endemic area has meant a big increase in demand for surveillance testing. Consequently, several large biomedical companies have developed TSE assay kits which they plan to market for rapid and/or high-throughput diagnosis of CWD, aimed not only at state diagnostic and wildlife laboratories who may not have the facilities to perform immunohistochemistry, but potentially also at game farms and even individual hunters. USDA licensing of such kits for sale in the U.S. requires data from field validation tests. The WSVL has performed such tests for two assays...
now licensed and on the market—a sandwich-ELISA (the Bio-Rad test) and a dot-blot ELISA (VMRD test). Dr. Williams and her group will be participating in similar ways with other CWD tests currently being developed, in the interests of seeing that rapid and accurate tests in addition to immunohistochemistry become available at reasonable per-sample costs.

Drs. Beth Williams and Jean Jewell
WSVL CWD Research Laboratory

CANINE PARVOVIRAL IN-CLINIC TESTS VERSUS EM OR VIRUS ISOLATION

Many of you use the in-clinic antigen detection kits for canine parvovirus, such as the IDEXX CITE or Synbiotics’ WITNESS™ or ASSURE®/PARVO tests. They are marketed with language like “100% sensitive and 97.4% specific when compared to hemagglutination as a gold standard.” These antigen detection assays are good screening tests during the 4 peak antigen shedding days concurrent with clinical symptoms. But clinical symptoms extends over 10 - 12 days with canine parvoviral enteritis. This gives a window of false negativity on the front end for 3 - 4 days and at the back end for 3 - 4 days.

![Graph showing CPV antigen in feces over time](image)

The false negative at the front end is due to not enough virus being shed. At the back end the false negative is probably due to secretory antibody from the gut binding to viral particles, which precludes the assay’s antibodies from binding. This is the major reason why you may get a disparity between an in-clinic test (negative) and the laboratory result (positive on EM or virus isolation). If you get back a result from the laboratory that says “Parvoviral particles seen on EM” and the history fits, go with the laboratory result. If you remain doubtful about the dog’s status, we can confirm it by virus isolation. The accuracy of the in-clinic test depends on catching infection at the peak of viral shedding, and before an early immune response blocks the kit from working.

As an aside, be wary of using PCR routinely to establish whether a dog’s acute diarrhea is due to CPV. PCR detects both clinical and subclinical carriers/shedders. It therefore is not a good diagnostic test for disease status. Commercial kits may give a misleading positive result when dogs were vaccinated recently (within 10 days).

VET LAB STUDENT HONORED

We are happy to announce that one of our long time student employees, Erin Fulton, was recently named the University of Wyoming Student Employee of the Year. Erin is a native of Powell, Wyoming, and has been working at the WSVL for more than three years, performing tours of duty in the Bacteriology, Rabies, Federal Serology, and Histology laboratory sections. Erin, an Animal and Veterinary Sciences (Pre-Vet option) major, was judged the top student employee based upon a variety of criteria, including reliability, creativity, quality of work performed, significant contributions, and attitude/disposition.

Erin has been involved in many student organizations and honorary societies during her tenure as a student, including the Gamma Sigma Delta National Honor Society, Golden Key National Honor Society, the Pre-Vet Club, and the Ag Council. This award is among many others that Erin has received during her time at UW. We are proud of Erin and our many other talented and hard working student employees. Erin was accepted into veterinary school at Colorado State University this spring. She also plans to be married this summer. We will miss Erin and our other graduating senior student employees. We wish Erin and the other students the best success in their future endeavors.

WEB PAGE UPDATES

- [Eradication Scheme for BVD in Beef Herds](#)
- [Nematodes Important for Wyoming Cattle](#)
- [Update on malignant catarrhal fever (MCF)](#)
- [Testing for BVDV](#)
- [West Nile Virus Update Spring 2003](#)
- [Handout on BVDV (Front Back)](#)
- [Drought and Livestock Disease](#)
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To: