# Wyoming State Veterinary Laboratory Newsletter – April 2006


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## MESSAGE FROM THE DIRECTOR

### NEW FACES

In this newsletter you will meet three important additions to the Wyoming State Veterinary Laboratory and the Department of Veterinary Sciences.

**Dr. Ana (Nicky) Bratanich** is our new virologist. She came in this week from Argentina. She worked for the past few years as professor of virology in Department of Virology, School of Veterinary Science at the University of Buenos Aires. Nicky is in charge of the virology, EM and diagnostic serology sections. She has a strong research and molecular diagnostic background, and it is my hope that she will take a hard look at the range of "classical" versus molecular diagnostic tests we offer. In the last few years we have seen a lot of new methods come on line, particularly on the molecular side. We have to be sure that we go with tests that are validated, specific, sensitive and cost effective.

**Dr. Majid Ghoddusi** is an Australian pathologist from Tasmania. Since Drs. Cornish and Montgomery were slowly sinking under a flood of research and diagnostic cases, I hired Dr. Ghoddusi to help us out for several months until a permanent faculty hire is in place. Majid will be here until the end of June 2006.

**Dr. Leslie Woods** is a board-certified PhD pathologist who will join us from the California diagnostic laboratory system in July. Leslie's background is in food animal and wildlife pathology. She represents an excellent fit for the department. I am pleased we have been able to winkle her out to UC Davis laboratory, where she worked for many years. As part of her start up, and with a generous gift from Beth Williams' family, we are modifying laboratory animal rooms into a wildlife disease research laboratory that will be Leslie's base. The laboratory will be named for Dr. Beth Williams. Leslie discovered and characterized a highly pathogenic adenovirus of deer, which we see in Wyoming. One of her goals in coming here is to continue and expand that adenoviral work.

When Dr. Woods is here in July, the laboratory will be back to full strength for the first time in a year and a half. I appreciate the patience you have shown with us, particularly the logjam in pathology. Dr. Montgomery and Dr. Cornish have put in a lot of hours. I am currently attempting to secure two additional positions from the university to strengthen the laboratory: a tenure track position for a prion biologist, and a slot for a molecular diagnostician. I will know by June whether we secured these.

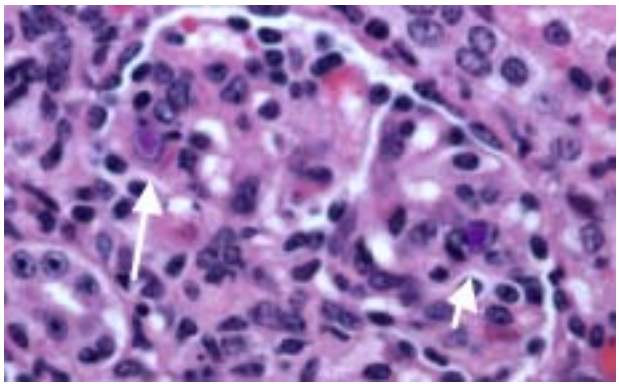
Donal O'Toole
April 8, 2006

## DIAGNOSTIC CASES OF INTEREST

### Fatal adenovirus in a Nebraska calf

A 10-day old heifer Hereford calf with a history of diarrhea was treated unsuccessfully with Nuflor, electrolytes and B vitamins. It was one of 10 calves showing these signs out of a 400 cow herd. A range of tissues was submitted for histology and microbiology.

The calf had an unusual lesion: systemic vasculitis with thrombosis, associated with viral inclusions in endothelial cells. Other changes were necrosis in liver and spleen, interstitial pneumonia, and glomerular necrosis.



**06002629:** *Adenoviral inclusions (arrow) in presumed endothelial cells in glomerulus of a 10-day old calf. Initial indication is that it is bovine adenovirus-5.*

Intestine and spleen were positive on FA for bovine adenovirus 5. An adenovirus was grown on culture. The isolate has been sent to Dr. Howard Lehmkuhl's laboratory in Ames. Dr. Lehmkuhl has done some of the work in North America defining the pathogenicity of adenoviruses in domestic and wildlife species.

There are 9 recognized bovine adenoviruses (BAV-1 - BAV-9). For the most part they induce subclinical infection, and serological studies indicate that a high proportion of cattle are infected without untoward effects. Three serotypes (BAV-4, 7 and 10) are known to infect endothelial cells and cause systemic illness. In some countries, specific types are associated with disease (e.g., in Northern Ireland, disease tends to be in adult cattle on grass, and to be BAV-10). The clinical syndrome is characterized as hemorrhagic enteritis or "pneumo-enteritis." Calves that show clinical signs have high mortality rates. Interestingly, it is difficult to reproduce the full blown spontaneous disease in experimentally infected cattle.

Dr. Don Montgomery/Dr. Donal O'Toole

Lehmkuhl HD, Hobbs LA, Woods LW: 2001, Characterization of a new adenovirus isolated from black-tailed deer in California. Arch Virol 146(6):1187-1196.

Lehmkuhl HD, DeBey BM, Cutlip RC: 2001, A new serotype adenovirus isolated from a goat in the United States. J Vet Diagn Invest 13(3):195-200

Smyth JA, Benko M, Moffett DA, Harrach B: 1996, Bovine adenovirus type 10 identified in fatal cases of adenovirus-associated enteric disease in cattle by in situ hybridization. J Clin Microbiol.; 34(5):1270-4.

### Small stature Angus calves with fractures in Nebraska beef herd

A producer in Western Nebraska had 6 calves born that were small (35 lb; typical birth weights in herd are 85 - 90 lb), with domed heads. Two of six calves developed fractures of the pelvic limbs and were euthanized. There did not seem to be a traumatic component to the fractures - they appeared to be "spontaneous." The owner had not seen this in previous years. The family was consciously breeding for small calves.

Tissues from one calf with fractures of both metatarsal bones were submitted by Dr. Lynn Steadman for histology and other laboratory tests. Dr. Steadman has a remarkable eye for unusual diseases, and we have characterized and published several reports with Lynn in the past few years.

Histologically there was marked osteoporosis (osteopenia) in the long bones. There were some changes in the growth plates, but interpretation of this awaits access to age-matched control tissue.

*Presumptive osteogenesis imperfecta. This 4 week old Angus calf is small (24 inches at shoulder) with short limbs and a domed head. No fractures were present at the time this photograph was taken.*

The owner agreed to donate two affected calves to the WSVL for workup. One calf (above) developed a fracture one day after arrival. Our goal is to define whether this is indeed osteogenesis imperfecta, and if so whether it has a genetic or some other basis. Blood and hair was taken from the bulls, dams and affected calves. If the affected calves trace to one bull, this increases the chances of it being a genetic disease.

Osteogenesis imperfecta is reported in Holsteins in the US, and in the Charolais breed in other countries. In Holsteins it is an autosomal dominant trait, associated with fractures in

ribs and long bones and abnormal scleral, dental and tendenous tissue. There is a large range of similar diseases in children, many due to mutations in type I pro-collagen genes. The two calves we examined had normal teeth, but the scleral tissue did appear to be distinctly, abnormally blue. We are doing electron microscopy on tissues to characterize this disorder.

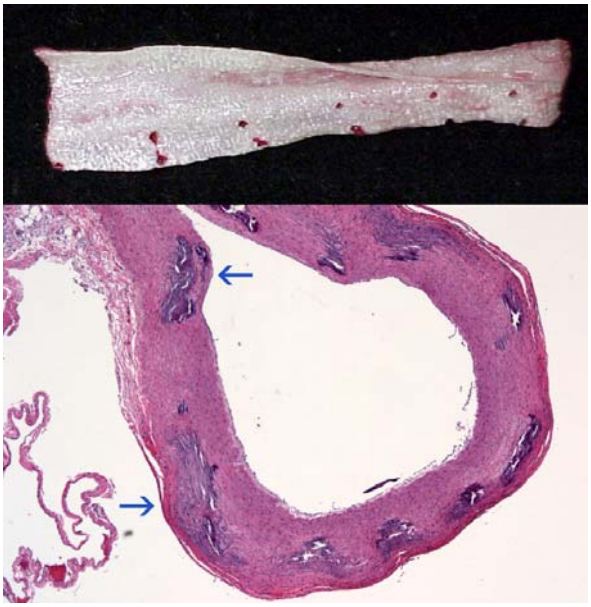
If any of you has seen a syndrome of perinatal fractures in small, full term cattle, particularly Angus cattle, please contact one of us.

Dr. Don Montgomery/Dr. Donal O'Toole

Agerholm JS, Lund AM, Bloch B, Reibel J, Basse A, Arnbjerg J: 1994, Osteogenesis imperfecta in Holstein-Friesian calves. Zentralbl Veterinarmed A. 41(2):128-38.
Denholm LJ, Cole WG: 1983, Heritable bone fragility, joint laxity and dysplastic dentin in Friesian calves: a bovine syndrome of osteogenesis imperfecta. Aust Vet J. 1983 60(1):9-17
Plotkin H: 2004, Syndromes with congenital brittle bones. BMC Pediatr 4:16.

## Vitamin D poisoning in rabbits

We have seen several recent cases of vitamin D toxicosis in rabbits. A typical case was submitted by Dr. McFarland in Rawlins. The history was that the rabbit's body condition deteriorated over a 6 week period. It presented to the veterinarian thin and with a rapid respiratory rate. Pneumonia was suspected. Another case was submitted by a faculty member in the college, who saw a similar syndrome in his pet rabbits. His rabbits lost weight, appeared to have respiratory signs, and died.



**05016587:** *Vitamin D poisoning in a rabbit. The intimal surface of the aorta is dull and granular due to mineralization (top). This is corroborated on histology where there are (arrows) areas of mineralization in tunica media (lower image)*

Gross and microscopic changes in the rabbits were similar. There was extensive mineralization of tissues, particularly the aorta ("barber pole" lesion), stomach, skeletal muscle, kidneys and heart. Respiratory signs were referable to heart failure.

The rabbits' diets were being supplemented with probiotics and vitamins, including vitamin D. This was the presumed source of the problem - too much of a good thing.

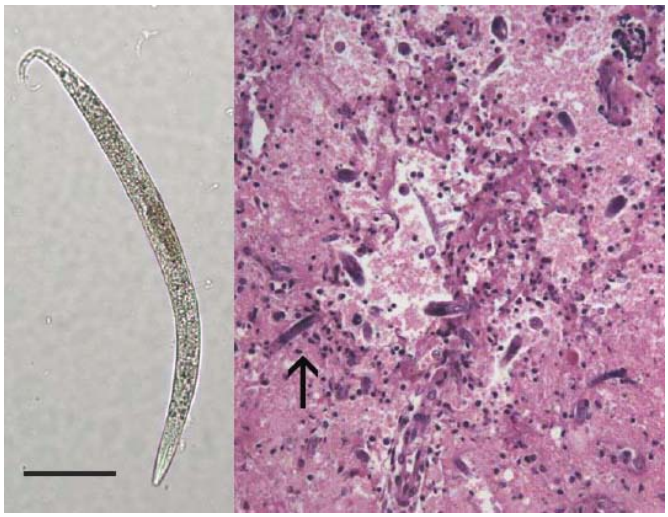
If rabbits are on a proprietary chow, they do not need supplementation with vitamin D. As in this case, it may kill them.

Dr. Don Montgomery/Dr. Donal O'Toole

## Neurological disease in a cow in Big Horn Basin due to a free-living nematode

A three-year-old black Angus cow became blind with signs of head pressing, ataxia, strabismus and nystagmus. Dr. Tharp ministered to the cow. As is his wont, a short time later we received her brain.

Scattered throughout the brain there was severe encephalitis with a perivascular orientation. Numerous small nematode larvae, many degenerate and partially mineralized, were scattered in neuropil and in perivascular areas. The parasites were long and thin, approximating the size of *Halicephalobus* (Micronema).



**0501782:** *Halicephalobus gingivalis-like nematode in brain of adult cow. Left: digest preparation from fixed brain. Right: angiocentric encephalitis with larval nematodes (arrowheads)*

As far as we are aware, this is the first time this free-living nematode has been found in cattle. Additional studies, including electron microscopy, are ongoing to lock in the diagnosis.

*Halicephalobus gingivalis* is a free-living saprophytic nematode that causes disease in horses and children. Animals are infected orally, with hematogenous spread to other tissues and organs. Common sites of involvement in horses are the oral cavity, kidneys, and brain. Diagnosis is determined on the basis of histological findings so when the nematode invades bone, kidney, or brain, antemortem diagnosis can be difficult. Treatment involves resection of affected tissue where possible. Parenteral use of fenbendazole and ivermectin has been attempted unsuccessfully. In a recent report in JAVMA, ivermectin and enrofloxacin were used successfully to treat a donkey with renal infection.

Dr. Don Montgomery/Dr. Donal O'Toole

Kinde H, Mathews M, Ash L, St Leger J: 2000, *Halicephalobus gingivalis* (*H. deletrix*) infection in two horses in southern California. J Vet Diagn Invest 12(2):162-165

### *B. OVIS*
### *Back in the Saddle Again*

As you know, we withdrew offering this serological test for *B. ovis* because of inconsistency in reagents offered by the National Veterinary Services Laboratory. For the past several months, Becky Wills has worked with NVSL and 16 other laboratories to establish whether the new regents are sufficiently robust to use for clients samples.

Sixteen of the 17 laboratories, including ours, are now willing to re-offer the test (one laboratory still has concerns). The test is now cleaner and more consistent - not perfect, but it is certainly a lot better that what we had in the recent past.

Please contact Becky Wills is you are interested in having this test run.

Dr. Donal O'Toole/Dr. Kenji Sato/Becky Wills

### Pooled ear-notch testing for BVD by reverse transcription PCR

A drawback for testing herds for BVDV persistently infected cattle has been cost of individually testing animals - $4/sample using the antigen-capture ELISA.

Recently a method to screen ear notch samples in batches by reverse transcription PCR was described by Kennedy et al in *J Vet Diagn Invest* **18**: 89 - 93, 2006. This makes it cost-effective to do PCR testing on large groups of samples.

Our virology laboratory is offering this RT-PCR method. Supernatant from separately submitted ear notches will be tested in batches of 92 (the odd number is because we have to run known positive and negative controls). If a batch is negative, there is no need to test each sample, reducing the cost to $0.54/sample. That should be the case for ~9 out of 10 batches. But if samples are positive, individual samples would need to be tested to find the PI (cost: $0.54/sample to screen by PCR + $4.00/sample tested by AC-ELISA).

Please request a **POOLED BVD PCR TEST** if you are interested in this method to identify PIs. It will not be cost effective if you have small numbers to test (<12). The method makes most sense if you have a herd or large groups you wish tested. Ear notch samples still need to be submitted individually identified.

Our laboratory, along with that in 5 other states, recently put in a grant proposal to the USDA to begin a voluntary BVD control program, premised on use of this test. If this is funded, we will be looking to work with a small number of your clients who are willing to test their herds to detect and remove PI calves.

Dr. Donal O'Toole/Dr. Nicky Bratanich
April 8, 2006

### BVDV in New World camelids

If you have any clients who are alpaca breeders or owners, you may be aware of a frisson of concern about BVD in New World camelids. This is because there is a report from Canada that alpacas can be infected. The paper was published in the Journal of Veterinary Diagnostic Investigation. There were other, earlier scattered reports of BVDV infections, with or without abortion, in llamas and alpaca.

The recent report by Dr. Carman and her group describes the recognition of a persistently infected cria in a small alpaca herd in eastern Ontario. This followed the introduction of a chronically ill cria and his dam onto the property. This PI cria had a low birth weight, had a hairy coat, chronic diarrhea, and was euthanized at 46 days. One alpaca aborted and BVDV type 1b was found in this 5.5 month fetus. Positive alpaca crias have also been identified in New York and New Jersey. One of the animals in the eastern Ontario traced back to Colorado, which may be why there is so much local concern.

If owners request that animals or their herd be tested for BVDV, particularly if they want to know if they have a persistently infected animal in the herd, the best test appears to be the PCR test. We need a purple top tube for this from each animal. To establish whether any positive animals are PIs, they should be re-tested 3 or more weeks later, again by PCR.

Please talk to Dr. Bratanich or Jackie Cavender in Virology if you have a herd that needs to be tested. Some owners are developing BVD control programs, and the principals are the same as for the disease in cattle:

- Buy in only animals that have tested negative for BVDV
- Isolate incoming animals for at least 3 weeks
- Keep the isolation area separate from any pregnant alpacas.
- Reduce or eliminate contact with other alpacas and cattle, particularly at shows

We posted a link on the WYOVET site to Dr. Carman's recommendations for testing and control. Go to:

http://wyovet.uwyo.edu/Diseases_2006.asp

and then click on the alpaca link.

Carman S, Carr N, DeLay J, Baxi M, Deregt D, Hazlett M: 2005, Bovine viral diarrhea virus in alpaca: abortion and persistent infection. J Vet Diagn Invest 17(6):589-93.
Belknap EB, Collins JK, Larsen RS, Conrad KP: 2000, Bovine viral diarrhea in New World camelids. J Vet Diagn Invest 12: 568 - 570.
Goyal SM, Bouljihad M, Haugerud S, Ridpath JF: Isolation of bovine viral diarrhea virus from an alpaca J Vet Diagn Invest 14: 523 - 525
Wentz PA, Belknap EB, Brock KV et al.: 2003, Evaluation of bovine viral diarrhea virus in New World camelids. J Am Vet Med Assoc 223: 223 - 228.

**Dr. Leslie Woods**

Dr. Leslie Woods graduated from the University of San Diego with a degree in Chemistry in 1977, and obtained her DVM degree from UC Davis in 1982. She became board certified by the ACVP in 1993, and got her PhD in pathology in 1996.

Leslie has worked for most of her career in the California veterinary diagnostic laboratory system, after spending 3 years in veterinary practice.

Her interests are broad. They include the lesions induced by toxic plants (vetch and summer pheasant's eye), adenovirus in deer and moose, West Nile virus, and circoviral infections of birds.

Her husband will join the college at the same time; Dale is a plant pathologist and he accepted a position with the department of plant sciences.

**Dr. Ana Bratanich**

Dr. Ana Bratanich is a native Argentinean who got her DVM degree in 1979 from the School of Veterinary Science at the University of Buenos Aires. Her PhD is from the University of Nebraska at Lincoln.

She worked as a post-doctoral fellow in the early 1990s in the Department of Veterinary Microbiology in the Western College of Veterinary Medicine in Saskatoon, Canada, working on bovine herpesvirus-1, and in the Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada working on HIV. She was a diagnostic virologist with the North Dakota veterinary diagnostic laboratory for 3 years before she returned to Argentina to accept a position as professor of virology in the University of Buenos Aires.

Dr. Bratanich's interests are in molecular diagnostics, bovine herpesviruses-1 and 5, and porcine circovirus, the cause of Post weaning Multisystemic Wasting Syndrome.

April 8, 2006

**Dr. Majid Ghodussi**

Dr. Majid Ghodussi was born in Iran and is an Australian citizen. Majid got his DVM from the University of Urmia in 1989. He did his pathology training in the University of Queensland with Dr. Roger Kelley, and obtained his PhD in 1998, studying the effects of pyrrolizidine alkaloids in liver.

For three years he was a senior research officer with a children's medical research institute where his focus was on using light, immunofluorescent, confocal laser scanning, and transmission electron microscopy to study muscle disease.

Majid will be with us until the end of June. He plans on taking and passing the ACVP board examinations in 2007. He retains an appointment with the Animal Health Laboratories in Tasmania, where he is a veterinary pathologist.

April 8, 2006

***Spirocerca lupi*, a newcomer to Wyoming or just a visitor?**

Last September, a stool specimen from a 13-year-old greyhound in central Wyoming contained a few ova from a roundworm never before seen at the WSVL. The dog's origin and travel history are unknown. It is unlikely that the dog was born and raised in our state.

The worm is commonly found in tumors in the wall of the esophagus, stomach, aorta and a few other locations in dogs, foxes, coyotes and wolves in warm zones of many countries throughout the world. In the USA, it has been reported in Alabama, Alaska, Arkansas, Florida, Georgia, Illinois, Louisiana, Michigan, Mississippi, New Mexico, New York, North Dakota, Oklahoma, South Carolina, Tennessee and Texas.

The canid natural hosts are infected by ingesting infected intermediate hosts, including dung beetles and a few other arthropods, or transport hosts including birds, rodents, rabbits and other prey animals. After ingestion by the canid, the worm larvae migrate into the gastric wall, enter small arteries, in the walls of which they migrate to the upper thoracic aorta. Some mature to adults and remain in the aorta, while others migrate to the walls of the esophagus and stomach. The period from infection to adulthood (egg-production stage) has been variously reported from 120 to 170 days in the dog.

Light infections may cause few/no signs of disease, whereas heavy infestations, depending on the location of the worms, result in granulomatous, inflammatory reactions that lead to fibrosis and the formation of pedunculated masses that protrude into the lumen of the organ. Persistent vomiting, emaciation, dyspnea and/or fainting are common in heavy infections of the esophagus, stomach or aorta, respectively. Aortic aneurisms sometimes rupture, causing hemorrhage and sudden death, and infections near the bronchial nerves sometimes affect the central nervous system, resulting in paraplegia, paralysis, convulsions and other reactions.

Diagnosis is usually based on finding eggs in a fecal flotation. They are elongate, with parallel sides and thick walls, ranging from about 22-30 um long and 9-13 um wide. On necropsy the nodules, from which the adult worms can be isolated, are conspicuous and distinctive. The adult worms are quite large, with the males ranging from about 3-5 cm and the females 6-8 cm in length, and reddish-colored when fresh.

As common as these worms are in other states, it is highly likely that other *Spirocerca*-infected canids enter Wyoming for short- or long-term periods, with the influx of tourists, hunters, workers and others. Although probably not as important as the canine heartworm, *Dirofilaria immitis*, in terms of numbers of infected animals and health risk, *Spirocerca lupi* may warrant some consideration by companion animal veterinarians in Wyoming.

Dr. Bill Jolley
April 1, 2006

**Canine heartworm in Wyoming—a closer look**

The WSVL has been watching for canine heartworm, *Dirofilaria immitis*, for many years. Infected dogs have been found sporadically by necropsy, serotest and/or microscopic examination of blood samples. An unknown number of veterinary clinics in Wyoming also test for the worm, but their results are not reportable, therefore the exact prevalence in the state is unknown. The logistics of sampling, trapping and otherwise acquiring specimens of, or from potential

reservoir mammals for testing are formidable, unacceptable to certain segments of society and inefficient. Considering the number of potential hosts, including dogs, coyotes, wolves, cats, foxes, ferrets, and possibly other animals in our state, the likelihood of the worm being resident here is reasonably high. Another suggestive factor is the known endemicity of the worm in all of our neighboring states.

In all of the years during which WSVL Parasitology has been watching for the worm, infected canines have been regularly found, most of which were known to have come from a heartworm-endemic state, or were taken to an endemic area during the mosquito season, when transmission risk is high. Only 4 infected dogs have been identified as hosts possibly or probably infected within Wyoming. One was near a state border adjacent to a neighboring state known to harbor endemic *D. immitis*. Two of the dogs were from more central locations, but were transported to areas of the state near borders, for hunting or general family purposes. Recently, from 2000 to 2004, 8 dogs have tested positive at the lab, 1 each from Crook, Laramie and Sheridan counties, 2 from Washakie and 3 from Albany County. None of the 8 is suspected of having acquired the infection in the state.

Recently, the lab has examined blood collected from Black-footed ferrets, from Wyoming and other states. Thirty samples from Wyoming ferrets have been tested, with 3 positives. One of those positive samples was rated very weak, and therefore possibly falsely positive. The two solid positives suggest that canine heartworm does, in fact reside in Wyoming. The finding of infected wild ferrets in our state was a surprise, and an indication that we do harbor one or more endemic sites.

A graduate research project has just begun in the Department of Vet Science, to survey mosquitoes, collected from areas where suitable mosquito vector species are known, or believed to reside. The project involves collecting, identifying and sorting mosquitoes trapped in 5 areas thought to be optimal for habituation by the vector species. A polymerase chain reaction (PCR) analysis will be performed on batches of 200 mosquito heads for detection of the L3 larvae of D. immitis, present in the salivary glands of the vectors. The test has been shown to be capable of identifying 1 infected mosquito head in a batch of 200. It is very specific for the canine heartworm.

Graduate student, Roy Fenoff is preparing for the mosquito trapping phase of the study, which will continue through 2005 and possibly 2006 mosquito activity seasons, after which the laboratory testing will begin. Whether or not D. immitis is prevalent, scarce or absent from Wyoming should be revealed within the next year or two, after which we will inform you of the results.

Much of the data for this report were extracted from piles of diagnostic reports in files in the Diagnostic Parasitology lab at WSVL, by a prized preveterinary student, Ms. Laura Linn. Her effort in this project was greatly appreciated.

Bill Jolley

**Ostertagia's effect on cattle production: the clear, complete story**

The detrimental effect of the small Trichostrongylid nematodes, especially *Ostertagia ostertagi* (the Brown Stomach Worm), on grazing herbivores has been explained regularly in this newsletter and over the phone for nearly 20 years. The "*O.o*" worm has been the special focus of precautionary emphasis, because of its ability to overwinter in the abomasal lining of cattle and in pasture forage, and because of its ability to kill, debilitate and retard development of young cattle. It is especially significant because of its survivability, capability in limiting or preventing digestion of protein in infected animals and the difficulty in treating/controlling the worm with drugs or management methods.

Cattle producers and large animal veterinarians wanting a clear, complete, but concise explanation of the biology, epidemiology and methods effective in controlling this worm now have access to information that can be put on the shelf for future reference. The May 2005 issue of the Angus Journal, (vol. 26, no. 10, pg 94-95) contains an article entitled, "Don't Get Played", devoted to this roundworm. The article was written by Ms. Corinne Patterson, associate editor of the Angus Journal, with input from Dr. Louis Gasbarre, a well-known research scientist with the Agricultural Research Service (ARS) in Beltsville, MD., Dr. Thomas Craig, a distinguished professor of pathobiology at Texas A & M University in College Station, TX and me. Drs. Gasbarre and Craig explain the life cycle of the worm, including its talents and abilities to thrive in the various climatic regions and conditions where the worm is significant. My contribution was focused on the factors pertinent to the northern "cold" regions, which I have explained in numerous classes, seminars and phone consultations.

Anyone wanting to acquire an edition of the Angus Journal containing this article may contact the American Angus Association at 3201 Frederick Ave., Saint Joseph, MO 64506; phone (816) 383-5100; fax (816) 233-9703; e-mail angus@angus.org.

Dr. Bill Jolley
April 1, 2006

**Trichomoniasis in Wyoming**

Dr. Jolley has written an update on venereal trichomoniasis and posted a map of confirmed cases that are on the WYOVET web site.

Please go to:

http://wyovet.uwyo.edu/Diseases_2006.asp

From: Wyoming State Veterinary Laboratory
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**To:**