Colorado State University Veterinary Diagnostic Laboratories

LABLINES

Volume 7, Number 2 **Fall 2002**

Letter from the Director

Many changes have occurred in the Laboratory since spring; some of these are detailed in this issue. We ended the fiscal year on June 30 with a 13% increase in accessions, compared to the previous year. We filled our open pathologist position with Dr. E.J. Ehrhart, who arrived in August. His expertise is in surgical pathology, oncology, and immunohistochemistry. At Rocky Ford, Dr. James Kennedy joined us as the new branch laboratory director. New staff in the Laboratory include Audrey Galm in sample entry, Ronni Hani in Virology, Todd Bass in Histopathology, research associate Phillip Mendoza, Wade Clemons in Necropsy, and Marsha Eilert as our "runner." New residents include Drs. Melissa Schutten and Michelle Dennis in Pathology, and Kristy Pabilonia in Microbiology.

In August, a site visit team from the American Association of Veterinary Laboratory Diagnosticians (AAVLD) visited our laboratory for our five-year accreditation renewal. We will hear the outcome of that visit in February 2003. In collaboration with the Colorado Division of Wildlife, Colorado Veterinary Medical Association, and Colorado Department of Agriculture, we embarked on a new statewide testing system for chronic wasting disease. This fall, we received $2 million from the USDA to establish our laboratory as one of five nationwide Core Animal Disease Diagnostic Laboratories, designed to protect our agriculture industry against foreign animal diseases introduced either intentionally or accidentally. This is the beginning of a National Animal Health Laboratory Network, a concept initiated by AAVLD. The majority of the funding will be used to obtain a BL-3 modular unit with equipment for high volume testing. In September, we were pleased to see many of you at the Colorado Veterinary Medical Association Annual Conference. We hope to see you again at the Annual Conference in January.

Barbara E. Powers

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STUDIES OF DISTAL SESAMOID LIGAMENT INSERTION SITES IN RACEHORSES WITH LIGAMENT RUPTURE AND SESAMOID BONE FRACTURES

--Robert Norrdin, Karl Hoopes and Chris Kawcak

Rupture of the distal sesamoidean ligaments (DSL) is one cause of acute breakdown in racehorses. The DSL are the functional continuation of the suspensory ligament from the proximal sesamoid bones (PSB) to the palmar aspect of the proximal (P1) and middle (P2) phalanx. The short ligaments that insert on the proximal medial part of P1 are the ones that usually rupture. Extreme over-extension of the fetlock is the likely cause for disruption of the suspensory apparatus and probably occurs in mid-stance of the gait phase when the forces are maximal. We have seen three cases of DSL rupture in a collection of 49 racehorse necropsies. In one of the cases, we found activated remodeling with osteoclastic resorption and erosion with loss of bone at the insertion site of the ruptured DSL (Figure 1).

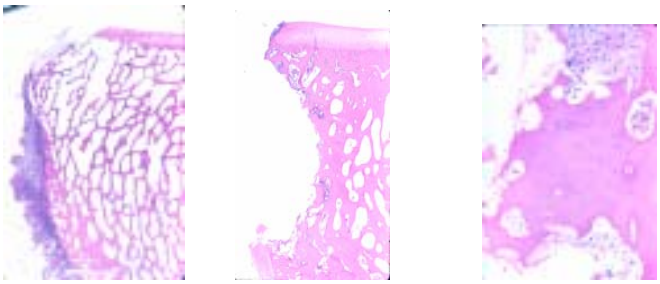


Figure 1. Histologic sections of the palmar proximal surface of P1. Control at left shows smooth surface at distal sesamoid ligament insertion site. At center is the same site from a race-horse with a ruptured DSL. Extensive erosion with bone loss can be seen. At right is a magnified image of P1 in center. It shows Howships lacunae and osteoclasts in resorption cavities at the inner surface of the area of bone loss.

From this observation, it was hypothesized that accumulated microdamage at the insertion site led to the activation of remodeling. During the removal of bone by osteoclasts and its replacement by osteoblasts, there is a state of rarefaction in which loosening of the ligamentous attachment could occur, thereby predisposing the ligament to rupture. Although the mechanism is not entirely clear, DSL rupture is associated with "end of race" fatigue, in which increased laxity and spikes in mechanical stress, in instances of transient misalignment, must play a role. A similar mechanism was hypothesized for fractures of the PSBs, a far more common lesion, and this condition was studied as well.

Post-mortem fetlock samples were obtained from racehorses with DSL ruptures (n=5), PSB fractures (n=6), and a third group of age and breed matched controls (n=10). NOTE: Three cases each of DSL rupture and PSB fractures were graciously donated by the Veterinary Orthopedic Research Laboratory at the University of California/Davis. The DSL insertion sites of the affected limb and the contralateral limb were examined to see if changes were present in both. Two parasagittal slices were taken from the DSL insertion sites of each P1 (Figure 2). One sample was decalcified and examined using the H&E stain. Semi-quantitation of irregularity indicative of bone erosion and proliferation sites along the surface and subsurface remodeling was done on these samples. The other sample was block-stained in basic fuchsin to prepare 120µm thick ground sections for morphometric evaluation. Microdamage, in the form of microcracks and diffuse basic fuchsin staining of bone matrix, is the result of mechanical fatigue and can be quantified in these undecalcified sections. Statistical analysis consisted of paired T-tests to compare affected and unaffected sides and analysis of variance and least significant difference tests to look for differences between test groups and the controls.

At the end of the study, focal deep erosion of the P1 insertion site was only seen in the single DSL rupture case which was the source of the original observation. It was probable post-traumatic remodeling induced focally and resulted in avulsion of the ligament at the insertion site. Morphometrically, there was no significant difference in cortical bone area/porosity or vascularity between groups. Nor was there any difference in the amount of subsurface remodeling in the H&E sections evaluated semi-quantitatively. This indicates that there was no increased remodeling activity in the groups that had ruptured DSL or fractured PSBs. There was a trend for cortical thickness to be less in the ruptured DSL group and in the fractured PSB group as compared to controls ($P<0.10$). In the decalcified sections, there was greater surface irregularity indicative of erosion and/or proliferation in these groups as well. This suggests that there was more adaptational modeling in response to mechanical stresses in these groups. It is also possible that there was less thickening during development or genetically thinner cortices in these groups.

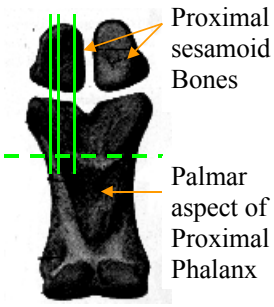


Figure 2. Section planes through the DSL insertion site for histologic studies.

In summary, we found no evidence of increased remodeling in bone at the insertion sites of sesamoid ligaments in racehorses with ruptured distal sesamoid ligaments or fractured sesamoid bones. There was some evidence of more surface remodeling at the site and thinner cortices.

WELCOME TO DR. JAMES KENNEDY-- NEW DIRECTOR AT ROCKY FORD

On September 1, Dr. James Kennedy began his new position as director of the Rocky Ford Branch Laboratory. Dr. Kennedy received his DVM from the University of Missouri, practiced as a feedlot consultant veterinarian in Kansas, and was an educator at the University of Nebraska's College of Technical Agriculture in Curtis, Nebraska. He brings his expertise in food animal diseases to manage the Laboratory, and provide extension services and consultant services to veterinarians and producers in the area. The position is a joint appointment with the Colorado Department of Agriculture. Dr. Kennedy will assist in regulatory affairs for the CDA and maintain a watchful eye for any foreign animal or emerging disease outbreaks. Dr. Kennedy is a member of the Colorado Veterinary Medical Association and already has visited many of you in the Rocky Ford area. If you have a chance, stop by and introduce yourself and feel free to provide Dr. Kennedy with suggestions to improve service to those in the Arkansas Valley. Special thanks to the Rocky Ford transition team for their assistance in the selection process for this most important position. Welcome Dr. Kennedy!

DEVELOPING A BIOSECURITY PROGRAM, PART I

—James A. Kennedy

Events of the past two years have served to reiterate the need for the development of functional biosecurity programs at the producer level. It is important to understand that bioterrorism is only a component of biosecurity, and that we should concentrate as much on managing our livestock resources as we do on concerning ourselves with some clandestine activity. Sixteen percent of the U.S. GNP is derived from agriculture—what impacts agricultural productivity also impacts the entire country. If we review the Veterinarian's Oath, it is plain to see how the veterinary profession and biosecurity are intimately intertwined. Veterinarians should be key players in the development of agricultural biosecurity programs.

The first step in the development of a biosecurity program is education. Key components of client education include: educating clients and producers about methods of disease spread; initial clinical signs; appropriate methods of handling diseased animals; who to contact when an infectious disease is suspected; and proper channels of communication to report suspected contagious diseases and breeches in biosecurity. Clients and producers should understand how disease is transmitted, whether by aerosols, ingestion, direct contact, indirect contact, vectors, biological hosts, fomites, or other carriers. Biosecurity is an area where the veterinarian must assume the role of educator. Following are some thoughts about what might be shared with clients and what producers should know.

In order to prevent infection, the risk of infection must be minimized. "All in/all out" is an ideal method of minimizing risk of infection but may not be a realistic approach for every producer. Quarantine of newly arrived animals is a commonsense practice that will aid in stopping disease spread. However, producers need to understand that merely putting animals in an isolation pen is not enough. Feeding schedules must ensure that disease-causing agents are left with the newly arrived animals. Communal feed bunks and water tanks should be eliminated. Equipment such as tractors and trucks should not be freely moved from isolation areas to other areas of the farm unless appropriate measures are taken. These measures may include thorough disinfection or, at a minimum, washing and exposure to sunlight for a period of time. The concept and principles of isolation should be extended to livestock that are diagnosed as diseased. Some current thinking suggests that animals will recover more rapidly if they are not separated from their pen mates. That concept often is supported by the argument that the pen or pasture mates already have been exposed. But we should not forget that even a healthy animal will become sick if given a heavy enough challenge. It is important that we realize when isolation is needed and when it no longer benefits.

Another method of diminishing disease spread is through vaccination. Vaccination is a valuable tool in preventing disease and should be an integral part of every biosecurity program. However, we need to remember that vaccination has its limitations. No vaccine is 100% efficacious in preventing disease and some vaccines may carry potential adverse reactions that exceed the risk of the disease itself. Clients and producers need to understand the proper selection and use of vaccines as there are literally hundreds of vaccines on the market, each with a manufacturer hailing

their worth. The veterinarian needs to be actively engaged in the development of vaccination programs, and must do so with the attitude of what is best for the client's operation.

Another component of a biosecurity program concerns who is allowed to visit an operation, and maintaining visitor records. Signing a guest registrar helps the producer keep their livestock healthy. Included in this list of visitors should be wildlife that frequent the farm, feedlot, or ranch. Wildlife can serve as reservoirs and vectors for infections. Minimizing livestock and wildlife interactions will aid in minimizing risk of exposure to disease. A case in point is this year's battle with West Nile virus. Could we have done a better job of keeping the mosquitoes and birds away from our horses?

So far, the crux of this discussion has been to educate clients and producers, and to evaluate management schemes. Eventually, it will be necessary to look at what clinical signs we should concentrate on to avoid overlooking the possibility of an infection from some catastrophic disease, and how disease-causing agents could be introduced into an operation intentionally. This article is entitled Part I for two reasons – it gives a place to start when asked again, but more importantly, it points out that a biosecurity program is an on-going project that requires constant revision.

CHRONIC WASTING DISEASE TESTING FOR HUNTER-SUBMITTED GAME

Through a large cooperative effort with the Colorado Division of Wildlife, Colorado State University Veterinary Diagnostic Laboratory, Colorado Department of Agriculture, Colorado Veterinary Medical Association and the hunters of the State of Colorado, the first rapid test for chronic wasting disease in the United States passed field validation trials and was granted a license by the USDA. The license applies to mule deer, elk, and white-tailed deer. In the testing system set up by the State of Colorado, hunters submitted their deer and elk harvest to Colorado Division of Wildlife sample collection sites or to participating veterinarians from the Colorado Veterinary Medical Association. Samples were extracted and sent to us at either Fort Collins, Grand Junction, or Rocky Ford for diagnosis using a rapid test for chronic wasting disease. This ELISA-based test is marketed by Bio-Rad Laboratories of California. The field validation trials were performed on more than 6,000 samples, and the results were compared to immunohistochemistry. The Bio-Rad rapid test successfully identified all positive animals. Moreover, the test showed that lymphoid tissue was the tissue of choice for diagnosis instead of brain samples. These findings are consistent with previous studies indicating that abnormal prions accumulate in lymphoid tissue prior to entering brain tissue. All animals determined to be positive by Bio-Rad continue to be compared to immunohistochemistry.

The rapid test is an efficient procedure able to diagnose large numbers of samples in a short period of time, requiring only five hours to complete. Up to 1000 samples can be processed in a day. Depending on the volume of samples received on a daily basis, hunters can expect results in three- to 14 days. This compares to immunohistochemistry which takes four- to five days to complete, and is limited to approximately 250 samples per day. The rapid test is cost-effective and does not require board-certified pathologists to interpret.

To-date, more than 23,000 hunter-submitted deer and elk samples have been tested for chronic wasting disease and approximately 1% of these tested positive. The cooperative efforts of multiple Colorado state agencies and the hunters of Colorado enabled the successful completion of this field validation study. This allowed the approval of the first rapid test for chronic wasting disease for use by other USDA-approved laboratories in the United States. The results of this testing program also gave the state new, very valuable information on the distribution of chronic wasting disease in Colorado.

*****PRICE INCREASES*****

Most unfortunately, due to state-mandated budget cuts to our state appropriation, we have had to raise prices on many of the tests we perform. We will continue to strive to meet our service mission in the most cost-effective manner we can.

*******ON-LINE RESULTS*******

For on-line access to antimicrobial sensitivity results and All Lab Results, please call us at 970-491-1281 and speak with Jay or Carrie.

DIFFERENTIAL DIAGNOSES FOR EQUINE NEUROLOGIC CASES

—Dan Gould, Dwayne Hamar, and Hana Van Campen

Please see the following table to aid you in consideration of other causes of neurologic diseases in horses, other than West Nile virus. The preferred diagnostic tests are listed in order of (1) the first and sometimes only choice, (2) the second choice, and (3) the third choice.

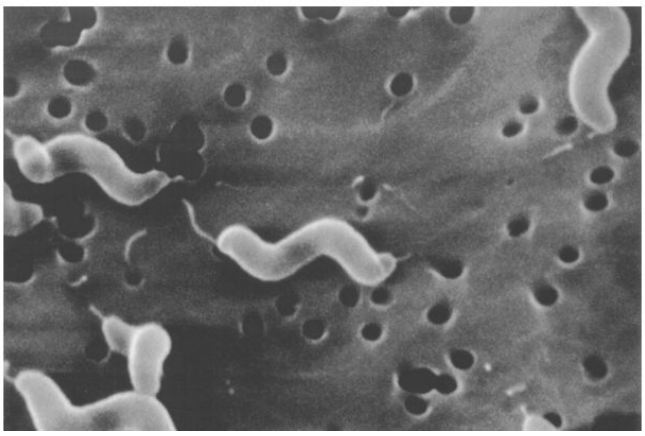
AGENT	DIAGNOSTIC TESTS			
	Tissue Submitted	**Choice 1**	**Choice 2**	**Choice 3**
Trauma				
Fracture, cranial, vertebral	Carcass	Gross lesion		
Viruses				
Rabies	Brain	FA	Histo	
EHV-1 (Rhino)	Serum,brain	Acute and conv samples serology	Blood, brain, PCR	Histo
West Nile Virus	Serum,brain	IgM ELISA (DPHE,CDA) serology	Brain, PCR	
WEE	Serum	HI,SN(NVSL) serology		
EEE	Serum	HI,SN(NVSL) serology		
VEE	Serum	HI,SN(NVSL) serology		
Borna disease	Exotic to the US			
Bacteria				
Meningitis, septicemia	Swab,blood,tissue	Culture	Histo	
Abscess, brain, vertebral	Swab,aspirate,tissue	Culture	Histo	
Osteomyelitis, spine	Swab,aspirate,tissue	Culture	Histo	
Granuloma	Tissue	Histo		
Toxins				
Locoweed	Plants,brain	Plant ID	Histo	
Senecio (other hepatotoxic spp.)	Plants,liver, brain	Plant ID	Histo	
Moldy corn (*Fusarium* spp.)	Brain, feed	Histo	Referral Lab toxicology	
Star Thistle	Brain	Histo		
Botulism	Blood	Bio-assay Referral Lab		
Sudan grass	Plant	Plant ID	Histo	
Nutritional/metabolic				
Equine motor neuron disease	Brain, spinal cord	Histo		
Hepatic encephalopathy	Liver, brain	Histo		
Parasites				
Equine protozoal myelitis	Spinal cord, brain, Serum & CSF	Histo	Referral Lab	
Visceral larval migrans	Brain	Histo		
Cryptococcus	Brain	Histo		
Tumors				
Lymphoma	Tissue	Histo		
Melanoma	Tissue	Histo		
Schwannoma	Tissue	Histo		
Cholesterol granuloma	Tissue	Histo		
Degenerative				
Cervical instability, compressive myelopathy, OCD	Carcass	Gross with histo confir-mation of spinal cord		
Genetic				
Cerebellar cortical abiotrophy	Brain	Histo		
Congenital encephalomyelopathy	Brain	Histo		
Other				
Polyneuritis equi/Cauda equina syndrome	Carcass	Histo		

FROM THE WESTERN SLOPE-- AN UNUSUAL ABORTION STORM

—Darrel Schweitzer

This past spring, a group of 600 ewe lambs in western Colorado experienced an unusual abortion outbreak. These lambs were part of a large range operation and were destined to be replacement ewes in this operation. These lambs were vaccinated against Campylobacter abortion, BVD, and clostridial diseases. They were bred to lamb earlier than the adult ewes, and were confined to lambing corrals while the ewe bands were still on winter range. At about 90 to 100 days gestation, the owner noticed aborted, very autolyzed fetuses. He sought help from us at the Western Slope Animal Diagnostic Laboratory.

Necropsy and culture of the first of these autolyzed fetuses were unrevealing. A second attempt at diagnosis of more developed, less autolyzed fetuses a week later revealed the presence of swollen livers, scattered hemorrhages in various organs, and the presence of organisms having swimming characteristics typical of *Campylobacter* sp. on darkfield examination of abomasal fluid. Bacterial culture of liver and abomasal fluid yielded *Campylobacter coli*.



Scanning electron microscope image of *Campylobacter jejuni*, illustrating its corkscrew appearance and bipolar flagella. (Source: VA-MY Regional College of Vet Med/Blacksburg, Virginia).

Abortions continued over the next few months into the late gestational stage, so an attempt was made to control the abortions by use of antibiotics. Unfortunately, this organism is slow-growing and not amendable to antibiotic susceptibility testing with an acceptable degree of reliability. The choice of antibiotic was accordingly based on the clinician's experience in other situations, with acknowledgement that this situation was unlike any other previously encountered. Another complicating factor was that, sheep being a minor species, most drugs are extra-label. Accordingly, whole-herd antibiotic therapy was initiated to no effect on the condition. After lambing started in this group of ewes, the abortion rate dropped. Strict sanitation in lambing pens and prompt cleanup of aborted fetuses and fetal membranes likewise had little effect on the disease. In all, approximately 150 ewes aborted.

During this time, the adult ewe bands were on desert range. Even though they had been previously vaccinated against campylobacteriosis, the owner was concerned that they also would contract the disease when brought into the lambing corrals. The owner selected 10 pregnant ewes that required attention for other reasons, and placed them in the herd of ewe lambs to act as sentinel ewes. One of these ewes aborted, but not from campylobacteriosis. Eventually, the adult ewe bands were brought into the corrals for lambing and none of these ewes aborted from this cause. An autogenous vaccine was prepared from the isolate and will be used on all herd members prior to the 2003 lambing season.

Campylobacteriosis (Vibrionic abortion) in ewes usually is caused by *Campylobacter fetus* subsp. *fetus*. Infection by this organism is common in unvaccinated sheep and usually results in late-term abortion. This case is unusual in that the causative organism is *Campylobacter coli*, and the disease was occurring initially at a much earlier stage of pregnancy. According to Prescott, *Campylobacter coli* is a normal inhabitant of swine intestinal tract. Isolation of this organism was of particular concern because of its potential as a cause of gastroenteritis in humans. The source of this outbreak has not been identified. Swine operations are few and far between in western Colorado, so it is puzzling as to how this organism occurred on this sheep ranch.

NEOSPOROSIS FOUND IN A COLORADO DAIRY HEIFER REPLACEMENT HERD

—John Cheney

Neospora caninum, a protozoan parasite, was identified for the first time in 1988 in dogs. Earlier, it was probably diagnosed as *Toxoplasma*. The first abortion associated with *Neospora* in cattle was reported from a New Mexico herd in 1988. Numerous reports across the world have since indicated *Neospora* as an important cause of abortion in cattle. It's estimated that neosporosis accounts for approximately 40,000 abortions annually in California alone at an estimated cost of $35 million. Though considered primarily a "dairy disease," a study by Texas A&M University determined that the disease cost the Texas beef industry $37 million in 2001.

Dogs have been reported to be the definitive host for *Neospora*, and oocysts are passed in their feces. *Neospora* can be transmitted through the ingestion of oocysts from dogs (horizontal transmission) or transplacentally from congenitally infected cows to their offspring (vertical transmission). Transmission may be apparent in successive generations of infected cows from the dam to the calf. It is thought that horizontal transmission is not the most common route of infection since dogs pass very few oocysts in their feces. Transmission may result in aborted fetuses, infected calves, or healthy calves. Abortions usually occur in the fifth- or sixth month of gestation.

Herd History—

This case was from a dairy heifer replacement operation in western Colorado. Heifers were obtained by the first owner from several Texas dairies at 4- to 5 weeks of age. These heifers were fed milk until 10-to 12 weeks of age, then moved to group pens and fed until they weighed 600 pounds. The second owner then purchased these 600 pound heifers and moved them to dry lots in an old dairy facility. Heifers were synchronized with MGA and bred once by artificial insemination (AI) at 14- to 15 months of age; then bulls were turned into the breeding pens. Heifers were examined for pregnancy at 45-days post-breeding and the pregnant heifers were moved to different pens until sold at 7 months of gestation. This group of pregnant heifers was moved from their home pen to another pen for one week while their original pen was used to group a batch of heifers for sale.

Abortions—

Five days after the bred heifers were returned to their home pen, 10 heifers aborted. Within three weeks, 25 of 94 heifers had aborted. Of these 25 abortions, 16 were observed (fetuses found), seven were palpated open, and one heifer each was found with a pyometra and a mummified fetus. All abortions occurred in heifers from this one pen, and occasional abortions continued.

Vaccination History—

The vaccination history prior to purchase is not known. On arrival, all heifers received Vira Shield 5 (killed vaccine against BVD, cytopathic and non-cytopathic), IBR, PI-3, and BRSV. Two boosters of Bovishield 4+L5, modified live IBR, PI-3, and BRSV plus five strains of Lepto were given within the next two months. After the heifers were 600 pounds, no more vaccines were administered.

Laboratory Results—

From the first 10 heifers to abort, nine serum samples were taken and sent to the Diagnostic Laboratory for testing. These samples were tested for antibodies to five strains of Lepto, Brucella, IBR, BVD, and *Neospora* antibody (ELISA). All samples were negative for antibodies to Lepto and Brucella. Titers for IBR varied from 1:4 to 1:16, and titers for BVD varied from 1:128 to 1:512 for seven of the heifers. One heifer had a BVD titer of 1:1024 and another had a titer of 1:2048. The *Neospora* OD (optical density) readings were from 0.0 in one heifer, and from 1.451 to 6.432 in the remaining seven heifers. Second blood samples taken from these same heifers 2- to 3 weeks following abortion were re-tested. All the Lepto and Brucella antibody titers remained negative. The IBR titers ranged from 1:4 to 1:16, and the BVD titers from 1:128 to 1:1024, which is not a significant change. The *Neospora* OD readings ranged from 0.737 to 5.7208 on these second samples.

Interpretation of Findings—

The negative Lepto and Brucella antibody results would eliminate these as a cause of the abortions. The titers for IBR and BVD are what you would expect to see post-vaccination with MLV vaccines and cannot be distinguished from those due to natural infection. The *Neospora* OD ELISA antibody readings are the highest we have reported.

ELISA OD values are associated with the following probabilities of infection:

- <0.2=5% probability of infection, interpreted as negative
- 0.2-0.45=9% probability of infection, interpreted as negative
- 0.45-0.7=68% probability of infection, interpreted as positive
- >0.7=100% probability of infection, interpreted as positive

Since all nine heifers tested had OD ELISA antibody readings higher than 0.7, it was concluded that *Neospora* was the cause of these abortions. It was also thought that the heifers had become infected after arriving at the replacement lot. There were more than 12 free-roaming dogs belonging to the workers and the owner of the lot, and these dogs were thought to be the source of the infection. The dogs also had access to uncovered silage pits from which the heifers were fed.

Neospora serology: Submit .5ml serum. Fee=$10 (1-10); $7 (11+).
Neospora IHC: Submit fetus, or brain and heart. Fee=$15.

NEW PCR TESTS AVAILABLE STARTING OCTOBER 1, 2002

Test	Sample	Special Media	Shipping/Handling	Day Test is Run	Turn Around Time	Fee
Feline Calicivirus (FCV)	Oral, nasal tissue or swab	None needed	On ice (4°C) or frozen (-20°C)	Wed	1 week	$30
West Nile Virus (WNV)	Brain	None needed	On ice (4°C) or frozen (-20°C)	Wed	1 week	$30
Psittacine beak and feather disease (PBFD)	Feather pulp, EDTA blood	None needed	On ice (4°C) or frozen (-20°C)	Thurs	1 week	$25

A CASE OF RABIES IN A BOBCAT

—Dan Gould and Kristy Pabilonia

On October 29, 2002, two adult men were chased by a small female bobcat while working on a ranch in Del Norte, Colorado. The men reported that the bobcat was exhibiting strange behavior and was very aggressive. The men escaped into a house while the bobcat continued to stalk them outside. The bobcat even threw herself at the door in an attempt to attack them. Unable to leave the house, the men shot and killed the bobcat. The Colorado Division of Wildlife was notified and a preliminary necropsy was performed at the Frisco Creed Wildlife Hospital and Rehabilitation Center. The bobcat was then transported to the Diagnostic Laboratory for a rabies examination.

Necropsy revealed few significant lesions. Abnormal encounters with other animals were suspected because the carcass had the odor of a skunk, and there were porcupine quills in the skin of the lower neck and chest. Fat stores were depleted. Stomach contents indicated recent food consumption and contained numerous nematodes (*Physaloptera* spp.). Histologically in the brain, occasional eosinophilic inclusion bodies were evident in the cytoplasm of neurons. Leukocyte infiltration of the brain tissue was not present on histologic examination.

The cerebellum, medulla, and hippocampus were used to make impression slides and were stained for rabies virus by direct immunofluorescence. Fluorescent inclusions were observed, signifying that the bobcat was positive for rabies virus. The brain tissues were sent to the Colorado Department of Public Health where direct immunofluorescence was repeated and a mouse inoculation test was performed. Both tests were positive, confirming the diagnosis of rabies. Brain tissues were submitted to the Centers for Disease Control and the virus was determined to be a bat strain of rabies by monoclonal antibody and polymerase chain reaction testing.

Rabies is one of the oldest and most lethal of all known infectious diseases. All warm-blooded animals are susceptible to rabies and almost all cases result in death. Six rabies virus variants are prevalent in terrestrial animals in North America. The distribution includes most of the eastern and central United States and parts of California, Texas, Arizona, New Mexico, Alaska, Canada, and Mexico. The terrestrial reservoir host species include skunks, raccoons, coyotes, and red, gray, and arctic foxes. There are many variants of rabies present in various species of bats in North America.

Colorado has not had a case of terrestrial (non-bat strain) rabies since an outbreak of rabies in skunks from 1988-89. The last case of rabies in a terrestrial animal was a bat strain in a fox in 1996. Diagnosis of rabies must be performed by qualified personnel at an approved laboratory. Rabies-suspect animals can be submitted to the CSU Veterinary Diagnostic Laboratory for necropsy and direct immunofluorescence. Since January of this year, we have received over 120 submissions for rabies testing and have diagnosed four cases of rabies. One case was the bobcat detailed in this article, and the other three cases were bats. The bat cases were from the Fort Collins area and Craig, Colorado on the western slope.

Rabies FA: Submit fresh brain, or head. Fee=$60.

MEET YOUR LABORATORY/Chemistry/Toxicology, Parasitology, and Special Serology

Cathy Bedwell joined the Chemistry/Toxicology section of the Laboratory in 1991, shortly after earning a MS in Chemistry from Colorado State University. She performs all of the tests (from alpha-mannosidase to zinc) submitted to this section, and still finds time to keep an eye on Dr. Hamar. She enjoys the variety of samples submitted and the analytical challenges they pose.

Glenda Taton-Allen has worked in the Parasitology Laboratory for 15 years. From Colorado State University, she obtained a BS in Microbiology and a MS in Chemical Pathology. She is responsible for performing tests on all diagnostic parasitology samples submitted to identify internal and external parasites and runs all parasitology samples submitted from the VTH. She is heavily involved in teaching diagnostic parasitology to the veterinary students during their freshman and junior years. She also teaches undergraduate classes, community college students, independent study students, and continuing education students which may include technicians as well as veterinarians. Glenda is available to consult with anyone who might have a question about parasites – give her a call at 970-491-1281!

Melissa Brewer is the head research associate and laboratory manager for the Special Serology section, otherwise known as Dr. Michael Lappin's Laboratory. She has been in Lappin's Laboratory since graduating in 1992 from Colorado State University with a BS in Biological Sciences. The laboratory performs serological tests for *Toxoplasma gondii*, Rocky Mountain Spotted Fever, Lymes Disease, and all endocrine samples submitted. The laboratory is also involved in many research projects investigating various small animal infectious diseases such as *Mycoplasma haemofelis* and *haemominutum, Bartonella henselae, Cryptosporidium parvum, Ehrlichia* spp., and *Toxoplasma gondii*.

DROUGHT AND NITRATE IN FORAGES

—Dwayne Hamar and Cathy Bedwell

This fall, for the first time, we have provided a field test kit for nitrate to Cooperative Extension agents. This test can be used to determine if there is nitrate in the stalk of forages, but will not determine the amount of nitrate in the forage. A quantitative test performed by a testing laboratory is required to determine the relative potential toxicity of the forage.

As we are all aware, the amount of precipitation this year is well below average. This has resulted in lower than normal forage growth of many of the crops in Colorado and surrounding states. When drought results in a decreased growth of the plant, the nitrogen from the soil is absorbed by the plant and accumulates in the plant. Nitrate accumulates in higher concentrations in the stalk of the plant than in the leaf. Additionally, the concentration of nitrate in the stalk decreases as the distance from the ground increases. Nitrate can accumulate in almost all plants but it does not accumulate in the grain of the plant. Another important consideration is that the nitrate concentration in the plant varies markedly within a field. Therefore, collecting samples from several areas in a field or stack of forage is very important.

If the field test is positive for nitrate, or if you want to send a sample without field testing, submit a representative sample of the forage for quantitative nitrate analysis. In some cases, it may be advisable to submit more than one sample from a field or stack of forage because there may be variation in the nitrate level within a field. For baled forages, a forage probe is the best method of obtaining a representative sample. Most extension offices loan out core samplers, as do we. Standing forage may be sampled by clipping plant stems from several plants in different areas of the field.

For additional information about nitrate toxicity, consult the Fall 1998 issue of LabLines. If you don't have a copy, it may be found online at the Diagnostic Laboratory Home Page: http://www.cvmbs.colostate.edu/dlab.

Quantitative nitrate analysis—Submit forage, Fee=$8.

NEW APPLICATION FOR BVD ANTIGEN-CAPTURE ELISA USING BOVINE SKIN SAMPLES

—Hana Van Campen

Cattle that are persistently infected (PI) with bovine viral diarrhea virus (BVDV) are the main source of infection for other cattle. An important part of any BVDV control program is the identification and removal of PI animals. A new option for screening animals for PI status has been

approved by the USDA. PI animals have large amounts of BVDV in their skin. BVDV antigen, solubilized from skin or ear notch samples in saline solution, can be detected by a BVDV antigen-capture ELISA (Syracuse Bioanalytical, Inc.). This AC-ELISA is currently used in our Virology section for the detection of BVDV in serum. If you are interested in using skin or ear notch samples to screen cattle for PI animals, please submit 1x1cm skin or ear notch samples placed in individual, labeled (snap cap) tubes. Ship on ice overnight. Please freeze samples if it will take more than one day to submit them to the laboratory. Tests are set up daily and results are reported on the following day. Please call Kathi Wilson or Ronni Haney at 970-491-1281 in advance if you are submitting large numbers of samples.

BVD capture ELISA in skin--Submit sample as described above. Fee=$7 (1-10 samples); $5 (11-50 samples), $4 (50+ samples).

Corynebacterium pseudotuberculosis ON THE RISE AGAIN IN COLORADO

—Doreene Hyatt

Corynebacterium pseudotuberculosis infections cause a disease in equids commonly referred to as "pigeon fever," "pigeon breast," or "dryland distemper," and are the most common cause of ulcerative lymphangitis in equids. As mentioned in our Spring 2000 and Fall 2001 issues, before 1999 we annually reported approximately one isolation of *C. pseudotuberculosis* from horses statewide. However, it appears that the disease caused by this bacterium may be more common in Colorado.

Since January 2002, 73 samples have been positive for *C. pseudotuberculosis*; 72 of which have been isolated since July 30. The table below gives the number of isolations of *C. pseudotuberculosis* from horse samples in the past four years. It is unknown whether the increase in isolations this year is from an increase in the number of cases in Colorado or from an increased awareness of the disease, but this does represent a trend that we should continue to monitor.

Isolations of *C. pseudotuberculosis* from samples taken from horses in the years 1999 to current

Year (Jan to Dec)	# Positive Samples	Months Submitted
1999	15	Sept-Dec
2000	7	Jan,Mar,Oct,Dec
2001	12	Jan,Jun,Aug-Dec
2002 (Jan to Nov 1)	72	Jan,Jul-Oct

Unfortunately, without bacterial culture, there is no way to definitively rule out strangles or other non-contagious infections causing abscesses. Because *C. pseudotuberculosis* is contagious, it is crucial for veterinarians to accurately diagnose these cases in order to tailor treatment and control. Infected equids need to be isolated and, once the abscess is opened and draining purulent exudate, the pus needs to be collected into a disposable container and then discarded. If the abscess is draining pus onto a concrete floor, the floor needs to be cleaned and disinfected. Veterinarians should avoid having the abscess drain onto dirt. Although not definitively determined, an insect vector is suspected in transmission of this bacterium. Interestingly, most suspected cases were seen during the summer and fall months when insects are most active. Therefore, insect control is important especially for those horses that currently are infected.

POSSIBLE IONOPHORE TOXICOSIS IN DOGS

—Hana Van Campen, Randy Basaraba, and Dwayne Hamar

CASE REPORT—Over a period of five months in 2001, Colorado Humane Society and SPCA, Inc., personnel examined four dogs that presented with acute weakness, apparent joint pain, and paralysis of the rear limbs. The dogs shared a history of being found in or near cattle feedlots. All were initially treated for kennel cough or pneumonia. Clinical biochemical findings available for one dog included high normal creatine kinase activity, 259 IU/1, (CSU normal values, 50-275 IU/1), and phosphorus; and low sodium, chloride, BUN, creatinine, albumin, and total protein levels. Because of the association of these dogs with feedlots, ionophore intoxication was suspected.

In previous reports, dogs that consumed contaminated feed developed clinical signs within 6 to 12 hours after ingestion. The LD_{50} for monensin in dogs is 10- to 20mg/kg. Males are more severely affected than females. Treatment with chloramphenicol, erythromycin, and a variety of other antibiotics worsens clinical signs. Dogs may survive intoxication if treated aggressively with IV fluids and nursing care.

The differential diagnosis should include polyradiculoneuritis, myasthenia gravis, tick paralysis, and botulism. Monensin was not detected in dog food samples submitted from the Colorado Humane Society or SPCA shelter. It is assumed that dogs ingested ionophore-contaminated material in the feedlots.

A fifth dog was reported by the owners to exhibit hindlimb pain and weakness immediately after adoption, which became progressively worse. The dog had difficulty negotiating stairs and fell out of the car. She was taken to a veterinarian who treated her for kennel cough, and was returned to an emergency clinic with fever, panting, and stiffness in the forelimbs. The dog was treated with subcutaneous fluids and Baytril. She died 10 days after

onset of clinical signs with severe dyspnea terminally. The dog was submitted for necropsy.

Previous reports of ionophore toxicosis in dogs do not include gross or histopathologic findings. In this case, the lungs were congested and oozed blood from cut surfaces. There were no gross abnormalities of skeletal or cardiac muscle. Microscopically, segmental skeletal myofiber degeneration and increased numbers of satellite/nurse cells were observed. Necrosis of smooth muscle cells was found in the intestinal wall and in the walls of small arteries. Axonal degeneration was evident in peripheral nerves. Hypercellularity of glomeruli and moderate degeneration of renal tubular epithelium also were noted.

Postscript—The fourth dog that presented with this syndrome was successfully treated by the staff at the Colorado Humane Society and SPCA's clinic. She was adopted and is doing well.

*****REMINDER*****

LARGE VOLUME TESTING SAMPLE SUBMISSION

Please notify the laboratory at 970-491-1281 in advance of any large volume testing so that we have proper media and supplies available, and can adjust our schedules to meet your needs and provide you with the most efficient, timely service. For *Trichomonas* samples, call Glenda directly at 970-491-1233 two to four days before sending 50+ samples.

NEW STUDY ANNOUNCEMENT HEMANGIOSARCOMA VACCINE TRIAL

Study Description--This study is designed to determine whether either one of two new tumor vaccines can prevent the development of tumor metastasis in dogs with hemangiosarcoma. Dogs that have been diagnosed with hemangiosarcomas without metastatic disease, and are in good general health, are eligible for entry into this study. Dogs that enter the study will receive chemotherapy treatment and a series of eight vaccinations administrated over a 21-week period. This new vaccination protocol has not been found to induce toxicity or unacceptable side effects. The study will pay for the cost of treatment and vaccination as well as examinations, radiographs, and ultrasound. The owner will be responsible for the cost of the staging diagnostics. Animals can be enrolled and treated at either one of two treatment centers—Animal Cancer Center/CSU (970/221-4535) or Veterinary Cancer Specialists/Denver (303-874-2054).

NEW STUDY ANNOUNCEMENT MELANOMA VACCINE TRIAL

Study Description—This study is designed to determine whether either one of two new tumor vaccines can prevent the development of tumor metastases in dogs with melanoma of the oral cavity. Dogs with Stage II or III melanoma of the mouth or lips are eligible for entry into the study, provided there is tumor tissue available for biopsy. Dogs that enter the study will receive a series of eight vaccinations administered over a 19-week period. This new vaccination protocol has not been found to induce toxicity or cause unacceptable side affects in dogs that have been vaccinated to date. The study will pay for a portion of the cost of tumor surgery to remove the tumor, as well as follow-up examinations and radiographs for one year. Animals can be enrolled and treated at one of two treatment centers: Animal Cancer Center/CSU (970-221-4535) or Veterinary Cancer Specialists/Denver (303-874-2054).

WHAT'S IN THIS ISSUE

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