Colorado State University Veterinary Diagnostic Laboratories

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Letter from the Director

Our Fall edition of LabLines is here! You will find interesting articles inside and we would appreciate any comments you may have about these or any other topics you would like to see discussed.

At the end of June, our fiscal year ended with our case accessions being 20.1% above the previous year, and the total number of tests performed increasing 45.6% compared to the previous year. Due to this increased workload, we have hired new office personnel, JoAnn Paille and Janet Tamarak, to continue to provide you with efficient service. We are sad to lose Tina Sund and welcome Lee Ann Mitchell to assume her duties. In order to upgrade our computer programs and data acquisition, we have added a new program analyst, Don Davis. In July, our new pathology residents, Jeremy Johnson and Sean Troth, and our new microbiology resident, Michelle Jay, joined us. Faculty searches for a new pathologist and another microbiologist are nearing completion.

Did you know that as a part of our quality assurance/quality control program, we participate in a number of proficiency "check tests" provided by the National Veterinary Services Laboratory? In our most recent check tests for Bovine Leukosis and Equine Infectious Anemia, we passed with 100% correct responses! We also have annual proficiency tests for pseudorabies, Johne's disease, Brucellosis, Bluetongue and Equine Viral Arteritis, all of which we routinely pass with 98-100% correct responses. This is just one part of our quality control program that assures you of our accurate diagnostics.

We greatly enjoyed the Annual Colorado Veterinary Medicine Association Conference in September and had fun providing the session in Food Animal Medicine. We look forward to seeing you again at the Annual Conference at Colorado State University this January!

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Salmonella typhimurium DT104

Claudia Gentry-Weeks and Robert Jones

Salmonella typhimurium phage-type DT104 recently has been recognized as an emerging pathogen in livestock and humans. This strain was first identified in the United States in 1985. *S. typhimurium* DT104 recently has gained more attention since it is associated with increased morbidity and mortality in livestock and humans, is resistant to multiple antibiotics, and is being identified with increasing frequency from livestock. The characteristics of this strain include:

- •resistance to ampicillin, chloramphenicol, tetracycline, streptomycin, and sulfonamides
- •serotype *typhimurium*

- •susceptible to a specific bacteriophage (bacterial virus) designated DT104

The most common clinical signs of infection in livestock include watery diarrhea, loss of appetite, and loss of condition. In severe cases, the disease will progress to bloody diarrhea. The mortality rate in cattle is ~40%, with a higher fatality rate in calves.

Salmonella typhimurium DT104 has caused epidemics in both livestock and humans in Great Britain. The first isolates in 1984 were traced to sea gulls in Great Britain, and exotic birds imported from Indonesia and Hong Kong. In 1990, 259 cases in humans were caused by *S. typhimurium* DT104 with an increase to 4006 cases by 1996. In Great Britain, this is the second most common *Salmonella* strain isolated from humans, exceeded only by *S. enteriditis* phage-type 4. Direct contact with animals results in 30% of human cases. Seventy percent of human cases are acquired through food (not limited to unpasteurized milk).

A retrospective study of *Salmonella* isolates from cattle in the Northwest by C.C. Gay and co-workers at Washington State University indicated that *S. typhimurium* DT104 was absent from specimens obtained before 1986. However, between 1986 and 1991 this strain accounted for 13% of 83 isolates and was identified from 64% of 51 isolates obtained since 1992. This strain was isolated from horse, goat, emu, cat, dog, elk, mouse, coyote, ground squirrel, racoon, chipmunk, and birds (pigeon, starling, pine siskin).

Salmonella typhimurium DT104 has been isolated from five bovine (of 120 isolates), one equine (of 68 isolates), and one elk specimen submitted to us between July 1, 1997, and June 30, 1998. This bacterium can be transmitted via ingestion of fecal material following contact with animals or via unpasteurized milk. It causes severe disease with ~36% of infected humans in Great Britain having required hospitalization for an average of 5 days. It is important to advise persons with affected animals to wash hands and clothing after handling sick animals and to not drink unpasteurized milk from affected animals. We routinely identify and serogroup all *Salmonella* isolates. All *Salmonella* isolates are referred to Ames, Iowa, for serotyping. If the serogrouping, serotyping, and antibiogram suggests a DT104 isolate, then the isolate is automatically subjected to phage typing to confirm *S. typhimurium* DT104.

For identification of Salmonella, place fecal specimens for transport in a screw-capped, leakproof, sterile container. Label the specimen container and place it inside a biohazard specimen bag. Tie intestinal segments at the ends and place them in a whirlpak bag or in a sterile, screw-capped container for transport. Wrap specimens in absorbent paper, place on cold packs and ship to arrive to us within 24 hours for optimal recovery of *Salmonella* species.

Aerobic culture: Submit sample as described above. Fee=$11.00 ($20.00 with antimicrobial susceptibility).

Antimicrobial Susceptibility Trends for

***E. coli* Isolated from Cattle**

SPECIES-Bovine ORGANISM-*Escherichia coli*

Antibiotic	# Tested	#Susceptible	% Susceptible
Ampicillin	56	28	50.00%
Cephalothin	;	11	54.55%
Enrofloxacin	7	7	100.00%
Erythromycin	;	56	0	0
Spectinomycin	45	30	66.67%
Sulfonamides	45	21	46.67%
Tetracycline	56	17	30.36%
Trimetho-sulfa	45	30	66.67%
Ceftiofur	46	31	67.39%
Tilmicosin	45	15	33.33%
Pirlimycin	11	0	0
Florfenicol	45	25	55.56%
Penicillin/ ovobiocin (mastitis)	11	0	0

CAT SKIN INFECTIONS

Pat Schultheiss

Occasionally, cats develop skin infections caused by unusual organisms that present diagnostic and therapeutic challenges. These infections often have serious consequences for the cat and may pose a hazard to the owners and veterinarian. The cats may present with firm nodules or abscesses, while draining tracts are variable. Since the cat's body serves as an excellent culture medium for the pathogens, draining lesions are a human health hazard because of the high numbers of organisms present in the exudate, even if the organisms are not usually considered highly virulent.

Possible infectious agents include various Mycobacteria that are not obligate pathogens, fungi, and anaerobes. These will not be detected with routine cultures so we must be informed that special cultures are needed. Submit a punch biopsy of skin or a swab of exudate in a Portacul when anaerobes or Mycobacteria are suspected. Submit a skin biopsy for fungal culture in saline or a Portacul. We can use one sample for the variety of cultures that will be set-up in the Laboratory. Cats that have been treated with antibiotics may have L-forms of bacteria. These organisms have a defective cell wall and pose a challenge to us trying to identify them. Information on the submission form about the patient's treatment history will help us.

With some skin lesions, an infectious agent is not suspected until we histologically identify patterns of inflammation, particularly in nodular lesions. We will then prepare special stains for acid-fast organisms and fungi on the formalin-fixed tissue and we may recommend submission of fresh tissue for cultures.

Most cases of bacterial pyoderma will respond to antibiotics and any predisposing factors such as pruritic conditions should be sought. Most abscesses will respond to conventional treatment. However, when lesions do not respond or when the problem is recurrent, then consider unusual organisms.

Dermatohistopathology: Submit at least 3 punch biopsies of skin in 10% formalin. Fee=$22.00.

Dermatology Cultures: Submit samples as described above. Fee=Aerobic culture=$11.00, aerobic/anaerobic culture=$30.00; fungal culture=$11.00; antimicrobial susceptibility=$9.00.

SHOULD I WORM MY CATTLE THIS FALL? John Cheney

Evaluation of bleeding patients in a herd setting is a serious situation that warrants a rapid diagnosis to prevent loss of animals. Accurate diagnosis also is required to alter management of the herd or flock to prevent future losses. The following two histories are examples of entirely different causes of bleeding in large groups of animals where an accurate diagnosis allowed for a successful outcome. These cases also exemplify how we can aid in solving these divergent problems.

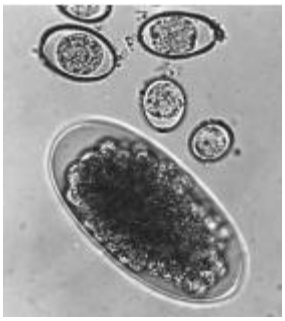
Case 1: A cow/calf herd in eastern Colorado had calves become pale and weak, and then die at 7-10 days of age. They did not have diarrhea or respiratory difficulty, and the cows remained healthy. Necropsy of two calves revealed massive hemoperitoneum. None of the calves had histologic evidence of liver or intestinal disease that would affect absorption of Vitamin K or synthesis of coagulation factors. We found no anatomical abnormalities that could account for the hemorrhage. We tested the liver for Vitamin K-antagonists and found dicumarol.

Case 2: A flock of 400 ewes in South Dakota had 20% of their lambs born in the first week of lambing die within 24 hours of birth. These lambs had subcutaneous hemorrhage, hemarthroses, massive hemorrhage around the umbilical arteries, and death was caused by exsanguination through the umbilical artery. The liver and gastrointestinal tract were normal histologically, and we did not find Vitamin K-antagonists in the liver. The ewes and rams were all normal and did not bleed.

The first case represents a case of dicumarol intoxication, but was unusual because the cows were unaffected clinically while the calves were bleeding severely. The source of the dicumarol was moldy sweet clover hay. Mold converts non-hazardous coumarin in sweet clover to dicumarol which inhibits Vitamin K cycling. The reason that the cows were not bleeding while the calves were dying is that calves (as well as most other neonates) have low Vitamin K and Vitamin K-dependent coagulation factor concentrations when they are born.

It is time again when many beef producers will be weaning and processing their cattle. Prices in the cattle market are depressed again this fall, therefore, producers do not want to spend money processing their cattle unless they are fairly sure their cost can be returned by increased performance in the cattle.

One question often asked is "should I treat my cattle for internal parasites when processing at weaning time?" Most internal parasitism in cattle is sub-clinical so usually there are no clinical signs associated with gastrointestinal parasites. The best method to determine if the animals are parasitized is a fecal exam. A fecal exam does not need to be done on many animals to determine if the herd has a parasite problem. If all the animals are the same age (calves/yearling/adults), and have been grazing similar pastures (mountain pastures, irrigated pastures), usually 5 to 10 individual samples from each group will give you a good idea if parasites are a problem. Collect these samples fresh and run individually using a centrifugation technique to recover the parasite eggs and coccidia oocysts. You will probably recover a few eggs in some of the samples but if you see no more than 20-30 eggs per gram of feces in a few samples, worming usually doesn't pay for the cost of treatment. If any one of the individual animals counts are over 50-80 eggs per gram of feces, treat the entire age group. You should always start by checking the calves and yearlings first and if you determine that internal parasites are not a problem, there is no need to test the adult cows.



Large strongyle egg with smaller coccidia oocysts.

Coccidiosis also can be a problem in newly weaned stressed calves. If these calves are going to be confined in a drylot after weaning, it usually pays to include a coccidiostat, such as decoquinate or amprolium. Give these two products to the calves for the first 3-4 weeks following weaning and confinement. If a protein supplement is fed, some coccidiostats can be mixed in with the supplement.

Fecal exam: Submit fresh feces. Fee=$8.00.

IMMUNOHISTOCHEMISTRY IN TUMOR DIAGNOSTICS

Barb Powers

We are using immunohistochemistry as an aid in tumor diagnostics with increasing frequency. This staining technique is especially useful for anaplastic cancers when the cell of origin is in question. The most common stains are for intermediate filaments cytokeratin and vimentin. Cytokeratin is a marker for epithelial cells and vimentin is a marker for mesenchymal cells, allowing us to distinguish between sarcomas and carcinomas. Factor VIII antigen is useful for determining if the tumor is of endothelial origin (hemangiosarcoma), and also is useful in assessing tumor vascularity as a potential prognostic factor to predict degree of malignancy. We also may use T and B cell stains to distinguish if tumors are of lymphoid origin, and as prognostic indicators since T cell lymphomas have a worse prognosis compared to B cell lymphomas.

Many other immunohistochemical stains are available. These include S100, originally thought to be specific for melanomas. More recently, however, this

Dicumarol is passed in the milk from cow to calf, and the calves bled first because they naturally had low concentrations of these coagulation factors. The cows would eventually have bled if left untreated and continued to consume the hay. Vitamin K is the treatment of choice for these cattle, but it may be expensive and even unnecessary to treat all animals. Vitamin K also can be hazardous as anaphylactic reactions can occur after intravenous administration. Optimal management would require identification and treatment of at-risk cows or calves with Vitamin K. To determine if these animals are at risk of bleeding, an activated coagulation time (ACT) is performed. This can be done easily in the field, and requires only an ACT tube, a 37C heating block, and a stopwatch. These tubes are commercially available, or can be obtained from us (Clinical Pathology Section). Any animal with an ACT time greater than 1.5 times normal would be treated with Vitamin K.

In the second case, the sheep were fed good quality alfalfa hay, and did not have detectable Vitamin K antagonists in the liver. Occasional bleeding lambs were born with a twin, and twins were not affected. Vitamin K was administered to one bleeding lamb without effect. These findings suggested that the lambs have a congenital defect of coagulation rather than an acquired defect. Further analysis of this flock demonstrated a hereditary carboxylation defect of all Vitamin K-dependent coagulation factors which was amplified in the flock due to inbreeding.

We are available to discuss any case including those involving disorders of hemostasis. We can recommend appropriate tests for accurate diagnosis, and provide the appropriate sample containers or simple test methods for field use.

OXALATE *(Rumex venosus)* POISONING IN CATTLE Charles Dickie

A common source of plant oxalates is the genus Rumex. Included in this genus are the sorrels and docks. *Rumex venosus* is a dock and has the common names of veined dock, wild begonia, and wild hydrangea. This member of the buckwheat family reproduces by seeds and horizontal rhizomes. Scaling along the stem is characteristic. It's found in the eastern half of Colorado and in the Northwest.

Rumex venosus

Mature ruminants can usually consume large quantities of plant oxalates without harm, apparently because the oxalates are metabolized to a large extent in the rumen. If cattle graze slowly, they can safely consume amounts of toxic plant material that would be lethal if consumed more rapidly.

In early April, eight mature beef cows of mixed breed were found dead at various locations in a large pasture in Southern Colorado. Calves were not affected. The pasture was predominantly rolling sandhills with sagebrush, prickly pear, hairy grama, blue grama, buffalo grass, and large patches of *Rumex venosus* comprising most of the flora. The grasses were still brown and dry, but the rumex was green and succulent.

protein has been found in a number of nervous system tumors. Muscle markers include desmin or actins and a histiocytic stain is lysozyme. Chromogranin is useful for tumors of neuroendocrine origin and a wide variety of specific stains for endocrine products such as insulin or thyroglobulin are available.

Immunohistochemistry, however, does not replace routine good diagnostics and is only an aid to an accurate diagnosis. For example, many cross reactions can occur and very poorly differentiated tumors may be negative with all stains. This technique also does not distinguish normal from neoplastic tissue. Fixation, processing and staining artifacts may affect staining quality. However, we always use control tissues to ensure that the procedure is working properly. As this staining technique is specialized, our turnaround time is longer (48 hours) compared to routine special stains (24 hours).

Tumor diagnostics: Submit biopsy sample in 10% formalin. Fee=$22.00 plus postage/courier service.

JOHNE'S DISEASE IN CATTLE

Gary Mason, Claudia Gentry-Weeks, and Frank Garry

Johne's disease is a chronic granulomatous enteric disease of ruminants caused by infection with *Mycobacterium paratuberculosis*. Although the disease is most commonly associated with dairy herds; beef cattle, small ruminants, and cervids also become infected and suffer clinical disease.

Johne's disease is well-known to veterinarians but the importance of the disease is often poorly acknowledged by producers. This lack of recognition is largely due to the long incubation period, chronic nature of the disease, and conception that the economic impact is limited to the loss of individual animals in the dramatic terminal period of the disease. The disease is widespread and more costly than often realized. The NAHMS Dairy '96 Study reveals that approximately 22% of the United States dairies contain at least 10% animals infected with *M. paratuberculosis*, with little regional variation. This study also revealed an estimated $235 per head per year loss in dairies in which 10% of cull animals show clinical signs of the disease. These losses are due to decreased milk production, decreased slaughter value, and premature culling with an increased cull rate, both of which slow potential progress in genetic improvement. Additionally, the known presence of the disease in a herd adversely affects the price of saleable seed stock. The economic impacts in a herd increase for each year the infection is allowed to progress.

There is some evidence that *M. paratuberculosis* may be involved in the pathogenesis of Crohn's Disease in humans; a chronic inflammatory disease of the lower bowel which is often quite severe. Although there is no conclusive evidence that animals infected with *M. paratuberculosis* constitute a public health threat, it is easy to envision potential ramifications if the available scientific information is reported in a biased manner.

Awareness of these facts and issues may provide the stimulus for producers to embark upon a Johne's disease herd control plan. A Johne's advisory committee has been formed in Colorado and has representatives from each of the livestock groups. The committee will help develop coherent state policies and programs that help producers deal effectively with the disease. A test and cull strategy is an integral element in such efforts. Several extremely important aspects of the developing Colorado Johne's program are:

At necropsy, the rumens were packed full of rumex, making it difficult to find any other kind of ingesta. Ecchymotic and petechial hemorrhages were prominent on visceral and parietal peritoneum. Thin, yellowish fluid was present in the abdominal and thoracic cavities. Mesenteric lymph nodes were enlarged and edematous. Other findings included catarrhal abomasitis, enteritis, edematous kidneys, and congested lungs.

Histologically, we found birefringent crystals of calcium oxalate in the renal tubules. With acute poisoning in some individuals, oxalates combine in the systemic circulation with calcium ions to form insoluble calcium oxalate. This results in functional hypocalcemia and death. Many of these more acute cases do not have calcium oxalate in the renal tubules. Presumably, this is because there is insufficient time for them to develop in the kidneys prior to death.

In Colorado, *R. venosus* is usually among the first plants to green in March and April; and, especially if other forage is depleted, cattle will consume large quantities of it. Plant oxalate concentrations on a dry weight basis were 9.2% in April and 13.9% in June. These are toxic amounts if rumex constitutes a large amount or all of the ration. As the grasses green and grow, the danger of rumex poisoning decreases. Usually by mid-July or August, the above ground parts of the plant have shriveled and dried, and it's no longer a danger. This pasture was rendered usable in early spring by providing dicalcium phosphate supplementation. Oxalate was complexed in the rumen to the extent that little was absorbed.

SWINE CORNER-POST-WEANING MULTISYSTEMIC WASTING SYNDROME

Barb Powers

Post-weaning Multisystemic Wasting Syndrome is a recently recognized disease causing poor growth, wasting, and even death in weaned pigs. Originally identified in Canada and California, it also has been reported in Indiana. Pathologic findings are generalized lymphadenopathy, interstitial pneumonia, interstitial nephritis, hepatitis, and a unique granulomatous inflammation of lymphoid tissues. Viral inclusions are found in the cytoplasm of macrophages. This virus has been identified as a circovirus. However, porcine circovirus infection is common in pigs and inoculation studies have failed to reproduce the disease. Regardless, extensive examination for other agents have failed to reveal other pathogens and the virus is consistently associated with the pathologic lesions. The virus can be identified by electron microscopy and immunohistochemistry. To date, we have not seen this disease in pigs in Colorado, but we will continue to watch carefully for it.

NITRATE/NITRITE TOXICITY

Dwayne Hamar

This fall, 12 of 75 head of cattle were found dead in the morning after being fed kochia/hay the previous evening. The kochia/hay was harvested from a new seeding of grass that had become overgrown with kochia. When the veterinarian examined the herd later and took post-mortem samples, the remaining animals appeared normal. The nitrate concentrations in the post-mortem ocular fluid and blood were 400ppm and 1000ppm, respectively, while the kochia/hay was 3.3% (DM). These results confirmed the suspected nitrate toxicosis as ocular fluid and blood greater than 40ppm is considered diagnostic and forage greater than 0.5% nitrate should be fed with caution. All suspect forage should be tested for nitrate prior to feeding. Since the amount of nitrate that plants accumulate varies across a field, several cuttings should be taken to provide a representative sample. Standing

- •Similar efforts are underway in other states and consistency will be sought between various state programs.
- •Producer participation in Johne's disease plans will be voluntary.
- •Herds that appear free of the disease will try to maintain that status by adopting appropriate management strategies.

A main thrust of the Colorado program will be to help identify Johne's-free herds and get this status recognized, which adds value to cattle in such herds. Herds with low levels of infection should work to achieve disease-free status as quickly as possible.

To test for Johne's disease in cattle, we use two methods--isolation of *M. paratuberculosis* from feces and an enzyme-linked immunosorbent assay (ELISA) for detection of antibodies against *M. paratuberculosis* in serum or plasma from cattle (IDEXX Test Kit).

Each method has its advantages and disadvantages. Fecal culture has historically been considered the "gold " standard since it is highly specific and has a sensitivity of ~45%. However, *M. paratuberculosis* is a slow-growing organism and fecal culture requires up to 16-20 weeks for detection of colonies of this bacterium. Since the ELISA detects antibodies against *M. paratuberculosis*, this test has the advantage of being a much quicker test, with processing time of one day. The ELISA we use has a sensitivity of ~45% (with some variation depending on stage of disease) and a specificity of 97%. The disadvantage to this test is that, for unknown reasons, occasionally fecal shedders are not detected by this method. The ELISA can be used in combination with fecal culture to identify animals that are infected with *M. paratuberculosis*. ELISA results should be used as a whole herd test to estimate the prevalence of infected animals in a herd and to plan control measures.

The ELISA assay provides an S/P ratio which is a ratio of the level of antibody present in the patient specimen to the level of antibody in the positive control (a known highly-infected animal). We report the S/P ratio and S/P values of <0.25 as negative and values >0.25 as positive. Test values near the suggested cutoff point of 0.25 have sufficient antibody response to be suspect of having the infection. All positive responses should be considered in the context of herd history for disease occurrence. Since this test was developed specifically for bovine specimens, interpretation of the results is limited to cattle.

For *M. paratuberculosis* culture, submit feces (1g minimum), lymph node, intestine, and rectal biopsy in a sterile, screw-capped, leakproof container. Place the specimen on a cold pack for transport to the Laboratory. For detection of antibodies against *M. paratuberculosis* using the ELISA test, submit bovine serum or plasma (0.5ml) in a sterile red top or lavender top tube.

Johne's diagnostics: Culture; Fee=$15.00. ELISA; Fee=$4.50 for 1-20 samples, $3.60 for >21 samples.

BREAKDOWN INJURIES IN RACEHORSES

Bob Norrdin

During the last four years, we have performed post-mortem examinations on horses that die or have to be euthanized under the auspices of the Colorado Racing Commission at the Arapahoe Park Racetrack. Dr. Tom Jones, the Racing Commission veterinarian at the track, initiated this program. We established a protocol for routine evaluation of musculoskeletal lesions in these horses to monitor their importance and provide material for in-depth studies. The program is funded by the Colorado Equine Research Fund with monies from a 0.025% apportionment from exotic parimutuel wagering.

forage may be sampled by grazing with a pair of scissors. For baled hay, a core sampler should be used. Most extension offices loan out core samplers, as do we. To inquire about borrowing the core sampler, contact us.

Dwayne Hamar with Core Sampler

Sources of dietary nitrate may be either water or forages. Normally, water nitrate concentrations are not elevated enough to cause nitrate poisoning, but could exacerbate the toxicity of high nitrate forages. Nitrate accumulates in plants when the soil nitrogen is high and water supply is inadequate for plant growth. Plants that are commonly high in nitrate include any cereal grain (cornstalks, oat hay, sorghum), weeds (pigweed, kochia, Canada thistle, Johnson grass), and grass hay. The seed part of a plant will not contain high concentrations of nitrate. Using wastewater from hog operations for irrigating alfalfa has resulted in nitrate accumulation.

Nitrate toxicity varies with dietary factors. For example, when the grain-to-roughage ratio is high, the toxicity of nitrate will be lower than when the diet is predominantly roughage. Also, the animal may adapt to dietary nitrate. Nitrate toxicosis usually occurs when animals are fed a low quality forage diet only. We recommend that forages greater than 0.5%, or water greater than 1,500ppm nitrate, be used with caution.

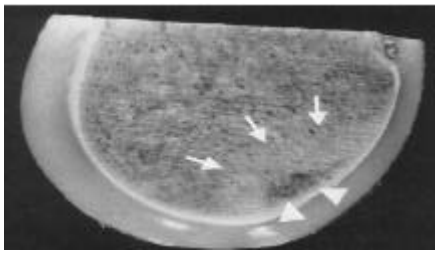
Clinical signs can develop within a few hours to several days after the animals have consumed excess nitrate. These signs may include muscle tremors, weakness, ataxia, and low tolerance to exercise. Late-term abortions have been associated with chronic consumption of high nitrate water and/or feed.

Confirming nitrate toxicosis usually involves forage and/or water analysis. Post-mortem ocular fluid or blood nitrate analysis of adult animals can confirm nitrate toxicosis. Fetal ocular fluid may be suggestive of possible nitrate-induced abortion, but this should be confirmed with forage and/or water nitrate analysis. Elevated fetal ocular nitrate concentrations also have been reported with concurrent *Actinomyces pyogenes* or other bacterial infection.

When nitrate is ingested by ruminant animals, microorganisms of the rumen reduce it to ammonia. In the process, nitrite is formed. When the amount of nitrate is high and conditions are right in the rumen, nitrite reduction to ammonia becomes rate limiting. Under these conditions, nitrite accumulates in the rumen and is absorbed into the blood. The absorbed nitrite undergoes an oxidation-reduction reaction in which the ferrous form of the iron in hemoglobin is oxidized to the ferric form, resulting in the formation of methemoglobin. Methemoglobin is unable to bind oxygen, thus decreasing the oxygen-carrying capacity of blood. When approximately 80% of hemoglobin is converted to methemoglobin, death results. Methemoglobin is dark brown or chocolate colored and results in a cyanotic appearance of the mucus membranes.

Nitrate/Nitrite testing: Submit blood, ocular fluid, feed, water. Fee=$6.00.

Most of these horses are euthanized with catastrophic musculoskeletal injuries. Carpal and metacarpal fractures are the most common injury, although we also see sesamoid, humeral, pelvic, and spinal fractures. Since these fractures are sometimes related to pre-existing microdamage in the bone, this provides valuable natural disease material to complement the studies of microdamage in the Equine Orthopaedic Research Program. In focusing on the carpal bones and metacarpal condyle in the fetlock joint, we found that failure was consistently associated with areas of thickened subchondral bone. To study this further, we examined the fetlock joints of 12 of the horses that had a spectrum of "traumatic osteochondrosis" lesions on the cannon bone condyle. This is a small, often subclinical lesion of subchondral bone collapse and cartilage infolding that occurs consistently at a site of mechanical overloading. Thickening or sclerosis of the subchondral bone was associated with the presence of non-viable bone cells. In some areas, we found debris-plugged vessels that may have interfered with circulation to these cells. Elsewhere, we saw osteoclastic removal of necrotic bone, suggesting activated remodeling in the area. Apparent fragmentation lines in the bone matrix indicated fragility in the sclerotic zone and in two cases, we found trabecular microfractures with hemorrhage and fibroplasia a few millimeters below the surface. Focal collapse of the overlying cartilage was usually at sites where breaks in the calcified cartilage layer had occurred indicating this had preceded the collapse.



Subchondral sclerosis (arrows), reddening and bone fragmentation (arrow heads).

While these observations provide a framework for the development of the lesions, they also raise questions about the precise sequence of changes and their causes. Is there progressive thickening of trabeculae that may lead to interference with circulation to bone cells? Is the failure preceded by measurable microdamage? To answer these questions, we examined histomorphometrically metacarpal condyles from horses run on a treadmill. We found significantly greater bone formation and a resultant greater amount of bone in the area where the failure occurs. We found increased diffuse staining with basic fuchsin, a measure of mechanical fatigue microdamage, in the area of the metacarpal condyles from horses where subchondral failure occurs (Norrdin et al., Bone 22:1339; 1998). In addition, the calcified cartilage layer was thinner with greater irregularity at the cartilage-bone interface, suggesting increased remodeling at the site.

These studies allowed us to characterize the site as a model of subchondral bone failure and gave us some insight into the mechanisms involved. Many more questions remain to be answered, however. Hopefully, the combination of spontaneous disease examples from our racehorse material and other studies will continue to shed more light on the pathogenesis. This, along with development of technologies for early detection, may lead to modifications in training, shoeing, and other management practices to prevent these catastrophic diseases.

PATIENTS WITH BLEEDING DISORDERS IN CLINICAL PRACTICE

Dale Baker and Dan Gould

Chronic Wasting Disease has been diagnosed in farm herds in South Dakota, Nebraska, Oklahoma and Canada this past spring and summer. This disease is present in free-ranging deer and elk in Colorado and Wyoming. Colorado has a mandatory surveillance program for chronic wasting disease for farmed elk herds. This program is coordinated by the State Veterinarian's office and the testing is done by us. So far, we have not had any positive cases in Colorado farmed elk. Call us for more information.