Wyoming State Veterinary Laboratory Newsletter – October 2006

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MESSAGE FROM THE DIRECTOR

PRESENTATIONS TO CLIENTS

Faculty and staff at the veterinary laboratory make regular presentations on disease items of interest to the WVMA, and to regional and national meetings. Toward the end of this newsletter are presentations made this past week at the AAVLD's annual meeting. We will make presentations at the winter meeting of the WVMA in early December in Casper.

What some of you may not be aware of is that we can also present to producers or small animal clients about issues of interest. For example, in the recent past, diagnosticians from the laboratory spoke at meetings in Wyoming on highly pathogenic avian influenza, canine influenza, BVDV control, trichomoniasis, West Nile virus, rabies, research on-going at the WSVL, and our pre-veterinary program offered here. The motivation is not purely altruistic. This is a land grant university. Part of our job entails getting out in front of Wyoming citizens and telling them what their tax dollars buy at UW. It is pertinent to the laboratory, animal owners, and wildlife managers. It enhances the visibility of the college.

So, if at some time you would like to have one of us present a talk to your clients, at an appropriate venue, please contact the WSVL. All we ask is that you give us reasonable advance notice (months, not days), that you do your best to ensure good attendance (>20 people; prime rib is an effective attractant), and that there is a mechanism to acknowledge that one of us spoke. A letter of acknowledgement/thanks after the meeting works fine. This goes in the speaker's personnel file. It is especially helpful for junior faculty when they come up for promotion. Topics we can cover include:

- Rabies
- Plague/tularemia
- Testing strategies for BVDV
- Current research in the Department of Veterinary Sciences
- What it takes to get into veterinary school
- Avian and canine influenza
- Brucellosis and the GYA
- West Nile encephalomyelitis in animals and people
- Toxicological investigations

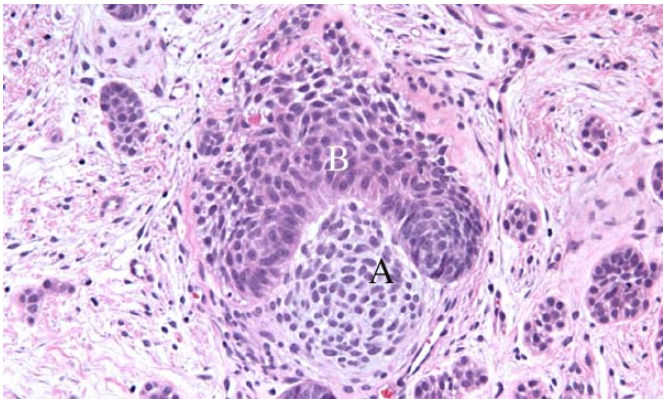
The list is not exhaustive. There is considerable expertise in the department. You may have topics you would like to see addressed. If you make the contact, we will try to supply the speaker.

Donal O'Toole

DIAGNOSTIC CASES OF INTEREST

Feline inductive odontogenic tumors

Tumors of tooth-forming tissues in dogs and cats can be challenging, particularly since the classification system is amended with greater frequency than pathologists relish. But some actually follow textbook descriptions. Dr. Don Montgomery was recently submitted a proliferating, space-occupying, radiolucent mass from the maxilla of a cat in Casper.



WSVL #06C13893. Inductive fibroamelobastoma arising in the maxilla of a cat. A characteristic feature of this tumor is it consists of mesenchyme forming dental pulp (A), capped by odontogenic epithelium (B).

This tumor, which has also been erroneously called inductive fibroameloblastoma, is a rare tumor. It most commonly arises from the dental arcade of the rostral maxilla. Although rare, it is the most common tumor arising in the oral cavity of young cats. Lesions present as osteolytic masses that interfere with mastication and/or result in facial deformity. Local recurrence of these destructive tumors may follow incomplete excision. The lesions consist of irregular cords and nests of ameloblastic epithelium forming caps over loose organized dental mesenchyme. Surgery can be curative.

Drs. Don Montgomery/Donal O'Toole

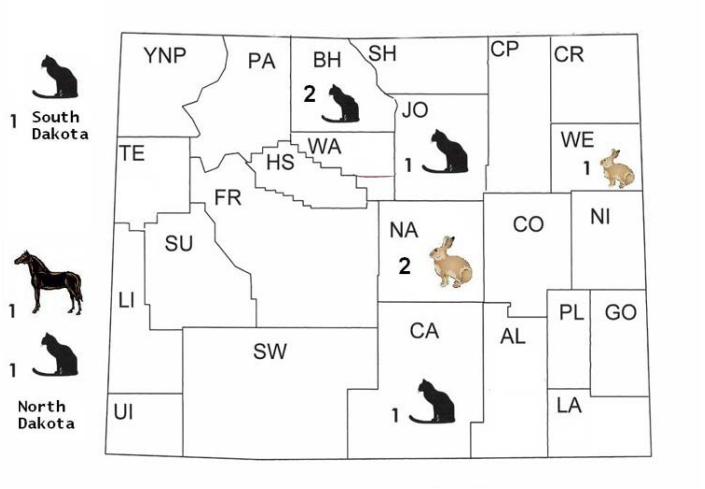
Gardner DG, Dubielzig RR: 1995, Feline inductive odontogenic tumor (inductive fibroameloblastoma)--a tumor unique to cats. J Oral Pathol Med.24 (4):185-190

Health official concerned about increased tularemia activity in Wyoming

A Wyoming Department of Health official today expressed concern about possible increased activity this year for tularemia, an infectious disease that animals and insects can spread to humans.

Tularemia, also known as "rabbit fever" or "deer fly fever," is a bacterial infectious disease that typically affects rabbits, hares and rodents. Other mammals, including domestic animals, can also become infected.

"We've had reports this year of large rabbit die offs in a number of areas around the state," said Dr. Jaime Snow, state public health veterinarian with the Department of Health. A woman in Baggs who was bitten by a cat and a male youth in Lovell who recently skinned a rabbit are both believed to have contracted the disease. In addition, three cats, one foal and one cottontail rabbit have tested positive for the disease at the Wyoming State Veterinary Laboratory.



Tularemia cases diagnosed in WSVL in 2006.

Cases have been seen in cats in Big Horn, Johnson and Carbon counties. Additional cases were confirmed in cases from the Dakotas in a foal and two cats

People typically acquire tularemia when bit by an infected tick, deer fly, horse fly or mosquito; by handling infected animals (especially rabbits or rodents); or through ingestion or contact with untreated, contaminated water or insufficiently cooked meat.

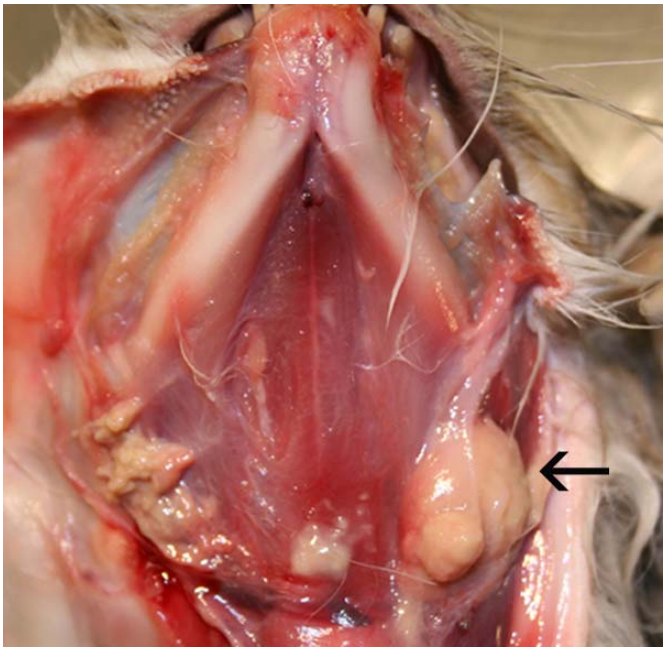
Human signs and symptoms of tularemia can include skin ulcers, swollen and painful lymph glands, inflamed eyes, sore throat, mouth sores, diarrhea or pneumonia. If the bacteria are inhaled, symptoms can include abrupt onset of fever, chills, headache, muscle aches, joint pain, dry cough and progressive weakness.

"Tularemia can be fatal if the person is not treated," Snow said. "People who become ill after an insect or tick bite, after handling a sick or dead animal should contact their healthcare provider."

Snow suggested several precautions that can help reduce the risk of tularemia infection:

- Avoid bathing, swimming or working in untreated water and avoid drinking untreated water.
- Avoid handling rabbits, squirrels or other animals that appear sick.
- Wear rubber gloves when skinning animals, especially rabbits and squirrels; skin animals in a well-ventilated area.

- Wash hands thoroughly with soap and water after handling sick or dead animals.
- Cook meat thoroughly before eating, especially rabbit and squirrel.
- Use measures such as insect repellent and protective clothing to prevent insect bites.



WSVL #06F12920. Four-month old kitten from Buffalo that presented with anorexia and fever. There was marked enlargement of mandibular lymph nodes (arrow), as well as tonsils and most cervical nodes. The kitten was tularemia positive. Photo: Dr. Todd Cornish.

Sept 5 2006 press release
Wy Dept Health

Hemorrhagic bowel syndrome in dairy cattle

In early September a 1500 lb Holstein cow in Cody WY was found depressed one day and dead the next. The veterinarian's post-mortem examination revealed an anemic carcass. His main finding was mid-jejunum filled with clotted blood. Histologically, Dr. Cornish found severe locally extensive enteritis with intraluminal bleeding. According to the history provided, another cow died on the property recently and had similar clinical signs.

Hemorrhagic bowel syndrome is a newly reported syndrome, predominantly of dairy cattle. It was originally reported by Dr. Glenn Cantor, a pathologist with the Washington animal disease laboratory Since Dr. Cantor's report there have been several peer reviewed publications describing the occurrence of the disease, risk factors, and possible association with *Clostridium perfringens* type A.

Mortality with the disease is high. Of 22 cases seen at CSU between 1997 and 2000, 17 died (77%). Cattle were more likely to survive if they underwent surgery to remove obstructing blood clots in the gut lumen, but even then mortality was high (5 of 9 cows died or were euthanized during or after surgery). A survey of producers in multiple states found that about 1 in 10 producers have recognized a case of HBS in the previous 5 years. Management practices designed to achieve high milk production, such as high energy diets, are thought to be risk factors. There may be an association between the identification of *Clostridium perfringens* A, with or without beta2 toxin, and the disease. *Clostridium perfringens* A, with and without beta 2 toxin, can also be found in the digestive tract of normal cattle. To date there have been no reports describing successful production of the disease using clostridia.

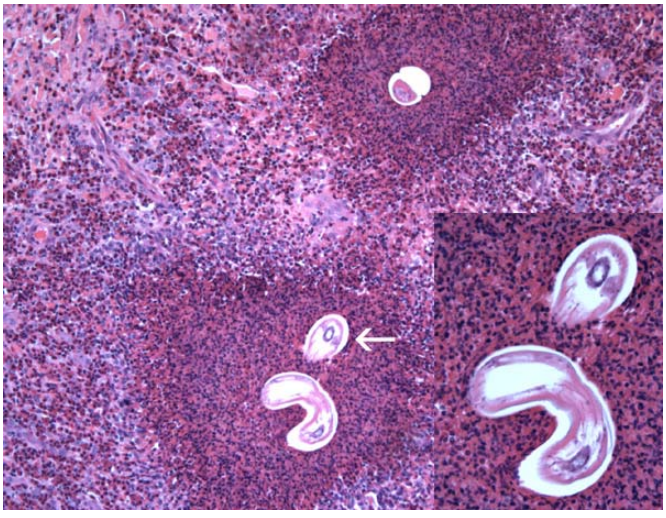
There is no vaccine currently available in the US for *Clostridium perfringens* A and/or for strains that produce beta2 toxin. Bacterin-toxoids for types C and D do not appear to be protective.

Dr. Donal O'Toole

Cantor, G: 1999, Jejunal hemorrhage syndrome: a new, emerging disease of dairy cows. Washington State Veterinary Medical Association Newsletter. July 1999.
Dennison AC et al: 2005, Comparison of the odds of isolation, genotypes, and in vivo production of major toxins by Clostridium perfringens obtained from the gastrointestinal tract of dairy cows with hemorrhagic bowel syndrome or left-displaced abomasum. J Am Vet Med Assoc. 227(1):132-138.
Berghaus RD et al: 2005, Risk factors associated with hemorrhagic bowel syndrome in dairy cattle. J Am Vet Med Assoc. 226(10):1700-1706.
Dennison AC et al: 2002, Hemorrhagic bowel syndrome in dairy cattle: 22 cases (1997-2000). J Am Vet Med Assoc.;221(5):686-689.

Cutaneous habronemisiasis (summer sores) presenting as a rapidly growing mass in a horse

In August a veterinarian in Big Horn Basin was presented with an adult AQH with a rapidly growing mass on the coronet extending to the fetlock. It had tripled in size over the previous 10 days. The mass was pruritic and necrotic. Calcified areas were throughout the mass..



WSVL #06E11021: Habronemiasis in a horse. Larvae are in the center of pools of eosinophils (arrowhead). Inset: higher magnification of degenerate larvae

Histologically there were multiple foci of eosinophilic granulomas with extensive fibrosis and necrosis. At the centers of some inflammatory foci there were profiles of degenerating larval nematodes. Dr. Cornish's diagnosis was pyogranulomatous dermatitis with intralesional parasites

Habronemiasis is a hypersensitivity reaction to the larvae of three species of nematode that have a horse-house fly (or stable fly) life cycle. Larvae deposited near the lips are swallowed and adult nematodes develop in the stomach. But larvae can also be deposited on wounds, where they elicit a strong inflammatory reaction.

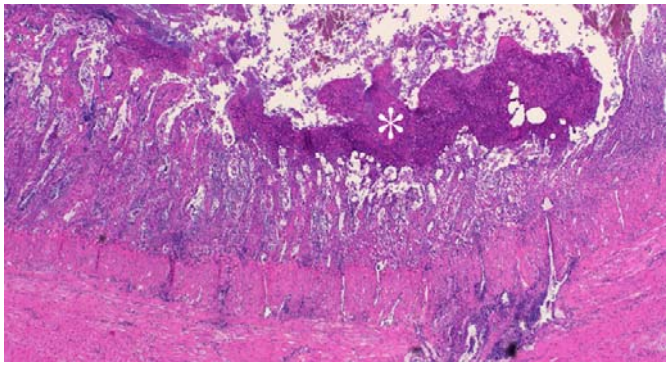
Typically lesions are on the distal aspect of limbs (as here), around the eyes, ventrum and prepuce. Treatment is by debriding affected tissue, combined with local and systemic treatment (glucocorticoids). The merit of using parasiticidal treatments to kill the larvae is questioned, but it may have merit in killing the adults in the stomach and thereby reducing the possibility of recurrent infections.

Drs. Todd Cornish/Donal O'Toole

Purple gut in trumpeter swans

Three trumpeter swans were received for necropsy. Two died from necrotic enteritis (*Clostridium perfringens*) and concurrent aspergillosis. The remaining animal had disseminated aspergillosis. Gross pathology of necrotic enteritis is classic (almost pathognomonic) so many times presumptive diagnosis can be made on necropsy and treatment can be initiated immediately. Antibiotics used to treat necrotic enteritis include lincomycin, bacitracin, oxytetracycline, penicillin or tylosin tartrate in the water. The disease is usually referred to as necrotic enteritis, but it the equivalent of purple gut in calves and young foals.

High levels of wheat, barley, fishmeal or rye in the ration can precipitate necrotic enteritis in a flock, as well as coccidiosis or contaminated feed or litter. In this case, the ration had been changed to wheat prior to the outbreak, and this may have been responsible for losses.



WSVL #06W13532: Histological appearance of necrotic enteritis in bowel of a trumpeter swan. The asterisk marks necrotic material in the lumen of this swan's gut.

Dr. Cynthia Tate/Dr. Todd Cornish

Observation on failure of VSV transmission from infected to uninfected horses.

VS-NJ infection was confirmed in a group of 4 equine suspects in Wyoming, based on serology and virus isolation testing conducted on 8/17/06. Initial serology and virus isolation results were consistent with recent clinical infection. All 4 horses eventually tested C-ELISA and CF positive for VS-NJ on 9/19/06 serial retest.

Over a 3 day period beginning 8/15, the 4 affected horses were removed from the premises where they had acquired VS and were transferred to a remote pasture 1.32 miles east of the original one. There, nine other resident horses were exposed to infection for over a month. All 13 horses ran jointly, shared a water tank, salt blocks with no separation/isolation or insecticide use of any kind.

All 13 horses were bled on September 19, when the 21 day countdown inspection was conducted. The 9 residents that were extensively exposed to the 4 infected horses for over a month tested serologically negative to equine VS.

The lack of direct transmission of VS-NJ to any of the nine residents of the remote pasture, despite extensive exposure to 4 infected equine in this natural experiment, support the notion that direct transmission of equine VS does not readily occur away from high risk environments. In other words, direct horse-to-horse transmission is rare.

Transmission of equine VS appears to be primarily vector driven.

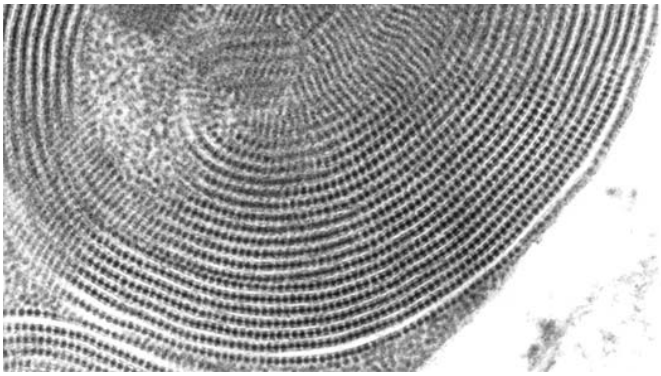
Dr. John Duncan, USDA VS
28 Sept 2006

Pigeon circovirus

A juvenile pigeon was submitted for necropsy with a history of high mortality in the juvenile pigeons in a loft. The pigeon had necrotizing pneumonia and marked lymphoid depletion in the bursa of Fabricius. Cytoplasmic inclusions typical of pigeon circovirus were absent.

Circovirus was detected in the bursa by *in situ* hybridization performed at the University of Georgia. Circovirus causes lymphoid necrosis and atrophy of the bursa and is associated with immunodeficiency. Pigeons infected with circovirus typically die of concurrent infections with fungi, bacteria and other virus such as paramyxovirus, herpesvirus and adenovirus. Diagnosis is by histopathology when cytoplasmic inclusions are present (definitive diagnosis can be made by electron microscopy (direct or transmission) or by in situ hybridization). *In situ* hybridization is necessary for a definitive diagnosis when inclusions are absent.

As far as we know, this is the first time that we recognized circoviral infection in pigeons in Wyoming.



Electron photomicrograph of a cytoplasmic inclusion in bursal epithelial cell. It shows circoviral particles (14-17 nm) in semicircular arrays.

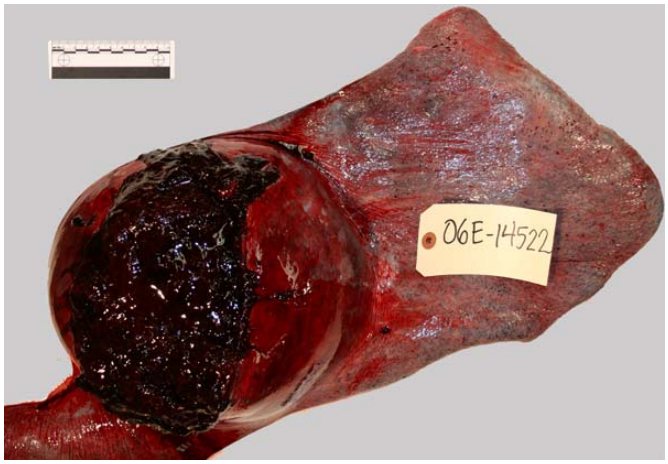
Dr. Leslie Woods

Note: Dr. Woods is the person who first recognized and reported circovirus as a cause of disease in pigeons.

Woods LW et al: 1994, A retrospective study of circovirus infection in pigeons: nine cases (1986-1993). J Vet Diagn Invest 6(2):156-164.
Woods LW et al: 1993, Circovirus-like infection in a pigeon. J Vet Diagn Invest. 5(4):609-612.

Fatal splenic disease in two horses

Sudden death in horses is a source of acute distress to owners, and can be a diagnostic challenge. Between one third and one half have no lesions at necropsy. It is assumed that at least some of these die of cardiac arrythmias. In terms of defined causes of sudden death, massive internal hemorrhage is a well recognized cause.

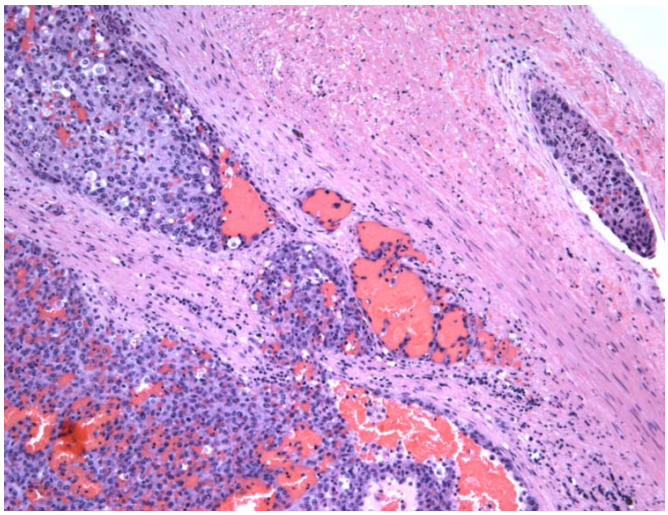


WSVL #06E14522: Intra-splenic hematoma in a horse. The mass ruptured, resulting in sudden death.

We were recently presented with a 10 year old Tennessee Walker that had been normal the previous evening. Necropsy by Dr. Montgomery quickly established the cause: a large mass in the spleen had ruptured, resulting in exsanguination. Histologically there was no evidence of neoplasia in the spleen.

Coincidentally, a short time later a veterinarian submitted samples from a 10 year old horse that had became acutely ill over the previous 3 weeks. The horse died of intra-abdominal hemorrhage. The veterinarian submitted samples from liver and spleen.

The intrasplenic mass was a hemangiosarcoma. It had metastasized to liver, and there were intra-vascular neoplastic emboli.



WSVL #06E14957: Primary hemangionsarcoma of spleen of 10 year old horse. The mass ruptured, resulting in exsanguination.

Unlike this tumor in dogs, where it is common, hemangiosarcoma is considered rare in horses. A recent survey of cases submitted to the New Bolton Center in Pennsylvania between 1986 - 2004 found 26 horses with hemangiosarcoma (of ~56,000 horses). Of these 26, 15 had a mean age of 11.2 years. Thirteen were euthanized. Most had disseminated hemangiosarcoma. Interestingly, the remaining 11 horses with hemangiosarcoma were young (<3 years of age) and three of these probably had the tumors from birth.

Drs. Don Montgomery/Donal O'Toole

Brown CM, Kaneene JB, Taylor RF: 1988, . Sudden and unexpected death in horses and ponies: an analysis of 200 cases. Equine Vet J 20(2):99-103.

Johns I, Stephen JO, Del Piero F, Richardson DW, Wilkins PA: 2005, Hemangiosarcoma in 11 young horses. J Vet Intern Med. 19(4):564-70.

Southwood LL, Schott HC 2nd, Henry CJ, Kennedy FA, Hines MT, Geor RJ, Hassel DM: 2000, Disseminated hemangiosarcoma in the horse: 35 cases. J Vet Intern Med 14(1):105-109.

The following talks were presented by WSVL personnel and UW Department of Veterinary Sciences graduate students at the annual meeting of American Association of Veterinary Laboratory Diagnosticians in Minneapolis MN 12 - 18 Oct 2006.

Survival and causes of mortality of free-ranging white-tailed deer (*Odocoileus virginianus*) in a high prevalence area of chronic wasting disease

D. R. Edmunds, F. G. Lindzey, R. G. Grogan, W. E. Cook, T. J. Kreeger, T. E. Cornish

Chronic wasting disease (CWD) is classified as a transmissible spongiform encephalopathy, a group of prion diseases that includes scrapie and bovine spongiform encephalopathy. Chronic wasting disease has been diagnosed in mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), and moose (*Alces alces*) within the historic endemic area, which includes northern Colorado, southeastern Wyoming, and Nebraska panhandle. Transmission and geographic spread of CWD are poorly understood. Our study, which began in 2003, will determine the effects of CWD on the behavior and survival of free-ranging white-tailed deer (WTD) and provide information on the spread of CWD. One of the specific objectives of this study is to monitor CWD-positive and CWD-negative WTD throughout their lifespan via radio-telemetry and global positioning system (GPS) collars to determine differences in survival rates and causes of mortalities. To meet this objective deer were captured as fawns, tested for CWD by tonsil biopsy immunohistochemistry (IHC), marked with ear tag radio-transmitters, and recaptured on a yearly basis to re-test for CWD and to replace radio-transmitters with GPS collars. All mortalities are subjected to complete necropsies with thorough CWD testing, including immunohistochemistry examination of tonsil, retropharyngeal lymph node, and obex region of the medulla oblongata as well as enzyme-linked immunosorbent assay (ELISA) testing of retropharyngeal lymph node.

In four years we captured, marked and CWD tested 152 WTD. The CWD prevalence for adult deer captured in 2006 is 29% (13/45), while the CWD prevalence for all deer including fawns is 23% (14/62). The yearly survival rates are lowest for male deer [50% (13/26)] and CWD-positive male deer [33% (2/6)]. For deer where cause of death was determined, hunter harvest (19), capture-related mortality (13), emaciation associated with clinical CWD (9), predation (5), vehicle collision (4), epizootic hemorrhagic disease (3), and entanglement in fence (2) are the major causes of mortality. We were unable to determine cause of death for 10 mortalities. The proportions of deer killed by hunter harvest (47%) and motor vehicle collisions (50%) that were CWD-positive was higher than expected, based on the CWD prevalence in the study population. These findings suggest that wildlife professionals and diagnosticians need to rule out CWD in otherwise apparently healthy deer killed by hunters, motor vehicle collisions, and other anthropogenic causes of mortality in areas where CWD is known to occur.

These findings support previously published reports that inclusion of hunter-killed and/or road-killed deer should be considered where applicable for CWD surveillance programs.

Long bone fractures in neonatal calves persistently infected with non-cytopathic bovine viral diarrhea virus

D. O'Toole, D. Montgomery, L. Steadman, R. Norrdin, J. Cavender, A. Bratanich, M. Raisbeck

Osteopetrosis of long bones with fractures is occasionally reported in association with transplacental bovine viral diarrhea virus infection[a, b] Here we report BVDV-associated osteopetrosis in a herd of beef cattle, of which three cases were confirmed histologically, and by virus isolation and/or immunohistochemistry. The mechanism whereby BVDV induces osteopetrosis was investigated. Five affected full-term calves were born in a 200-cow commercial Angus-cross herd in western Nebraska between February 17 and 22 2006. The herd was unvaccinated for BVDV since the owners sought to produce 'natural' calves. The owners were also attempting to breed for small adult frame size in order to produce small cows without loss of weaning weight. Two affected calves were out of heifers. The remaining three were out of cows.

Affected calves were small, abnormally stocky, with short limbs and slightly domed heads. Calf #1 developed a fracture of the diaphysis of the right metatarsus when 3 days old, and a fracture of the left metatarsus when 8 days old. Fractures were treated by fiberglass casts, yet after 3 weeks no healing of the bone occurred. The calf was euthanized by the owner and was unavailable for examination. Calf #2 developed compound diaphyseal fractures of both metatarsi when 16 days old. It was euthanized at 20 days. Tissues, including bone, were submitted for examination. Calves #3 and #4 were collected alive for laboratory characterization. The left tibia of calf #3 fractured one day after delivery to the WSVL. Both calves were euthanized when 41 days old, and examined post-mortem. Calf #3 had fractures of three ribs and the calvarium was abnormally thin; no fractures were present in calf #4. Growth plates were unremarkable. The dimensions of long bones were comparable to those of healthy 2 week old calves. Histological changes in the calves were consistent with cortical osteopetrosis. Noncytopathic BVDV was isolated from calves #3 and #4. BVDV antigen was detected in multiple tissues, including brain and cartilage, in calves #2, 3 and 4. Details on testing of the

rest of herd for persistently infected cattle, and radiographic and immunohistochemical features of the disease are in hand at the time this abstract was submitted, and will be presented.

a. Constable PD et al: 1993, Femoral and tibial fractures in a newborn calf after transplacental infection with bovine viral diarrhoea virus. Vet Rec 132:383-385.
b. Scruggs DW et al: 1994, Osteopetrosis, anemia, thrombocytopeneia, and marrow necrosis in beef calves naturally infected with bovine viral diarrhea virus. J Vet Diagn Invest 7: 555-559.

Pre-clinical and early clinical lesions of malignant catarrhal fever in bison following experimental inoculation with ovine nasal mucus containing ovine herpesvirus-2 (OvHV-2)

D. O'Toole, K. Gailbreath, L. Oaks, N. S. Taus, W. C. Davis, M. Ghoddusi, H. Li

Malignant catarrhal fever is a common and fatal disease of American bison. Recently our group demonstrated that MCF can be induced experimentally in American bison by aerosol exposure to nasal secretions derived from sheep experiencing intensive shedding events (O'Toole et al: 2004, *Successful experimental induction of acute malignant catarrhal fever in bison using aerosols of ovine nasal mucus containing ovine herpesvirus-2 (OvHV-2).* Proceedings p. 55, 47th annual conference, AAVLD, 2004). An infectious dose of >1 x 10^5 OvHV-2 DNA copies induces MCF in susceptible bison (O'Toole et al: 2005, *Defining the minimum intranasal infectious dose of OvHV-2 that is required to induce infection and MCF in bison.* Proceedings, P. 114, 48th annual conference, AAVLD, 2006).

In the current study (May 31 - July 9 2006), we wished to define preclinical and early clinical morphological changes associated with MCF following experimental challenge. This is part of a long term goal to define the pathogenesis of MCF, and to develop practical immunological and/or genetic strategies for control of the disease.

Eighteen bison that were negative for OvHV-2 based on PCR testing of peripheral blood leukocytes (PBL) were obtained from a commercial bison operation. Following acclimation, 16 bison were challenged with OvHV-2 by nebulizing 2 ml of an inoculum containing 1 x 10^7 OvHV-2 DNA copies; the remaining two bison served as uninoculated controls. Bison had evidence of infected PBLs by 19 days post-aerosolization (DPA). All 16 inoculated bison, and neither of the two controls, were positive for OvHV-2 by 22 DPA. Pairs of bison were selected for post-mortem examination beginning at 23 DPA. Gross lesions were detected in two asymptomatic bison at 26 DPA. Clinical signs were first evident at 28 DPA. The study is ongoing at this writing. All bison are scheduled to be examined post-mortem by 40 DPA.

We conclude that:

- Aerosol exposure of bison using 1 x 10^7 OvHV-2 DNA copies reliably induces MCF, with onset of clinical signs of 28 DPI, and detectable gross lesions at 26 DPI.
- Gross lesions of MCF in the pre-clinical/early clinical stages of the disease are found in bladder (hemorrhagic cystitis), cecum and colon (ulcerative typhlocolitis), and larynx-pharynx (laryngitis-pharyngitis.
- A consistent feature in inoculated bison is the presence of multifocal consolidation throughout all lobe of the lung, involving <1 - 5% of pulmonary parenchyma. In earlier studies these foci corresponded to bronchointerstitial pneumonia; histological studies in the current experiment are pending.
- This is the first report of pre-clinical lesions of MCF in any species.

Necrobacillosis in elk (*Cervus elaphus nelsoni*) on a winter feedground in Wyoming

T.E. Cornish, C.M. Tate, A.M. Boerger-Fields, B.R.A. Parrie, J. Miller, T.J. Kreeger

Necrobacillosis is a clinical syndrome of ungulates caused by infection with the Gram-negative obligate anaerobe bacterium *Fusobacterium necrophorum*. Outbreaks of this disease have been reported in free-ranging elk (*Cervus elaphus nelsoni*) on feedgrounds from Wyoming since 1944, and significant mortality events associated with this disease are not rare. In the winter of 2006 increased morbidity and mortality were observed in elk on a large feedground in Wyoming, with common clinical signs including lameness, lethargy, recumbency, variable loss of condition, and terminal loss of response to environmental stimuli. Complete necropsies were performed on 20 elk that died or were euthanized. Eight of these elk demonstrated gross and microscopic lesions of classic necrobacillosis (necrotizing stomatitis, necrotizing reticulorumenitis, necrotizing hepatitis, and/or necrotizing interdigital dermatitis or foot-rot with intralesional gram-negative filamentous bacilli), however we were unable to isolate *F. necrophorum* from tissues with lesions, in part due to overgrowth of contaminants, and sometimes due to decomposition of samples. The diagnosis of necrobacillosis was confirmed by PCR performed on formalin-fixed tissues containing appropriate gross and microscopic lesions using primers specific for the hemaglutinin-related protein gene of *F. necrophorum* subsp. *necrophorum* (*F. n. necrophorum*). We also are in the process of validating an immunohistochemical technique for the diagnosis of *F. necrophorum*

infection in formalin-fixed tissue sections. Both subspecies of *Fusobacterium necrophorum* (*F. n. necrophorum* and *F. funduliforme*) have been associated with lesions of necrobacillosis in ungulates, but F. *n. necrophorum* is considered to be more pathogenic. Necrobacillosis remains a significant (if sporadic) cause of morbidity and mortality for wild elk on winter feedgrounds in Wyoming. Potential risk factors, including artificial concentration of elk and presumptive heavy environmental contamination with *F. necrophorum*, undoubtedly contribute to outbreaks of this disease.

Immunohistochemical, microbiological, and molecular detection of *Brucella abortus* in Rocky Mountain elk

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Brucellosis, caused by the bacterium *Brucella abortus*, is endemic in bison (*Bison bison*) and Rocky Mountain elk (*Cervus elaphus nelsoni*) in the Greater Yellowstone Area of Wyoming, Montana, and Idaho. *Brucella abortus* is shed by infected elk during abortion or parturition and most often is transmitted through contact with infected fetuses, fetal fluids, or vaginal exudates. Current diagnostic methods used to identify infection in elk include serological testing and bacterial culture, but the correlation between serology and culture results is poor, serology cutoff values are challenging to determine, and bacterial culture is slow and likely to be positive only later in gestation or following abortion or parturition. There is a need for more informative, sensitive, and rapid diagnostic tests to detect brucellosis in elk. As part of the Wyoming Game and Fish Department's brucellosis surveillance program, elk were trapped using corral traps and serologically tested for brucellosis on the Grey's River elk feedground in 2005 and on the Dell Creek, Muddy Creek, and Grey's River elk feedgrounds in 2006.

Twenty-three seropositive cow elk aged ≥ 1.5 years from the feedgrounds were killed and necropsied. Paired tissue samples (routine organs, reproductive tissues including fetal tissues, and a variety of lymph nodes) were collected and frozen at –70 C for bacterial culture and PCR and fixed in 10% neutral buffered formalin for histopathology and immunohistochemistry (IHC). Tissue sections were examined for microscopic lesions indicative of brucellosis and stained with an anti-*B. abortus* polyclonal antibody for IHC. *Brucella abortus* was isolated from 45% (9/20) of the seropositive cow elk and 5% (1/19) of the fetuses. Tissues were collected during necropsy for evaluation with real-time PCR to identify the presence of *B. abortus* in fresh tissue. The assay will be developed further to distinguish *Brucella* biotype, and PCR and immunohistochemistry results will be compared to culture (gold-standard test) results to determine the sensitivity and specificity of each assay.

Toxicity of the lichen substance (+)-usnic acid in ruminants

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Identifying *Xanthoparmelia chlorochroa* as the culprit behind the 2004 Red Rim-Daley Wildlife Habitat Management Area (WHMA) elk die-off resulted in many questions regarding the toxicity of this lichen species from ranchers, wildlife managers, and concerned citizens. Unfortunately, most questions could not be answered with any certainty because the toxin(s) within the lichen remained unknown. A reference from 1939 attributes *X. chlorochroa* toxicity in domestic cattle and sheep to usnic acid, a lichen secondary metabolite. In both the historical report and the recent episode, clinical signs were recumbency, weakness, and paralysis.

To test the hypothesis that usnic acid causes the clinical syndrome seen in elk, domestic sheep were dosed with (+)-usnic acid utilizing an up-and-down procedure for acute toxicity testing. Sheep were examined for congruence of signs and lesions with the Red Rim elk, and clinical pathology was used to monitor for evidence of subclinical disease. Prior to dosing ewes with (+)-usnic acid, a lichen feeding trial was conducted to ensure domestic sheep were a valid model. Three adult Rambouillet ewes were fed *X. chlorochroa* collected from the Red Rim-Daley WHMA. All three ewes showed clinical signs in varying degrees of severity matching those seen in the Red Rim-Daley elk. Following the lichen feeding trial, nine adult Rambouillet ewes were dosed with (+)-usnic acid resulting in an ED50 between 485 and 647 mg/kg/day. The two ewes that received the highest doses (776 and 647 mg/kg/day) were the only ewes to display clinical signs, abnormalities in clinical pathology, and have appreciable lesions. The remaining seven ewes were not clinically affected, nor were any changes observed in clinical pathology or under the light microscope. The clinically affected (+)-usnic acid ewes displayed markedly different clinical signs compared to those seen in lichen affected elk and ewes. Clinical signs included anorexia, appendicular stiffness, and signs of a belly-ache. Serum creatine kinase, aspartate aminotransferase, and lactate dehydrogenase were considerably elevated in the two high dose (+)-usnic acid ewes. Similarly these were the only ewes with significant lesions, which consisted of a degenerative appendicular skeletal myopathy.

Based on clinical signs, clinical pathology, and light microscopy, it was determined that (+)-usnic acid is not the toxic component of *X. chlorochroa* directly responsible for the syndrome seen in the Red Rim-Daley elk or lichen affected ewes. However, it is possible that (+)-usnic acid is working synergistically or antagonistically with other compounds within the lichen to induce the specific syndrome.

Tracking the spread of skunk rabies in Wyoming (20+years)

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Wyoming, being a headwaters state with varied and physically separated environments, offers an interesting setting for tracking the spread of rabies in terrestrial species. The WSVL is charged with doing all rabies testing within the state which includes more than just human or animal exposure submissions. All specimens are examined using the fluorescent antibody staining. During the course of gathering this data the reagents have changed from fluorescent labeled polyclonal antibody to two sources of labeled monoclonal antibody. Skunk rabies entered the state in 1984 along the Powder River, a tributary to the Missouri River drainage into Montana. We tend to refer to river drainages when talking about skunk rabies because skunks prefer habitat found along rivers, streams or water sources. Rabies spread through skunks in the northeast part of the state and included animals along the Powder, Tongue and the Belle Fourche rivers. The number of skunk rabies case has been somewhat cyclic with as many as 221 cases in 1986, 1 in 2003 and 15 in 2005. In the early 1990s we had cases appear in the Big Horn River basin which also drains into the Missouri but separated from the original outbreak by the Big Horn Mountains. Rabies spread south but did not extend past the Wind River canyon, which has marginal skunk habitat. In 1999 we had cases south of that canyon which spread through the Riverton area and on south to Lander. In late 2000 we had cases appear further south in the Farson area which lies on a tributary to the Green River. This is important because the Green River drains into the Colorado River which has never had rabies established in its skunk population. After a very short burst of cases limited to a very small area there have been no additional cases of rabies around Farson. As mentioned, we currently have very few positive skunks in Wyoming and diagnose less than 6 rabid bats each year. Bat rabies can be transmitted to terrestrial species but generally does not establish a perpetuating cycle. Over the space of 20+ years we have had a few cases of rabies in skunks in isolated areas far away from any other cases but in each of these situations the virus was found to be of bat origin and did not establish itself in skunks. It has been generally believed that once rabies has infected a population of skunks it will be maintained at some cyclic level. This does not seem to be the situation in Wyoming in that skunk rabies seems to have completely disappeared from areas of the state.

Evaluation of toxicity of *Adonis aestivalis* in calves

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Toxicosis of *Adonis aestivalis* is well documented in horses but adonis toxicity is unknown in cattle. In fact, cattle have been suggested as an alternate consumer of adonis-contaminated hay based on incomplete information obtained in a study performed in 1942.

Adonis aestivalis (summer pheasant's eye) was fed to six calves in acute, high dose and chronic, low-to-high dose feeding trials in order to establish the toxicity of *Adonis aestivalis* in cattle. Four 300 lb Holstein and two 90 lb, pre-ruminating Jersey calves were administered 1% body weight of ground *Adonis aestivalis* via a stomach tube and monitored for clinical signs for two weeks and one week, respectively. The Holstein calves were then fed 0.2-1% body weight *Adonis aestivalis* either ground or as a 50% adonis:alfalfa pellet daily for 4-5 weeks. The Holstein calves exhibited no clinical signs during the feeding trial. No gross or microscopic lesions were seen on necropsies performed at the end of the study. The 90 lb, pre-ruminating Jersey calves developed diarrhea 2-3 days post-dosing and mild signs of cardiac disease, both of which were transient and had no gross or microscopic lesions on necropsies performed 1 week post-dosing.

Based on this trial, cattle do not appear to be susceptible to toxicosis from Adonis aestivalis when fed up to 1% body weight daily for up to 5 weeks.

Vetch Poisoning in adult Angus cows

L. W. Woods, D. M. Woods

An 880 lb. adult Angus cow was submitted for necropsy to the California Animal Health and Food Safety Laboratory System (CAHFS), Davis. Eight cows died within a week. All cows were on free range pasture and all cows affected had calves. Heifers on the same range were not affected and bulls on an adjoining pasture were not affected. Clinical signs which included dehydration, weight loss and alopecia over the head, neck, trunk and limbs developed over a week and all cows that developed clinical signs died. Histopathologic lesions included multifocal, eosinophilic and lymphohistiocytic myocarditis; multifocal eosinophilic, lymphocytic and granulomatous interstitial nephritis; severe, diffuse, lymphocytic and granulocytic adrenalitis; and moderate diffuse, lymphohistiocytic and eosinophilic dermatitis. Hepatitis, interstitial pneumonia, enterocolitis and splenitis were also seen on microscopic examination of tissues. A diagnosis of vetch poisoning was made based on histopathology and history of exposure to vetch pasture.

In 2000, a cluster of three separate cases of vetch poisoning in cattle was diagnosed at CAHFS. Vetch (*Vicia benghalensis*) from the associated pastures in three different regions of northern California was collected and examined for plant diseases. A plant disease,

leaf spot/stem spot of vetch was diagnosed on the vetch and the etiologic agent, *Ovularia* sp. was isolated from the vetch associated with lesions on the stems and leaves. In the current case, the plant disease was identified in the pasture with the cows but not the bulls. *Ovularia* was grown on plates and then inoculated onto grain. Inoculated grain and alfalfa hay was then fed to two adult beef cows that had been exposed to vetch pasture the previous year over a 24 day period. No clinical signs developed.

The pathogenesis of vetch poisoning is unknown. The reason why vetch that is commonly used as forage becomes poisonous is unknown. The inflammation associated with vetch poisoning suggests animals develop a hypersensitivity reaction but it is apparently not an individual animal hypersensitivity since multiple animals are typically affected and clusters of cases occur certain years with no apparent cases other years. In California, clusters of cases were diagnosed in 1990, 2000, 2005 and 2006. The epidemiology suggests environmental conditions may play a major role, and environmental conditions can be highly tied to plant diseases. Leaf spot/stem spot of vetch caused by *Ovularia* has been identified in many of the outbreaks seen in California. Cows fed *Ovularia*-inoculated grain did not develop clinical signs but as in some other plant toxicoses, the poisoning may occur as a result of response of the plant to the plant fungus. Further studies are needed to determine if *Ovularia*-infected vetch will elicit clinical signs and lesions in cattle.

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