Colorado State University Veterinary Diagnostic Laboratories

LAB LINES

Diagnostic news and trends from the Colorado State University Veterinary Diagnostic Laboratories
Volume 15, Number 2 Fall/Winter 2010

Guardians of Public Health

CSU VDL now houses wild bird influenza tissue reference archive

The Wildlife Services' National Wildlife Disease Program Wild Bird Tissue Reference Archive was initiated in 2006 as a partnership between USDA's Animal and Plant Health Inspection Services' Wildlife Services and the National Animal Health Laboratory Network (NAHLN). The archive consists of swab samples collected for avian influenza surveillance, and has grown to be a valuable repository now housing over 250,000 samples. This collaboration has been a critical piece of Wildlife Services' effort to establish a network for early detection of highly pathogenic avian influenza (HPAI) in the United States. While highly pathogenic H5N1 avian influenza has not been found in North America, many of the samples have been critical in characterizing the distribution and movement of low pathogenic avian influenza in North American wild birds, and in assay development.

The Wild Bird Tissue Reference Archive is open and accessible to other agencies, universities, and organizations. We believe it will prove to be an invaluable resource for a variety of studies including: Avian Influenza-related research, assay validation, emerging disease diagnostics, and retrospective disease studies. The archive was originally located at the National Wildlife Disease Program offices in Fort Collins, Colo. During the summer of 2010, the collection was relocated to the CSU VDL. The archive is jointly administered through a cooperative agreement between Wildlife Services and Colorado State University.

The CSU VDL is excited to house this valuable archive. We are already collaborating with the National Wildlife Disease Program on a research project focused on isolating and sequencing avian influenza viruses from these samples. ▲

— John Baroch, Wildlife Disease Biologist, USDA/APHIS/Wildlife Services, National Wildlife Disease Program; and Kristy Pabilonia, DVM, DACVM, CSU VDL Avian Diagnostics and BSL3 Operations Section Head

Inquiries and proposals for research using the collection are invited. To inquire about sample loans or place a request, please contact the archive supervisors: John Baroch at John.A.Baroch@aphis.usda.gov or Kristy Pabilonia at Kristy.Pabilonia@colostate.edu

YEAR END CONTRIBUTIONS WELCOME

Please visit our website and consider a donation to our foundation to allow us to buy new or replace old equipment, perform in-depth disease investigations of unusual cases or enhance our ability to educate the next generation of veterinary laboratory diagnosticians. www.dlab.colostate.edu

Case Study

An outbreak of aborted and weak calves associated with leptospirosis

Case information provided by the owner, veterinarian and the CSU VDL system helps provide relevant testing results and potential conclusions that might be drawn from the available data.

A well managed, mixed breed beef herd began experiencing early parturition. Calving began in the first week of March, 30 days prior to the earliest potential calving date established by bull exposure. During this time the mountain ranch received multiple wet snows that melted, resulting in standing water where cattle where kept. Calves were weak. In spite of nursing attempts, including oral electrolyte supplements, antibiotics and anti-inflammatories, 16 had died by mid-March. Most died within a few hours of birth and never rose to nurse. Death didn't appear to correlate with age of the mother; half were from heifers and half from cows.

NECROPSY AND OTHER OBSERVATIONS

After consultation with his herd veterinarian, the owner submitted a whole calf to the CSU VDL at Rocky Ford. Gross necropsy provided no insight into the cause of death nor the herd problem. Observations included:

- The calf's lungs were inflated, indicating live birth
- The gut contained a commercial electrolyte and glucose drench; however, no colostrum/milk was noted.
- Vaccine history indicated a well-vaccinated herd with repeated doses of MLV viral products, the last administered in November. *Campylobacter* and *Leptospira* vaccinations were given with the MLV product. Mid-January a multivalent killed vaccine for *Rota-Corona* viruses, *E. coli* and *Clostridium* type C and D toxoid was given to the pregnant animals.
- Animals were fed bailed native mountain meadow hay, supplied well water in a tank, and provided a commercial mineral supplement.

DIAGNOSTIC FINDINGS

- Feed and water analysis indicated normal ranges with no indications of toxins.

— *Jim Kennedy, DVM, MS, Director, CSU VDL Rocky Ford Branch.*

- Bacterial cultures from tissues submitted provided no significant specific growth.
- FA results for IBR and BVD were negative on the initial submission; however, a positive BVD FA was found on the Pfizer representative's submission. The presence of the virus could not be confirmed on PCR, though.
- Initial blood samples drawn from four affected cows on March 15 were paired with follow-up samples on the same cows collected on March 30. Single blood samples were also collected on March 21 with no paired samples. Samples from the initial collection were saved and tested in parallel with the convalescent samples (see table).
- Fecal examinations indicated a significant internal parasitic load in the samples submitted to the Rocky Ford Diagnostic Laboratory, while samples submitted to the Grand Junction Diagnostic Laboratory showed only a slight level of parasitism.

> ***'Results do not indicate any clear single cause for the problem. There are likely several factors contributing to the premature calves'***

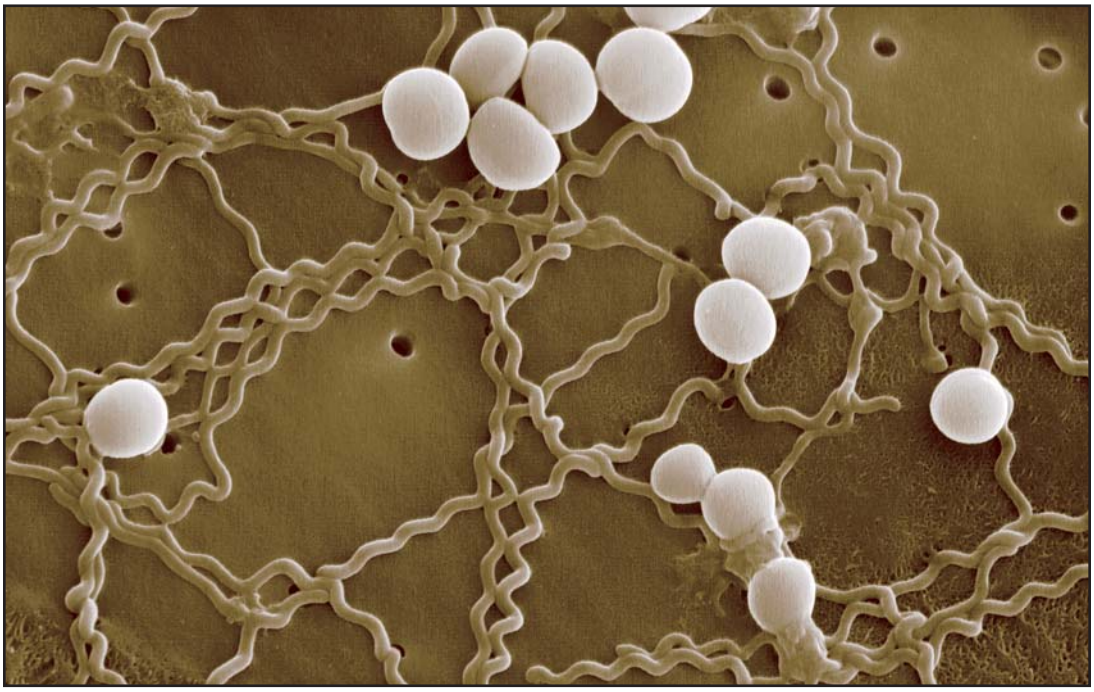
Based on laboratory results, a recommendation to de-worm the cattle and administer a sustained-release tetracycline to all cows and heifers was made to the owner. The owner agreed. Additionally it was suggested the owner provide an additional source of protein, which he did via incorporating a pelleted feed into his program.

At the time the treatment/prevention was carried out, a pharmaceutical-company representative collected additional samples and hand-carried them to the CSU Veterinary Diagnostic Laboratory at Grand Junction. The results of diagnostic studies are summarized from the initial report on that submission: "Laboratory results do not indicate any clear single cause for the problem observed on this ranch. There are likely several factors contributing to the premature and weak calves."

After further testing, an addendum read, "The relatively high titers in these three cows suggest fairly recent exposure to leptospiral antigens, probably through nat-

REFERENCE

1. Grooms DL, Bolin CA. Diagnosis of Fetal Loss Caused by Bovine Viral Diarrhea Virus and Leptospira spp. *Vet Clin North Am Food Anim Pract*. 2005 Jul;21(2):463-72.



© MicroAngela. Used by permission.

ural exposure." No follow-up diagnostics have been requested or completed.

DIAGNOSTIC INTERPRETATIONS OF RESULTS

With the exception of the FA positive for BVD and serological results, no diagnostic test confirms a specific etiological agent as the cause of this herd's weak calf/abortion storm. The FA positive BVD was not supported by PCR or by tissues from multiple calves. Serological evidence for BVD or IBR also does not support either of these two etiological agents, but indicates a well-vaccinated animal, as IBR and BVD titers are found in vaccinated cattle for extended periods when recommended vaccinations are carried out per label instructions. However, the titers for the various serovars of *Leptospira* are significant.

Titers associated with *Leptospira* vaccines are short term and do not reach levels seen in these serum samples.[1] In this case, multiple cows exhibited titers above 1:400, with the highest titer reported at 1:6400. These levels were seen with four-fold variations in acute and convalescent samples. Although no urine samples were collected from cows for culture or PCR for *Leptospira,* one kidney was tested by PCR, which failed to detect the organism. A second sample is being held for follow-up testing, although the need to confirm the etiological agent by culture or PCR is questionable based on the overwhelming serological evidence. Treatment with sustained-release tetracycline resulted as you would expect with *Leptospiral* infection. Little data support the effectiveness of *Leptospira* vaccines as being preventive for disease, in spite of widely accepted use. The most likely serovars associated with this herd problem are uncertain, although Pomona and Grippotyphosa did show consistent increased titers and are associated with the clinical history of abortion storms. ▲

SUBMISSION GUIDELINES FOR SUCCESS

Bovine Abortion Screen

- ❑ Submit fresh fetal lung, liver, kidney, spleen, stomach content, placenta, eyeball
- ❑ Submit fixed lung, liver, kidney, heart, thymus, spleen, brain, placenta
- ❑ Submit both acute and convalescent dam sera
- ❑ Whirlpak for fresh tissues; 10 percent buffered formalin for histopathology
- ❑ RTT for sera
- ❑ Refrigerate fresh tissue and sera
- ❑ Submit Monday through Friday
- ❑ Allow four days for results

Bovine Abortion Screen with Necropsy

- ❑ Submit whole animal with placenta
- ❑ Refrigerate
- ❑ Submit Monday through Friday and Saturday and Sunday mornings
- ❑ Allow five to seven days for results

Cow ID	LEPTO HARDJO	LEPTO ICTERO	LEPTO CANICOLA	LEPTO GRIPPO	LEPTO POMONA	BVD II	BVD	IBR
3030 ACUTE	1:200	Negative	1:400	1:800	1:1600	1:1024	1:1024	1:4096
3030 CONV	1:400	1:400	1:800	1:800	1:6400	1:1024	1:512	1:2048
7045 ACUTE	1:200	Negative	1:100	1:200	1:200	1:8192	1:8192	1:4096
7045 CONV	1:200	1:200	1:400	1:800	1:1600	1:16384	1:4096	1:4096
8007 ACUTE	1:200	1:200	1:800	1:1600	1:3200	1:1024	1:2048	1:4096
8007 CONV	1:1600	1:400	1:1600	1:1600	1:6400	1:512	1:4096	1:4096
8013 ACUTE	Negative	Negative	1:100	1:100	1:100	1:4096	1:512	1:4096
8013 CONV	Negative	1:100	1:200	1:100	1:200	?	1:8192	1:2048

Four-fold variation

Bacteriology Quality Assurance

Urine cultures: Are results time- or volume-dependant? Which is better?

— *Mike M. Russell, BS, bacteriology technician, and Doreene R. Hyatt, PhD, CSU VDL Bacteriology Section Head*

Any sample being submitted for culture should optimally be received as soon as possible; unfortunately, samples can't always reach the lab as quickly as we would like. Therefore, a common concern is urine stability. Clients sometimes worry their sample wasn't submitted in time or that the sample sat in the refrigerator for too long. Should they?

We performed a small investigation by re-culturing 17 urines, received at different times during a 10-day period, that had bacterial growth. The amount of time elapsed before re-culturing ranged from six days to 14 days. All samples were held at 39º F.

Re-culture showed 14 (83 percent) had the same organism(s) with the same amount of growth — even one that was two weeks old. Three (17 percent) had no growth. A possible explanation for that lack of growth could be that two of the three urines had only 0.25 and 0.5 mL submitted. We recommend sending at least 1 mL if possible. The third urine had such a small amount of growth the first time that perhaps the organism was unable to be detected because of low numbers in the second culture.

Based on this small-scale study, it may be fairly safe to assume that as long as you send enough sample and keep it refrigerated there shouldn't be a major effect on the results if the urine is held over the weekend. ▲

Amount Submitted	Species	Date Collected	Date Received	First Culture	Second Culture
<0.25 ml	Canine	7/13/2010	7/13/2010	*Enterococcus* sp. 20,000 cfu/ml	No growth 14 days
≈0.25ml	Canine	7/14/2010	7/15/2010	*E. coli* 500 cfu/ml 500 cfu/ml	Same 13 days
≈0.3 ml	Canine	7/15/2010	7/15/2010	*E. coli* >100,000 cfu/ml	Same 12 days
≈0.3 ml	Canine	7/14/2010	7/16/2010	*E. coli* >100,000 cfu/ml *Proteus mirabilis* 1,000-10,000 cfu/ml	Same 13 days
0.5 ml	Canine	7/13/2010	7/13/2010	*Staph. pseudintermedius* >100,000 cfu/ml	Same 14 days
≈0.5 ml	Canine	7/16/2010	7/16/2010	*Klebsiella pneumoniae* 1,000-10,000 cfu/ml	No growth 11 days
≈0.5 ml	Canine	7/19/2010	7/20/2010	Hemolytic *E. coli* 20,000 cfu/ml	Same 8 days
≈0.5 ml	Canine	7/20/2010	7/20/2010	*Enterococcus* >100,000 cfu/ml	Same 7 days
≈0.6 ml	Canine	7/20/2010	7/20/2010	*E. coli* >100,000 cfu/ml	Same 7 days
≈0.75 ml	Feline	7/15/2010	7/16/2010	*Enterococcus* sp. >100,000 cfu/ml	Same 12 days
≈0.8 ml	Feline	7/19/2010	7/21/2010	Hemolytic *E. coli* >100,000 cfu/ml	Same 8 days
≈1 ml	Canine	7/21/2010	7/22/2010	*E. coli* and Beta *Streptococcus* 300 cfu/ml	Same 6 days
1.5 ml	Feline	7/15/2010	7/16/2010	*Corynebacterium* sp. and CNS <1,000 cfu/ml	No growth 12 days
≈1.5 ml	Canine	7/19/2010	7/20/2010	*Staph. pseudintermedius* >100,000 cfu/ml	Same 8 days
≈2.5 ml	Canine	7/16/2010	7/19/2010	*Klebsiella pneumoniae* >100,000 cfu/ml	Same 11 days
≈2.5 ml	Canine	7/19/2010	7/20/2010	*Enterococcus* sp. 20,000 cfu/ml	Same 8 days
≈3.5 ml	Canine	7/13/2010	7/19/2010	*Acinetobacter* sp. 10,000-100,000 cfu/ml	Same 14 days

SUBMISSION GUIDELINES FOR SUCCESS

Urine Cultures

- ❑ Submit at least 1 ml in sterile container
- ❑ Refrigerate, unless using port-a-cul for transport media
- ❑ Deadline 4:30 p.m. weekdays, noon weekends
- ❑ Allow three to five days

CONCLUSION

In our small-scale study, we found no effect on delaying culture for samples stored at 39° F for most of the samples tested.

Canine Oncology Innovations

Expression of HES-1 in canine osteosarcoma

HES-1, a basic helix-loop-helix (bHLH) transcriptional repressor, is a downstream target of the Notch signaling pathway. Additionally, Notch-independent HES-1 expression has been reported in some human tissues. Notch signaling and HES-1 expression have been linked to growth and survival in a variety of human cancer types. Increased expression of HES-1 has been shown to be associated with increased metastasis and invasiveness in human osteosarcoma. Objectives for this study included confirmation and exploration of HES-1 expression in canine osteosarcoma (OSA).

Quantitative RT-PCR was utilized to quantify HES-1 gene expression in tumor and normal bone samples taken from dogs treated for appendicular OSA with surgical amputation of the affected limb and adjuvant chemotherapy. HES-1 gene expression was elevated in tumor samples relative to matched normal bone, but decreased in tumor samples from dogs with a disease free interval (DFI) of less than 100 days relative to those with a DFI of greater than 300 days.

FOCUS ON PCR

Immunohistochemistry was utilized to confirm translation of mRNA and expression of HES-1 protein in a subset of the same tumors analyzed by RT-PCR. Protein expression of HES-1 varied across tumors and within individual tumors, with neoplastic cells showing predominantly nuclear and less frequently diffuse cytoplasmic immunostaining. Immunostaining appeared to correlate with quantitative RT-PCR results. Changes in HES-1 gene and protein expression within these tumor samples suggest that alterations in the Notch signaling pathway occur in canine OSA. Furthermore, an inverse relationship of HES-1 expression and DFI warrants additional exploration of the correlation of HES-1 expression with patient survival in canine OSA. ▲

— *Deanna D. Dailey, DVM, VDL Pathology Resident; Liza O'Donoghue, CSU Graduate Student; Kristin P Anfinsen, Norwegian School of Veterinary Science; Dawn Duval, PhD, CSU Assistant Professor of Cancer Genomics; EJ Ehrhart, DVM, PhD, DACVP, VDL Pathologist; and Brad Charles, MS, Research Associate.*

PRESENTED AT

American College of Veterinary Pathologists™

Diagnostic Bacteriology

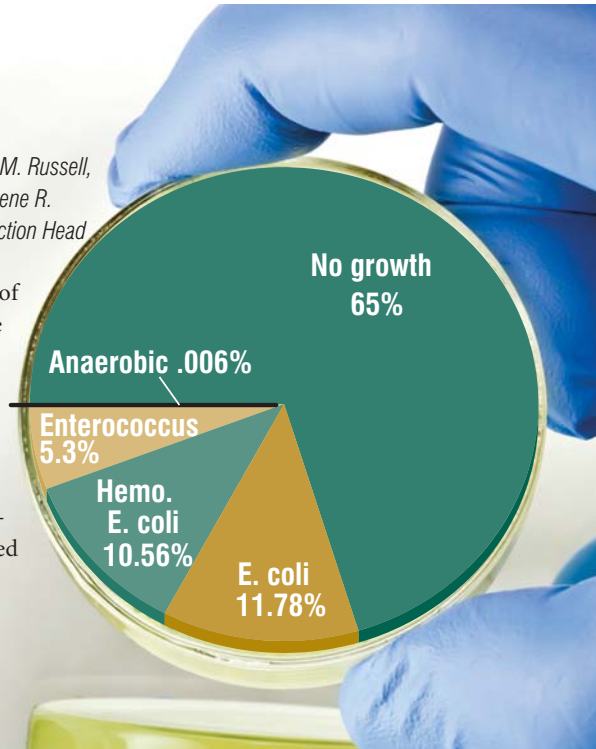
Should you request anaerobic cultures on urine?

Urinary tract infections pose a significant risk to animals, resulting in inappropriate urination, bladder stones or acute cystitis. So urine cultures are commonly performed.

We retrospectively studied all urine cultures submitted to the CSU VDL between January 2004 and December 2009. The 10,901 urine culture samples were submitted from bovines, equines, canines and felines.

Results showed more than 65 percent of urine samples were reported as no growth. The most common organisms isolated were *E. coli* (11.78%), hemolytic *E. coli* (10.56%), and *Enterococcus* spp. (5.3%). Although anaerobic cultures were requested on 1187 of the urines received by the VDL, only 23 urines had anaerobes isolated (0.006% of all bacteria isolated from urines).

At the VDL, anaerobic cultures of urine are requested and there are concerns as to whether this is a relevant test. This retrospective study clearly illustrates that the recovery of anaerobes from urine is very low, and a specific set of criteria should be developed to determine when urine should be cultured anaerobically. ▲



— *Denise S. Bolte, Microbiologist, Mike M. Russell, BS, bacteriology technician, and Doreene R. Hyatt, PhD, CSU VDL Bacteriology Section Head*

Bovine Virology

BVD Persistent Infection: Status and implications for control

FOR INFORMATION

For details on the Colorado voluntary BVD-control program offered in conjunction with CSU, visit our site at www.dlab.colostate.edu and click on the BVD Control Program button.

Data collected from recent surveys of beef cattle indicate the prevalence of BVD Persistently Infected (BVD-PI) animals in the U.S. cattle population is less than or equal to 0.3 percent. The prevalence of BVD-PI-infected beef herds, as defined by any PI animal detection within the herd is estimated at 4 percent for beef cattle. The prevalence of BVDV-infected dairy herds ranges from 15 percent, based on BVD-PI cattle detected in Michigan herds, to 1.7 percent in a national survey based on detecting BVD virus by reverse transcriptase-polymerase chain reactionin bulk milk samples. In that report, the percent of BVD positive dairy herds ranged from 0 percent in herds with less than 100 cows to 12.8 percent in herds with 500 cows.

Although the apparent prevalence of BVD-PI cattle is low in U.S. dairies, beef herds and feedlots, diagnosis by PI-animal detection is likely to be an underestimate of true herd prevalence. Some BVDV-infected beef herds may be misclassified as uninfected herds if there is no PI animal alive at the time of testing.

OBSTACLES TO CONTROL

Unlike several European countries, which are moving toward mandatory, systematic BVD control programs, the concept of BVD control by eradication has been slow to find acceptance in the United States.

Impediments include reluctance to institute a government-regulated control program, available data indicating a low prevalence of BVDV infection in beef and dairy herds, and uncertainty on the part of individual producers about the economic benefits of BVD-control. The lack of a clear danger is compounded by the "Gambler's" mentality among individual cattle producers. The low herd prevalence validates the general belief that the application of BVDV vaccines is

— Hana Van Campen, DVM,PhD,DACVM, CSU VDL Virology Section Head

A SENSITIVE BVD-PI BLOOD MARKER?

CSU VDL Virology Section Head Hana Van Campen collaborated on a study with Thomas Hansen and others from CSU's Animal Reproduction and Biotechnology Lab which infected naive pregnant heifers with noncyotpathic BVD Type 2 on day 75 to induce persistent infection. They found gene expression differed in maternal blood cells in the presence of PI vs. uninfected fetuses. PI adversely affected fetal development and antiviral responses, despite protective immune responses in the dam. Fetal PI with BVDV alters maternal immune function, compromises fetal growth and immune responses, and results in expression of maternal blood biomarkers that can potentially be used to identify cows carrying PI fetuses.

WHAT DOES THE AVERAGE BEEF CATTLE PRODUCER BELIEVE ABOUT BVD?

The USDA National Animal Health Monitoring System's latest *Prevalence and Control of Bovine Viral Diarrhea Virus on U.S. Cow-calf Operations, 2007-08* excerpts BVD-related questions from the third national NAHMS study of the U.S. beef cow/calf industry. The 24-state survey showed:

- **Only 12.3 percent of producers had never heard of BVD.**
- **Only 4.2 percent of operations had tested any calves for BVD Persistent Infection in the previous three years.**
- **Overall, 8.8 percent of operations had a BVD-PI calf; 0.12 percent of calves tested were BVD-PI positive.**
- **Overall, 46.6 percent of producers were unsure if removing calves that tested PI positive would affect the value of the remaining calves in the herd.**
- **Just more than 33 percent of operations vaccinated calves against BVD virus at 22 days through weaning; 25.1 percent vaccinated weaned replacement heifers through breeding; and 28.1 percent vaccinated cows.**
- **Of operations that vaccinated against BVDV, more than eight in 10 used a vaccine that included both type 1 and type 2 BVDV on all cattle groups vaccinated.**

SOURCES

Van Campen H. Epidemiology and control of BVD in the U.S. *Vet Microbiol*. 2010 Apr 21;142 (1-2):94-8.

Hansen TR, Smirnova NP, Van Campen H, Shoemaker ML, Ptitsyn AA, Bielefeldt-Ohmann H. Maternal and fetal response to fetal persistent infection with bovine viral diarrhea virus. *Am J Reprod Immunol*. 2010 Oct;64(4):295-306.

a "cure-all" rather than an aid in prevention of BVD infections. However, producers and veterinarians who have experienced losses due to BVDV-associated diseases are more motivated to adopt preventive measures. Producer and veterinarians' awareness of the potential impact is the impetus behind the BVDV testing recently adopted by bull sales, purebred stock sales, livestock shows and specific feedlots. The proliferation of BVD control and eradication programs in the United States is encouraging, and it highlights recognition of BVDV's importance to cattle health. ▲

Test	Submission	Container	Coolant	Deadline	Turn	Results as	Cost
Serology Type I and II	1 ml serum	RTT	Refrigerate	Mon and Thurs by 5 p.m.	4 days	SN test. Titers reported and interpreted	$7
Flourescent Antibody	Fresh lung, liver, kidney, spleen, lymph node, small intestine	Whirlpak	Refrigerate	Mon-Thurs by 5 p.m.	24 Hrs	Positive or negative	$6
Virus Isolation	Various tissues listed above, semen	Tissues in whirlpak/ semen straw in LN2 or dry ice	Refrigerate; semen in LN2 Dewar or dry ice	Tues. by noon	1-3 wks	Virus(es) are identified	$30
Antigen Capture Elisa	1 ml serum/ ear notch	RTT; RTT with 2 ml PBS	Refrigerate	Wed by noon	Same day	Positive or negative	$4-$7
PCR Type I and II	Tissues listed under FA and/or ear notch or PTT	Whirlpak	Refrigerate	Mon-Thurs by 5 p.m.	4 days	If positive, typing; positive pools/ individuals	$30; $75 Pool ≤50*
Bulk Tank Milk PCR	600 cc bulk tank	Screw cap bottle	Refrigerate	Mon-Thurs by 5 p.m.	1 week	Positive or negative. No typing	$36

** Pools more than 50 = $1.50 per sample. Pool testing done at Rocky Ford Lab only.*

Oncology Interventions

Clinical trials now underway at the Animal Cancer Center

Maintenance Therapy with Palladia Following Doxorubicin-based Chemotherapy for Canine Splenic Hemangiosarcoma

The purpose of this study is to evaluate the benefit of Palladia (toceranib phosphate) for the treatment of splenic hemangiosarcoma in dogs. In order to be eligible, dogs must have a diagnosis of splenic hemangiosarcoma, have undergone splenectomy, and have received five doses of single-agent Doxorubicin (one treatment every two weeks). Two weeks following the last dose of Doxorubicin, full staging is performed (thoracic radiographs, abdominal ultrasound), and if no evidence of metastasis is found, dogs are eligible to start treatment with Palladia. The study will cover the costs of Palladia, bloodwork, and restaging once enrolled.

Stereotactic Radiation Therapy for Feline Oral Squamous Cell Carcinoma (SCC)

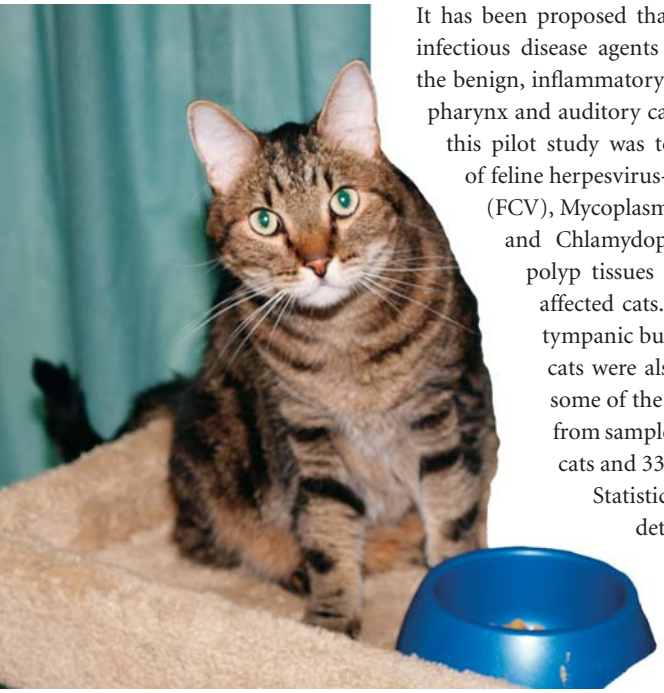
This study is designed to evaluate the benefit of stereotactic radiation therapy in the treatment of cats with oral SCC. A biopsy-confirmed diagnosis of squamous cell carcinoma is required, as well as appropriate staging tests (bloodwork, urinalysis, thoracic radiographs). A PET-CT will be obtained to determine the extent of the tumor and to generate a computerized treatment plan for radiation therapy. One dose of stereotactic radiation therapy will be delivered to the tumor. Measurements of tumor oxygen levels will be taken using oxygen probes prior to and the day following treatment. A brief recheck evaluation is required two weeks after treatment, and a second PET-CT will be performed four weeks following treatment. The client is required to contribute $1000 towards staging and treatment; the remainder of the costs will be covered by the study.

We also have several clinical trials available for dogs with osteosarcoma, soft tissue sarcoma, and lymphoma. For more information, a list of all currently available clinical trials, and contact information, please visit us at www.CSUAnimalCancerCenter.org.

CSU VDL In Press

A roundup of VDL faculty research

Klose TC, MacPhail CM, Schultheiss PC, Rosychuk RA, Hawley JR, Lappin MR. Prevalence of select infectious agents in inflammatory aural and nasopharyngeal polyps from client-owned cats. *J Feline Med Surg*. 2010 Oct;12(10):769-74.



It has been proposed that inflammation induced by infectious disease agents could trigger formation of the benign, inflammatory polyps that affect the nasopharynx and auditory canal of cats. The objective of this pilot study was to determine the prevalence of feline herpesvirus-1 (FHV-1), feline calicivirus (FCV), Mycoplasma species, Bartonella species and Chlamydophila felis nucleic acids in polyp tissues collected from 30 clinically affected cats. Samples collected from the tympanic bulla from 12 clinically normal cats were also assayed. DNA or RNA of some of the target agents were amplified from samples from 25 percent of normal cats and 33 percent of affected cats. Statistical associations were not detected for individual agent results or grouped results. The study documents that common oropharyngeal or blood borne agents can be detected in the tympanic bullae of normal cats; however, failure to consistently amplify RNA or DNA of the select agents from polyp tissues suggests the agents studied were not directly associated with the pathogenesis of this syndrome in the cats tested. Alternately, the inflammatory response may have cleared microbial nucleic acids to undetectable levels by the time of sample collection.

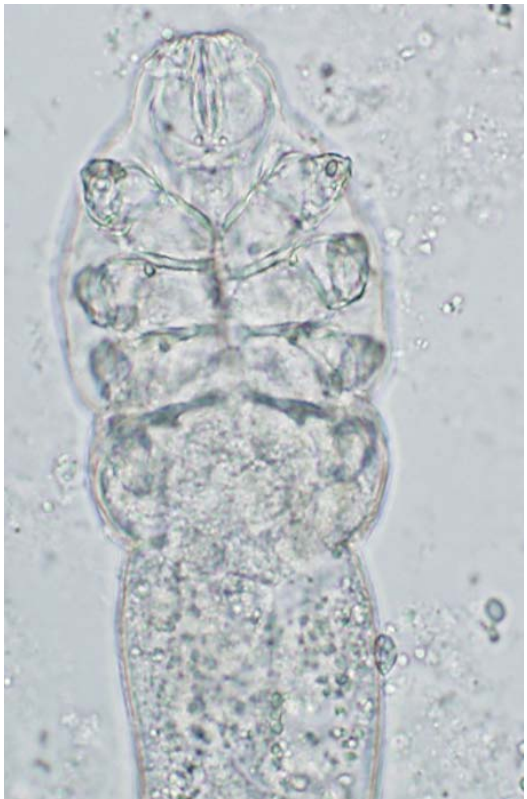
Desch CE, Andrews JJ, Baeten LA, Holder Z, Powers JG, Weber D, Ballweber LR. New records of hair follicle mites (Demodecidae) from North American Cervidae. J Wildl Dis. 2010 Apr;46(2):585-90.

Individuals of three species of cervids, with varying degrees of alopecia, were examined for ectoparasites: Rocky Mountain elk (*Cervus elaphus nelsoni*) and mule deer (*Odocoileus hemionus hemionus*) in Colorado and white-tailed deer (*Odocoileus virginianus*) in South Dakota. Hair follicle mites were recovered and identified as *Demodex kutzeri*, a species originally described from the European red deer (*Cervus elaphus*, from Austria) and the sika deer (*Cervus nippon pseudaxis*, captive in Germany).

These findings expand the geographic range of *D. kutzeri* to North America and extend its host range to include the genus *Odocoileus*. Thus, the host range for *D. kutzeri* spans two subfamilies of cervids. Additionally, *D. kutzeri* was identified in material from a white-tailed deer collected in South Carolina in 1971, indicating this parasite has been present, but unrecognized, on U.S. cervids for some time.

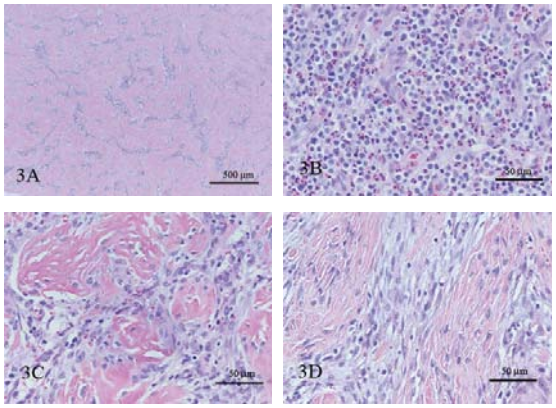
Halsey CH, Powers BE, Kamstock DA. Feline intestinal sclerosing mast cell tumour: 50 cases (1997-2008).Vet Comp Oncol. 2010 Mar;8(1):72-9.

This case series presents a unique and unreported variant of feline intestinal mast cell tumour recognized at the CSU VDL. Fifty cases of feline intestinal mast cell tumours described as having a significant stromal component were reviewed. Neoplastic cells formed a trabecular pattern admixed with moderate to abundant dense stromal collagen (sclerosis). Neoplastic cells had poorly discernible intracytoplasmic granules which demonstrated metachromasia with special histochemical stains consistent with mast cell granules. Additionally, a subset of cases stained for mast cell-specific tryptase and c-kit demonstrated positive immunoreac-



tivity. Eosinophilic infiltrates were moderate to marked in almost all cases. Lymph node and hepatic metastases were present in 66 percent of the cases.

In the 25 of 50 cases where treatment and clinical outcome was available, 23 of the patients died or were euthanized within two months of initial diagnosis. This is the first case series to characterize a sclerosing variant of intestinal mast cell tumour in the cat which appears to have a high propensity for metastasis and a guarded prognosis.



CSU VDL's previously unreported variant of feline intestinal mast cell tumours showed (clockwise from top left) the sclerosing component comprised at least 30 percent of the tumour in most cases, neoplastic cell morphology ranged from round, to polygonal, to spindle-shaped. A moderate to marked eosinophilic infiltrate was identified in 82 percent of the cases.

Lab Updates

To err is human; to really foul things up requires a computer

Barbara Powers, DVM/PhD/DACVP, CSUVDL Director

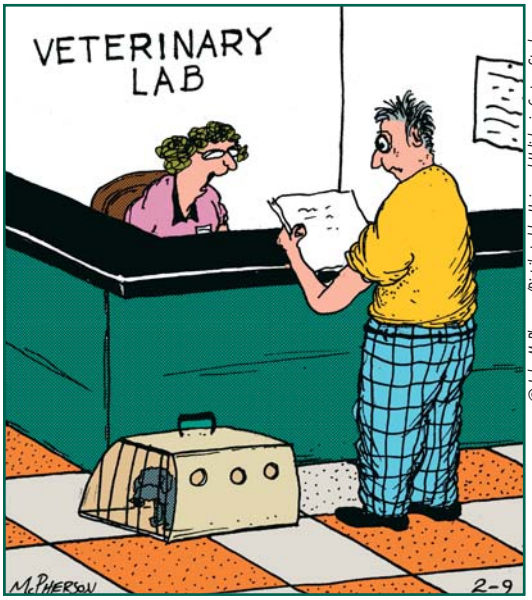
You have heard that old saying, "Change is never easy." Truer words were never spoken. On July 1, we implemented a new Laboratory Information Management System (LIMS). Despite what we thought was adequate preparation, soon we discovered many glitches that made it difficult for us to communicate with you, especially affecting invoicing.

The new system actually takes us longer to enter cases and send results, so while we could perform tests accurately, results were delayed. Then, the new system did not sync well with the fax system, so when we thought we sent results, they sometimes disappeared into cyber space. We have corrected these issues and are working on the results-page format to be more easily interpreted.

One of the biggest glitches was on the invoicing system. At first, it did not work well at all. Then, it had issues interfacing with the university. Now, it is difficult to make adjustments. This caused marked delay in billing. Then, it took us a while to get caught up with the backlog. These invoicing issues are now almost all resolved, we have a new format that is easier to interpret and we are nearly all caught up with sending invoices.

Finally, web access was interrupted for a few weeks, and while now available, doesn't have all the previous features, which will be returned and enhanced.

You may wonder why we embarked on this new system. Our old system was over 20 years old, "homegrown," poorly-documented and simply too old to handle the volume of information we need to operate. An upgrade was a requirement of the last audits by the American Association of Veterinary Laboratory Diagnosticians, as well as by the university system. The switchover certainly did not go as well as we hoped and we sincerely apologize for the inconvenience we caused.



"I'm sorry, does that say $777? It should be $111. Our new system mistakenly calculated your bill in dog dollars."

We realize this was difficult for you and greatly appreciate your patience.

We are continuing to modify the new system so it will be better than the old system. This requires time and interaction with the corporation that designed the system. Already, significant progress has been made, and we will continue to make more. We also appreciate the feedback many of you have given us to make the system more useful for you. We greatly appreciate your patience and apologize for the inconvenience we caused. ▲

IF YOU HAVE A COMPUTER-RELATED QUESTION, COMMENT OR COMPLAINT
Please don't hesitate to contact us:
Colorado State University
Diagnostic Laboratories
300 West Drake
Fort Collins, CO 80523
Phone (970) 297-1281
Fax (970) 297-0320
Email:
dlab@colostate.edu

E-services and other technology

Four Steps to Automate and Speed your Coggins Test Results

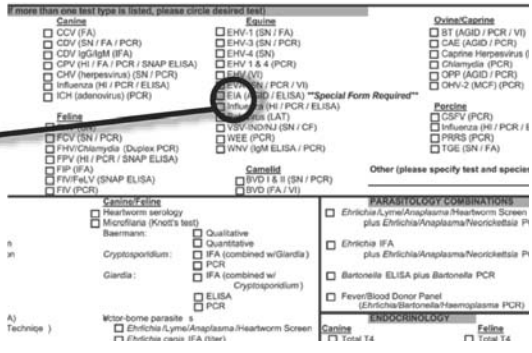
The Fort Collins and Rocky Ford laboratories now are approved to receive submissions of Equine Infectious Anemia (Coggins) test forms and report the results back to veterinarians electronically. Follow these four steps to begin submitting samples electronically:

– Hana Van Campen, DVM, PhD, DACVM, CSU VDL Virology Section Head

1 Obtain level 1 e-authentication by applying for a user ID and a password at: https://vsps.aphis.usda.gov/vsps/

2 After you have received approval for your user ID and password, then you may submit test requests though the following Web site: https://vsps.aphis.usda.gov/vsps/public/Login.do. Through this Web site, you can set up your name and address, a list of animals with owner addresses, and digital photos of the animals if desired. The electronic eEIA form is filled out automatically and sent electronically to us by selecting "Veterinary Teaching Hospital - Ft. Collins" toward the bottom of the pull-down list of laboratories.

3 Serum samples should be sent to us as you usually do with a regular accession form indicating that you are requesting the EIA AGID or ELISA and that you submitted the electronic form. This information will alert the technicians to look for your form on the Web site. You will receive the results electronically through the VSPS Web site.



4 If you require a copy of the form with the technician's signature, please indicate that on the accession form. The technician will print and sign the official form and the signed form will be faxed to you. Both the e-authentication Web site and the VSPS Web site have "help desk" links, if you have any problems. If you get completely lost, please call Hana Van Campen at (970) 297-1287 and she will be happy to help you or find someone who can. ▲

SUBMISSION GUIDELINES FOR SUCCESS

EIA testing

- ❑ **Submit 1ml serum**
- ❑ **Fees: AGID $8 ELISA $13**

Get to Know the Laboratory

New Members Join the Lab Team

Paula Schaffer received her DVM from the University of Tennessee at Knoxville, after which she completed an internship in small animal rotating medicine, surgery and emergency. Pathology has been her long-time passion; she is thrilled to be in CSU's anatomic pathology residency program. In her free time, she trail rides with her mustang and swing dances.

Alana Pavuk Garner grew up in eastern North Carolina, received her bachelor of science degree from Clemson University and her DVM from North Carolina State University. She then practiced small-animal medicine for one year before moving to Colorado. She is interested in naturally occurring diseases in wildlife and their interface with domestic species. Alana is looking forward to a career in academia. In her spare time, she enjoys running, hiking and exploring.

Clare Hoover grew up in central Pennsylvania. She received her undergraduate degree from Washington and Lee University, and her DVM from Ohio State University. Her research interests include infectious viral diseases and neurodegenerative diseases. In her free time, she likes to play tennis, cook, garden, and spend time with her cat, Oscar.

Diana Sierra Alzate comes from Colombia and has lived in Fort Collins for five years. A biologist, she has worked for 15 years with insects, specifically sand flies. She will focus on work involving nematodes, working with Parasitology Section Head Lora Ballweber.

Valerie Johnson grew up in New Hampshire and received her undergraduate training at Boston University and an DVM from Tufts University. Following veterinary school, she completed an emergency and critical care residency at the New England Animal Medical Center in West Bridgewater, Mass., and worked there in the ICU for several years. Having had enough excitement on the clinical front, she joined Colorado State as a microbiology resident and graduate student, where she is pursing an interest in studying the immune response during sepsis. She enjoys yoga, hiking, watching movies and cuddling with her dog.

Michelle McHugh, born in San Antonio, has lived in Fort Collins since 1983 and graduated from CSU with a bachelor of science in zoology. She is glad to be a part of the Veterinary Diagnostic Lab, and looks forward to learning all the services the lab has to offer to its clients and patrons. She enjoys fly fishing with her husband and their three boys, as well as running with her German shorthair pointer.

Brandy Nagamine, a Honolulu native and CSU alumnus, joins the VDL as a parasitology technician. She obtained her master of science degree in animal and veterinary sciences from the University of Wyoming's state veterinary laboratory. Brandy is excited and grateful to be back from Laramie to Fort Collins, working for her alma mater.

CONGRATULATIONS TO OUR 2010 ACVP DIPLOMATES

- **Greg Wilkerson**
  Anatomic pathology
- **Karen Fox**
  Anatomic pathology
- **Amy Miller**
  Clinical pathology

THE VALUE OF AAVLD ACCREDITATION

AAVLD Accreditation is based on the internationally recognized ISO/IEC 17025 standard and consistent with the World Organization for Animal Health (OIE) Quality Standard for Veterinary Laboratories. Accreditation is a formal recognition of the competency of laboratories and increases client confidence in diagnostic test results. In order to further demonstrate technical competence between accreditation assessments, personnel from accredited laboratories are also required to participate in relevant proficiency testing programs. Accreditation contributes to continuous improvement and is a management tool that can be used to increase laboratory efficiency, which is critical in times of emergency or limited funding.

Laboratories participating in the USDA's National Animal Health Laboratory Network may be involved in surveillance for early detection of foreign animal disease, surge testing during an outbreak, and testing samples during the outbreak recovery phase. As such, there must be a high degree of confidence in the quality of the laboratories and associated test results. USDA recognizes the value of quality management systems and requires that all NAHLN laboratories have a functional quality management system. Laboratories that are fully accredited by AAVLD are admitted to the NAHLN without additional requirements related to documentation of a quality management system.

Colorado State University
Veterinary Diagnostic Laboratories
College of Veterinary Medicine and Biomedical Sciences
Fort Collins, CO 80523-1644

Nonprofit Organization
US Postage
PAID
Fort Collins, Colorado 80523
Permit Number 19

Update from the Director

Please join us in expressing appreciation to the Colorado Cattlemen's Association for its generous donation of this atrium meeting table.

Welcome to the Fall/Winter issue of *LabLines*. It is snowing outside as I compose this, and the holiday season is upon us. Although economic times are tough, we are all hanging in there, and we actually have been quite busy. Our laboratories' accessions and tests were increased last fiscal year, reflecting the beginnings of the recovery in the economy. This increase has continued into the first half of the current fiscal year. We have a new group of residents that arrived July 1 and new staff that have replaced those that have left. See inside for these updates.

Also, see inside for an update on our new Laboratory Information Management Systems (i.e., computer system) which you undoubtedly noticed. We apologize profusely for the difficulty the change has caused and are working hard to refine the system to work for you and us. We greatly appreciate your loyalty and patience as we work through these issues.

BARBARA POWERS, DVM, PHD, DACVP
DIRECTOR

This new system has not affected our ability to perform quality diagnostic testing nor our desire to provide quality service you deserve. We continue to see unique and interesting cases and will share these with you. Please see inside for interesting articles on leptospirosis, urine cultures, BVD and oncology.

LAB NEWS

It was great to see many of you at the Colorado Veterinary Medical Association annual meeting in Loveland and the American Association of Veterinary Laboratory Diagnosticians meeting in Minnesota. We we look forward to our annual meeting with our external advisory committee in January.

Respectfully

Barbara E. Powers

INSIDE THIS ISSUE