

LAB LINES

Diagnostic news and trends from the Colorado State University Veterinary Diagnostic Laboratories
Volume 20, Number 2 Fall/Winter 2015

Advances in Molecular Diagnostics

Predicting the Unpredictable Using Evolving Molecular Technology

Characterization of infectious agents plays a crucial role in veterinary diagnostics. Not only is it critical for the diagnosis, but it also affects management, monitoring and control of infectious diseases. Historically, diagnosticians have demonstrated the microbial origin of diseases by demonstrating the presence of a specific pathogen in a given clinical sample. This approach was first dominated by the culture assay for bacteria and later adapted with virus isolation and electron microscopy for viruses. Within the diagnostic lab, these culture techniques often require specialized media—enriched or selective—culture conditions, defined oxygen tension, defined temperature and particular cell cultivars.

EXPANDING THE CLASSICAL OPTIONS

These specialized conditions and techniques suffer a number of limitations, including the need for dedicated and specialized staff and their intrinsic efficiency in the propagation of fastidious bacteria or slow-growing viruses, some requiring special immunologic stains to enhance recognition, for example, bovine viral diarrhea. Therefore, we have been progressively complementing these “classical” techniques with nucleic acid-based detection technology. Polymerase chain reaction (PCR) is most often used.

PCR is the nucleic acid test of choice in the diagnostic lab, as the advantages of this technique are clear: increased speed, automation, sensitivity and specificity. This efficient and high-resolution tool provides the veterinary diagnostic laboratory with the ability to undertake swift and flexible responses to emerging infectious diseases. But even targeted, pathogen-specific diagnostics still have their drawbacks. They beg the question: Are we really prepared to recognize and characterize unexpected or unknown pathogen variants?

PREPARING TO DIAGNOSE THE UNKNOWN

In order to circumvent the unexpected and unknown, several strategies have been developed to broaden the range of

—Christie Mayo, DVM, PhD, CSU VDL Virology Section Head

detection in veterinary diagnostics. Multiplexed PCR assays have been developed to detect a wide range of pathogens within one sample. Bacterial typing has been achieved by sequencing the 16S gene or other regions of the genome that are sufficiently conserved. It is tedious to identify primers conserved, yet sufficiently variable to allow for typing. However, assignment often stops at the level of genus, limiting the often necessary distinction to the species level.

At CSU’s Veterinary Diagnostic Lab, we have recently invested in matrix-assisted laser desorption/ionization (MALDI) technology, to expedite the identification of bacterial isolates after culture. This technology uses a principle component of mass spectrometry to analyze biomolecules—DNA, proteins, peptides, and sugars. Although the organism still must first be isolated by standard methods, this new technology is more accurate and can give final identification within hours after established growth on culture.

So, if detection of bacterial isolates by MALDI is still a targeted technique, does that limitation imply we are missing the unexpected or unknown?

An alternative strategy, next generation sequencing (NGS), captures genome-specific information and takes advantage of the speed and robust amount of data generated. NGS provides an increased resolution for characterizing pathogens without targeted enrichment or a preconceived idea of what the pathogen may be. Therefore, it is an excellent tool for undiagnosed diseases or cases in which the clinical picture does not completely match the diagnostic test result. This tool also strengthens our unique public mission to improve our understanding of pathogen evolution, adaptation and virulence determinants in the veterinary community.

See *THE NEXT GENERATION*, page 2



THE NEXT GENERATION
(Continued from page 1)



CSU VDL's Illumina MiSeq/Next-Seq expertise helps bridge this new technology with meaningful veterinary diagnostics.

The principle of NGS for whole-genome pathogen characterization brings diagnostic advantages: In particular, the need to design specific primers to pre-amplify or target pathogens disappears. However, these advantages come at a cost and have several drawbacks, including random amplification of both host and microbial nucleic acid material, making it difficult to tease out the correct amount of information in order to identify the pathogen of interest. Making

logical sense of the information also requires refined expertise in both the diagnostic lab and the computer lab.

CSU's VDL is one of few veterinary diagnostic laboratories investing in the equipment and appropriate expertise to bridge this new technology with veterinary diagnostics. As clinicians, private owners and livestock producers continue to navigate modern veterinary diagnostics, it is important to weigh the pros and cons of this new technology. Our ultimate mission is to combine classic techniques with these modern tools in order to provide an increased understanding of pathogens, their interaction with hosts such as livestock and impact on future disease prevention, control and management strategies. ▲

Lab Updates

New Residue Testing Available

Residues of the antimicrobials used in food-producing animals to treat, control or prevent disease and the growth promoting steroids used to promote muscle growth of food animals while using fewer resources are highly regulated to protect consumers from any possible adverse health effects. As part of the drug-approval process, FDA establishes withdrawal times to ensure a drug has sufficiently cleared the animal's system before its meat, milk or eggs enter the food supply. Established maximum residue limits represent safe residue levels for human consumption, and surveillance programs ensure foods with violative levels of residues do not enter the food supply. The FDA and USDA routinely sample milk and meat to verify producers are adhering to withdrawal requirements and that no violative residue levels remain in these products.

In partnership with the CSU Department of Animal Science, the Veterinary Diagnostic Laboratory is now offering food-animal residue testing using Randox Food Diagnostics' Evidence Investigator™ and Biochip Array

— Keith Belk, PhD, CSU Professor of Meat Safety & Quality; and Dwayne Hamar, PhD, CSU VDL Chemistry and Toxicology Section Head

Technology. The ELISA-based screening test is faster and less expensive than similar analyzers using mass spectrometry. Also, the biochip array technology allows for simultaneous testing of multiple analytes from a single sample.

Available screens include the beta-agonist ractopamine only, growth promoters and antimicrobials. USDA has approved Randox's Growth Promoter Array as a screening method for ractopamine in muscle, liver and kidney samples. This screening technology can assist producers as they access export markets and labeling claim programs certified by USDA.

All tests have a 48-hour turnaround time, but with advanced notice, wait times can be even shorter. Positive results are confirmed by an independent laboratory, with the added cost charged to the client. ▲

Array	Screens	Cost per sample*					
		Feed	Urine	Beef Muscle	Muscle, Liver, Kidney	Milk	Honey
Ractopamine	Ractopamine	\$140	\$195	\$110			
Growth Promoters Array	Beta-agonists • Boldenone Corticosteroids • Nandrolone Ractopamine • Stanozolol Stilbenes • Trenbolone • Zeranol	\$140	\$195		\$195		
Anti-microbial Array	Ceftiofur • Quinolones • Streptomycin Tetracycline • Thiamphenicol • Tylosin	\$140	\$110		\$110	\$140	\$110

For information about sample submission and cost, including volume discounts, call us at (970) 297-1281 or email DLAB@colostate.edu

Advances in Oncology Diagnostics

Tips to Better Ink Liver Margins

Surgical resection of massive hepatocellular carcinomas in dogs prolongs survival times and reduces local recurrence and distant metastasis.¹ However, both local recurrence and distant metastasis have been reported.^{2,3} In one study in 42 dogs following surgery for removal of hepatocellular carcinomas, there was no local recurrence and only a 4.8% metastatic rate. The median survival was over 1,460 days, compared to 270 days in six dogs that did not have surgery for removal of their tumors. Most of these tumors were well-differentiated, but even less well-differentiated hepatocellular carcinomas still resulted in good survival times. Therefore

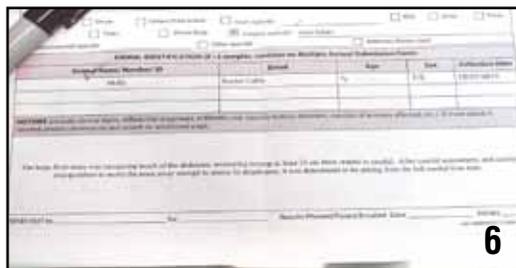
— Barb Powers, DVM, PhD, DACVP, CSU VDL Director; and Lee DeBuse, CSU VDL technician

if surgery is done it is important to try to achieve good margins.

When these tumor samples arrive in the laboratory after being in formalin and losing their natural color, it can be very difficult for us to determine the surgical margin unless it is marked. Using ink is most helpful. Ink will survive processing and be clearly evident on the histologic slides. See below instructions on how to ink margins. ▲



1. Margin to be inked
2. Types and colors of ink to be used
3. Inked margin, dried for 10 to 20 minutes
4. Ink stayed on after drying,
5. After inking and 24 hours of fixation, the sample is ready to be trimmed in or processed
6. Good diagnosis requires a great history like this one.



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2 Kosovsky JE, Manfra-Marretta S, Matthiesen DT, et al. Results of partial hepatectomy in 18 dogs with hepatocellular carcinoma. *J Am Anim Hosp Assoc.* 1989;25:203-206.

3 Patnaik AK, Hurvitz AI, Lieberman PH, et al. Canine hepatocellular carcinoma. *Vet Pathol.* 1981;18:427-438.

CSU VDL in the Field: Case Study

Possible Cystic Fibrosis-Like Disease in a Sumatran Orangutan

Sumatran orangutans (*Pongo abelii*) are a critically endangered species, with numbers believed to be less than 7,000 animals in shrinking and fragmented native habitats. Conservation of Sumatran orangutans is complex and involves many environmental and human factors, as well as rehabilitating healthy animals and maintaining valuable genetics in international zoos. The unfortunate fact is between 20% and 40% of captive orangutans suffer chronic respiratory disease, which is difficult to manage and can eventually be fatal.

In this case, a 31-year-old captive male Sumatran orangutan suffered classic recurrent bouts of sinusitis, laryngeal air sacculitis and pneumonia starting at about 14 years of age. The animal experienced intermittent periods of lethargy, bloat and diarrhea. Respiratory cultures showed infection with mixed Gram-positive and -negative bacteria, including *Pseudomonas aeruginosa*, which was responsive to antimicrobial and supportive therapy, but with eventual relapse and failure of complete resolution. Attentive zoo veterinarians and keepers consulted a human pulmonologist, Jennifer Taylor-Cousar, and an extensive respiratory examination was performed including pulmonary CT scan, rhinoscopy, bronchoscopy and bronchoalveolar

lavage showing sinusitis and bronchiectasis, with clinical and diagnostic findings determined to be suggestive of a cystic fibrosis-like disease. Pulmonologist, veterinarians and keepers began an intensive cystic fibrosis treatment regimen of nebulized antibiotics, mucolytic therapy and pancreatic enzymes, which the orangutan voluntarily accepted. With therapy, the patient showed marked clinical and behavioral improvement and stabilization of respiratory and gastrointestinal disease.

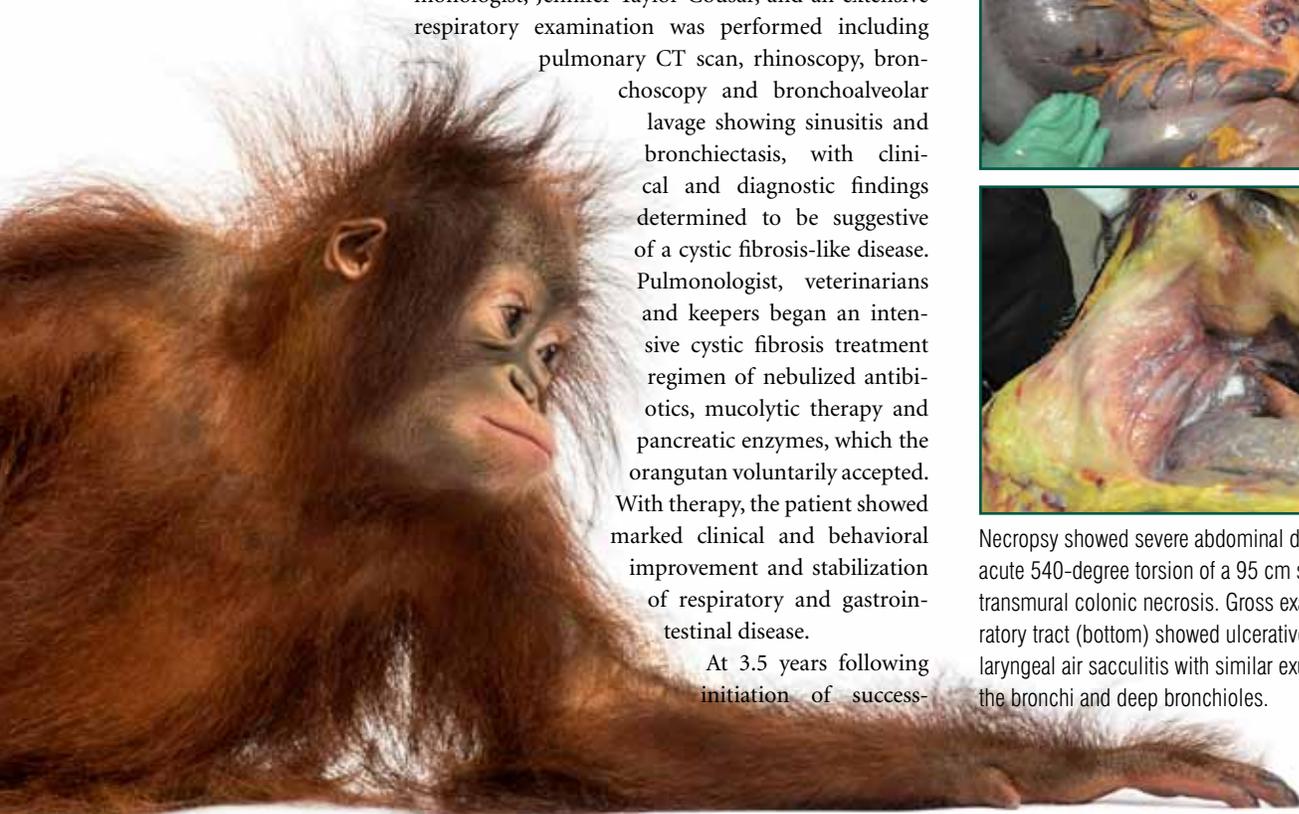
At 3.5 years following initiation of success-

— Elizabeth Stringer DVM, DACZM, Denver Zoo Staff Veterinarian; Cindy Cossaboon, Denver Zoo Curator of Primates; Sushan Han, DVM, PhD, DACVP, CSU VDL Pathologist; Jennifer L. Taylor-Cousar, MD, University of Colorado Denver Associate Professor

ful treatment, the orangutan died suddenly. Necropsy showed severe abdominal distention with acute 540-degree torsion of a 95-cm section of colon and transmural colonic necrosis. Gross exam of the respiratory tract showed ulcerative and mucopurulent laryngeal air sacculitis with similar exudate extending into the bronchi and deep bronchioles. Bronchioles were extensively occluded with inspissated mucus and circumscribed by prominent fibroplasia. Histologically, bronchioles were constricted by smooth muscle hyperplasia and goblet cell and mucus gland hyperplasia



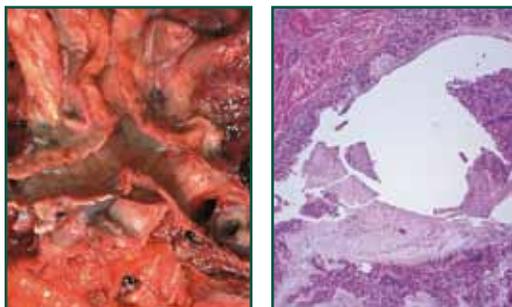
Necropsy showed severe abdominal distention (top) with acute 540-degree torsion of a 95 cm section of colon and transmural colonic necrosis. Gross examination of the respiratory tract (bottom) showed ulcerative and mucopurulent laryngeal air sacculitis with similar exudate extending into the bronchi and deep bronchioles.



and surrounding alveoli were multifocally disrupted by patchy and suppurative bronchopneumonia. Gross and histological lesions were impressively similar to chronic cystic fibrosis lesions described in people.

Cystic fibrosis in people is associated with mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, leading to disruption of membrane ion-channel function. This disruption affects mucosal surfaces, leading to thick mucus production of sinuses, upper and lower airways, the gastrointestinal tract and pancreatic ducts, with chronic disease resulting in bronchiectasis, recurrent sinusitis, pneumonia and pancreatic insufficiency with periods of diarrhea, bloat and constipation.

This male Sumatran Orangutan presented with chronic and debilitating respiratory and gastrointestinal disease, which is frequently diagnosed and difficult to manage in this species in captivity. With advanced diagnostics available to human patients, it was astutely determined that his clinical findings were strikingly similar to humans with hereditary cystic fibrosis. Through the dedicated care of veterinarians and keepers and the willingness and trust of this charismatic orangutan, he was successfully treated as a cystic fibrosis patient and showed significant



improvement in respiratory and gastrointestinal function. His sudden death by colonic torsion is thought to be a consequence of segmental bloat and ileus of the colon, a complication of pancreatic insufficiency and flatus. His case has opened the door to the study of possible cystic fibrosis-like disease in the Sumatran Orangutan, a disease which may be an important cause of morbidity and mortality in this critically endangered species. ▲

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A detailed manuscript on this subject has been accepted for publication in the *Journal of Zoo and Wildlife Medicine*.

Advances in Parasitology

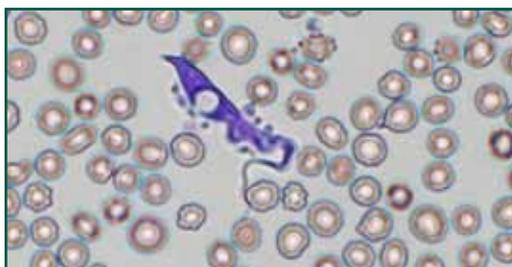
North American Trypanosomes Exist

Recent news and social-media reports about the possible spread of human Chagas disease, or American trypanosomiasis, beyond endemic areas of South America to North America, have raised a little-known fact: North American trypanosomes already exist! In fact, several are considered endemic in ruminants:

- *Trypanosoma theileri*, which parasitizes cattle and bison.
- *Trypanosoma melophagium* of sheep
- *Trypanosoma cervi* of deer, moose, elk and reindeer.

All are transmitted by insects – *T. theileri* and *T. cervi*, by tabanids; *T. melophagium*, by sheep keds. Efforts to demonstrate disease associated with these species have provided mixed results. In cattle, it has been suggested that immunosuppression or concomitant diseases may allow the parasite to exert pathogenic effects, resulting in issues such as abortion. Although peritonitis and suppurative meningoencephalitis have also been associated with *T. theileri* infections, these

— Lora R. Ballweber, DVM, MS, CSU VDL Parasitology Section Head



are extremely unusual sequelae. In sheep and cervids, diseases attributable to trypanosomes have not been identified. Although the parasites can be seen on blood smears, the extremely low parasitemias make finding them quite difficult. Should they show up, particularly in cattle, evaluation for immunosuppressive diseases may be in order. ▲



Diagnostic Interpretation

Five Tips to Use Titer Interpretation to Improve Clinical Decision-Making

— Christie Mayo, DVM, PhD, CSU VDL Virology Section Head

Over the last two decades, the veterinary profession has become more accepting of the need to re-evaluate standard vaccination practices. Several strategies have been proposed, including:

- Lengthening the interval between adult booster vaccinations to three or more years;
- Measuring serum antibody titers to determine the need for boosters;
- Separating booster vaccines to include fewer antigens given at one time; and
- Administering booster vaccines for only clinically important diseases of dogs and cats.

At CSU VDL, we routinely perform serum neutralization diagnostic assays to determine canine distemper and parvovirus titers for dogs, and panleukopenia, herpesvirus and calicivirus titers for cats. Serum titers are blood tests that measure whether or not your animal may be immune to a given virus. More specifically, a quantitative serum titer (e.g. 1:32) is a numerical value indicating the animal's actual degree of immunity to a virus. For most of these viral pathogens, the presence of serum antibody able to neutralize infectious virus and prevent infection provides an extremely strong correlate of protection, but it is important to recognize the limitations of serology and to provide correct interpretation of

the results. The following are some applications for the use of serological titers:

CONFIRM PUPPY RESPONSE AND PROTECTION



The use of serology can provide a simple measure of whether a puppy or kitten is protected after the initial series of vaccinations. Typically, final CORE vaccination occurs at 14-16 weeks of age. The puppy can be tested from 2 weeks after this vaccination

(~18 weeks). Seropositivity at this stage indicates that the pup has made an endogenous immune response to the vaccine, as there can be no maternal antibody remaining at this time. A puppy that is seronegative at 18 weeks should be revaccinated (perhaps with an alternate product) and then tested again 2 weeks later. A high titer result indicates protection. A second low titer or negative result may indicate that the pup is either a low responder or non-responder. A low responder may be protected from clinical disease but not from infection. Alternatively, the dog may

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- 1 Jensen WA, Totten JS, Lappin MR, Schultz RD. Use of serologic tests to predict resistance to Canine distemper virus-induced disease in vaccinated dogs. *J Vet Diagn Invest.* 2015 Sep;27(5):576-80. doi: 10.1177/1040638715602291.

lack antibody and be a genetic non-responder that is incapable of ever making an immune response to that particular antigen. It is to be noted that genetic non-responders are generally unable to respond to one (rather than all) CORE vaccine antigen and the estimated prevalence of non-responders in the US for CPV is 1 in every 1000 dogs and for CDV 1 in every 5000 dogs. Canine adenovirus non-responders are very rare (estimated <1 in every 10,000 dogs).

REFINE LAPSED ADULT-DOG VACCINATION



Owners may be offered serology rather than automatic vaccination in circumstances where an adult dog has lapsed in vaccination schedule or the dog was adopted without a vaccination history. These dogs may not actually require vaccination

because they have been previously vaccinated or have acquired natural immunity from field exposure to a virus, but it is important to note that if titers are low or non-existent, vaccination may still be recommended in addition to titer costs.

MANAGE RISK OF ADVERSE REACTION



Adverse reactions of a wide spectrum are recognized post-vaccination in dogs and cats. The prevalence of these is low and most are mild; however, some are potentially life-threatening and if there is suspicion that vaccination might have been a trigger

for a disease then such animals should be subject to rigorous benefit/risk analysis before revaccination is considered. For CORE vaccine antigens, this decision is now made simpler by performing serology. A dog with serum antibodies to CDV, CAV, and CPV does not require revaccination and serious consideration should be given to which non-CORE products this animal receives.

EXPAND ANNUAL HEALTH-CHECK OPTIONS

It is becoming increasingly popular to perform annual health checks for animals within the United States. Many practitioners are offering the alternative of triennial revaccination and instead are offering triennial serological testing using serology and titer interpretation. Animals that are seropositive are not revaccinated with CORE vaccines, as these are not required. In these cases, it is of particular importance to point



out serology only gives the practitioner an idea of circulating antibodies at that time of serum collection. Where this approach is used, it is recommended that the testing interval be reduced to annually for senior animals to ensure that immunosenescence (aging of the immune system) is not an issue.

MANAGE SHELTER DISEASE OUTBREAKS



One of the most valuable applications of serology has been in the management of infectious disease outbreaks in shelters (herd health medicine approach). This strategy is typically used for CDV, CPV, and FPV outbreaks. The ability to rapidly and cheaply test populations in order

to identify animals that are protected or susceptible has allowed many animals to live (animals that might have otherwise been euthanized as they were of unknown status). In the face of an outbreak, all animals currently resident within the shelter should be tested. Those that are seropositive are protected and will not become infected or die. The susceptible population should not be adopted out of the shelter until after at least 2 weeks for CPV/FPV or until after at least 6 weeks for CDV (typical incubation periods of diseases). The susceptible population should be retested after these intervals. The second population under consideration includes those animals that are wishing to enter the shelter. Seropositive animals may enter as they are protected from disease whereas seronegative animals should be revaccinated and then ideally sent to foster homes and not allowed to enter the shelter until they have seroconverted.

Immunity induced by vaccines depends on the development of an acquired immune response after vaccination. The response that develops is dependent on the type of vaccine used and the host response to that vaccine. Protective immunity is dependent on many factors and the duration of protective immunity varies among hosts and diseases. Vaccines rarely provide duration of immunity that is greater than immunity after recovery from natural infection or disease, so it is important to consider all of these factors before making decisions for accurate interpretation of serologic titers, especially when using in lieu of vaccination. ▲

SMALL-ANIMAL VACCINE TITER TESTING AT CSU

- Submit 1 mL serum
- \$17 per sample



Source: Flickr/Shannon McGee. Some rights reserved. Used under CC BY-SA 2.0.

CSU VDL in the Field: Case Study

Histophilus and *Mycoplasma* Septicemia Display Many Faces

— Don Kitchen, DVM, PhD, ACVP, CSU VDL Western Slope Branch Director

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Many veterinary diagnostic situations are complex, involving multiple causes or contributing factors in their etiology. For example, in early October 2015, a 7-month-old Limousin calf that had died suddenly was submitted to the CSU VDL Western Slope Diagnostic Laboratory. Several calves from this herd had died in a similar manner, and numerous yearling calves and mature cows were diagnosed clinically with polyarthritis or mastitis.

At necropsy, the yearling calf had gross findings of chronic heart failure, including a very enlarged round heart. The posterior vena cava was engorged and significant amounts of edematous fluid were present in the thoracic and abdominal cavities. The pericardial sac also contained fluid, and pericarditis was evident. Examination of the heart revealed a very thickened left ventricular wall with thinning of the right ventricular. The cardiac muscle of the left ventricle was mottled, pale and firm. The papillary muscles were affected most severely. The calf also had mild interstitial pneumonia. PCR results were positive for *Mycoplasma bovis*.

In late September, pooled, four-quarter milk samples of one cow from the herd had been submitted for bacteriological culture and polymerase chain reaction to detect *M. bovis* genetic material. No significant bacterial growth was observed; however, the PCR results for *Mycoplasma bovis* in that sample were also positive.

CLOUDED BY CONFUSING CONTRIBUTORS

Sudden death, particularly in feedlot cattle, has been associated with similar multifocal myocardial infarcts, necrosis and fibrosis and was first described as a syndrome associated with septicemic infections with *Histophilus*

somni in the late 1980s.¹ Additionally, pneumonia, mastitis, and polyarthritis have also been associated with *Histophilus* septicemia in beef calves and mature animals.¹ Other studies have incriminated *Mycoplasma bovis* as a cause of suppurative myocarditis, mastitis, arthritis, tenosynovitis, or chronic pneumonia and polyarthritis.^{1,2} Bacterial organisms associated with myocarditis in beef cattle would include *Histophilus somni* septicemia, embolic pyogenic bacterial infections and *Clostridium* myocarditis.³

An immunohistochemical (IHC) study of various bacterial organisms including *H. somni* and *M. bovis* indicate they may cause death losses due to myocarditis in feedlot cattle. *M. bovis* may play a role in causing this specific lesion; however, this bacterium was found in only a minority of hearts with myocarditis lesions.¹ In this study of 92 animals, 70 animals with myocarditis stained positively for *H. somni* and only four animals stained positively for *M. bovis*.

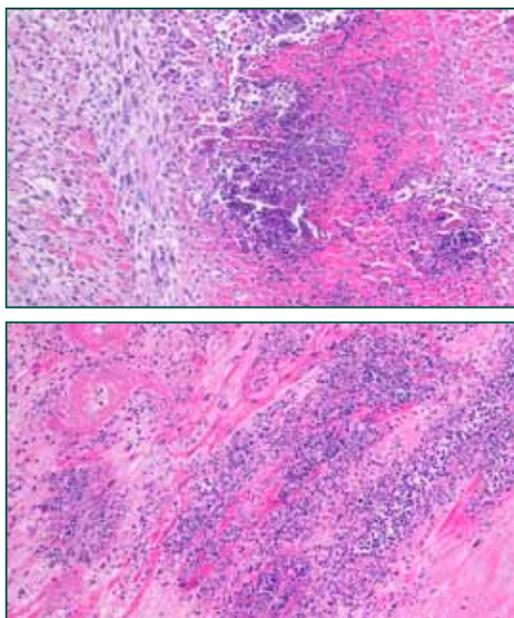
WHICH TO BLAME FOR THE INITIAL CAUSE?

So what role did *M. bovis* play in the development of myocarditis in this case? Histopathologic examination of the tissues in this case revealed mild interstitial nephritis, minimal to mild interstitial pneumonia with abscess formation and suppurative myocarditis. In addition to the suppurative myocarditis, there was myocardial necrosis with infarction, severe fibrosis and fibroplasia along with multifocal mineralization. Bacterial colonies were observed in the vasculature and within necrotic debris and suppurative materials.

Aerobic bacteriological cultures of cardiac and hepatic tissues were negative with no growth observed. Liver trace-mineral analysis from the calf found hepatic iron levels slightly were low and zinc levels slightly above the normal expected range. The other hepatic trace mineral levels fell within normal reference ranges.

It was concluded that the myocarditis in this case was likely due to *H. somnus* septicemia. The cardiac lesions were caused by *Histophilus* septicemia; however, with a herd history of *M. bovis* polyarthritis, mastitis and septicemia, a concomitant infection with both organisms is possible. The organism that actually initiated or caused the myocardial insult was *H. somnus*. IHC staining for the presence of *H. somnus* antibodies was positive, confirming the specific cause of this myocardial disease. IHC procedures for *M. bovis* are not as reliable as they are for *H. somnus*⁴ and were not performed in this case.

Many diagnostic situations we face each day in the veterinary diagnostic arena are complex in nature, and there may be multiple causes and contributing factors involved in their etiology. As veterinary diagnosticians, we must continually strive to provide accurate diagnoses which may include the unweaving of complex disease syndromes. ▲



Multifocal suppurative myocarditis (at top) with extensive fibrosis and fibroplasia, along with (at bottom) myocardial infarction and necrosis caused by bacterial septicemia were identified in this case.

Advances in Bacteriology

Now: Pathogen Identification in Minutes

CSU Veterinary Diagnostic Laboratories's new bacterial and fungal identification equipment, using MALDI-TOF technology, has been in use for nearly four months, and we have already used it to identify over 1,900 bacterial isolates.

What is MALDI-TOF? It is matrix-assisted laser desorption ionization time of flight. That mouthful stands for a specialized mass spectrometry that measures identification-specific ribosomal proteins. Our specific equipment, the Vitek MS IND,TM is made by a leader in bacterial identification for the last 50 years. It is the first MALDI-TOF to receive FDA fast-track approval. It is able to give bacterial and mold identifications in less than 20 minutes—for some as quickly as 5 minutes—from its database of over 29,000 different organisms. This is a significant advantage over old methods that often required overnight incubation.

The organism must first be isolated by standard methods, but this new technology shortens the final identification by over 24 hours and is more accurate.

Thus far, we have used the system to identify over 1,900 bacterial and fungal isolates on the same day they were isolated. We have even had clients wondering why they have the identification before their sensitiv-

—Doreene Hyatt, PhD, CSU VDL
Bacteriology Section Head

ity results. Best of all, this improved technology and faster turnaround comes at no additional costs for our clients!

- Based on Matrix Assisted Laser Desorption/Ionization Time of Flight technology
- Database contains over 690 species, including nine mycobacteria, 105 fungi, and two algae
- Provides greater than 99% correlation with sequencing data
- Database contains over 29,000 spectra for high specificity
- Affordable at \$20 per sample, with negotiable quantity discounts
- Intercampus sample transport speeds delivery of results
- Results within 30 minutes after isolation of organism. ▲



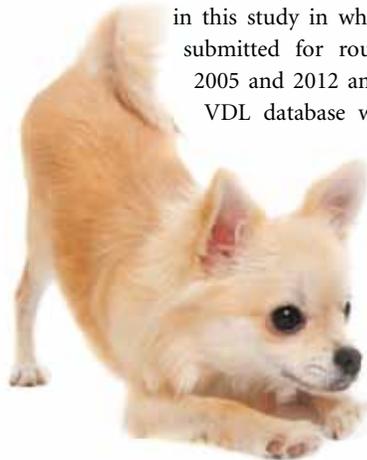
CSU VDL In Press

A Roundup of VDL Faculty Research

MORE MARKER OPTIONS FOR INTESTINAL TUMORS

Dailey DD, Ehrhart EJ, Duval DL, Bass T, Powers BE. DOG1 is a sensitive and specific immunohistochemical marker for diagnosis of canine gastrointestinal stromal tumors. *J Vet Diagn Invest.* 2015 May;27(3):268-77. doi: 10.1177/1040638715578878.

VDL Pathologists Deanna Dailey and E.J. Ehrhart, Lab Tech Todd Bass and Director Barb Powers participated in this study in which 55 canine tumors submitted for routine biopsy between 2005 and 2012 and housed in the CSU VDL database were used to test the value of a commercial human immunohistochemical marker for differentiating gastrointestinal stromal tumors (GISTs) from leiomyosarcomas in dogs. Canine GISTs are currently differentiated from other gastrointestinal sarcomas by positive immunoreactivity for KIT protein (cluster of differentiation [CD]117.) Because in humans only about 5% to 10% of GISTs are KIT negative or only weakly positive, other markers have been explored, including the one discovered on gastrointestinal stromal tumors protein 1 and thus dubbed DOG1.



For each of the 55 tumors, IHC for DOG1, KIT, and desmin was performed. A subset of tumors was additionally evaluated for reactivity for smooth muscle actin (SMA). Thirty-three tumors (60%) were diagnosed as GIST based on positive immunoreactivity for KIT or DOG1 regardless of reactivity for desmin or SMA. Most GISTs—32 of 33, or 97%—had similar staining for both KIT and DOG1. DOG1 expression was identified in only two tumors (one study tumor and one additional tumor) that was negative for KIT and desmin that had histologic features consistent with KIT-negative, platelet-derived growth factor receptor-alpha (PDGFRA)-mutant human GISTs.

The results of this study therefore suggest that

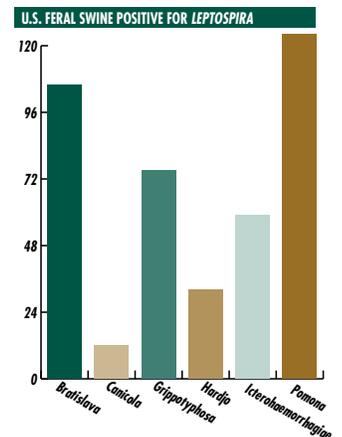
DOG1 has improved specificity and sensitivity to that of KIT for differentiating between canine GISTs and leiomyosarcomas. Including both DOG1 and KIT IHC in diagnostic panels will improve the accuracy of canine GIST diagnosis.

FERAL SWINE A HUGE LEPTOSPIRA RESERVOIR

Pedersen K, Pabilonia KL, Anderson TD, Bevins SN, Hicks CR, Kloft JM, Deliberto TJ. Widespread detection of antibodies to *Leptospira* in feral swine in the United States. *Epidemiol Infect.* 2015 Jul;143(10):2131-6. doi: 10.1017/S0950268814003148.

Populations of feral swine continue to expand into urban areas, overlapping in range with both domestic swine production and human activities, increasing concern over the possibility they may transmit pathogens like *Leptospira*. Noting no current existence of a nationwide comprehensive effort to determine the geographical distribution and apparent prevalence of this pathogen in the United States, CSU VDL Avian and Molecular Diagnostics Section Head Kristy Pabilonia participated in this study to test feral swine sera from across the country and to characterize the antibody prevalence of to the six serovars of *Leptospira* considered of agricultural or zoonotic concern.

Pabilonia and staff tested 2,055 feral swine serum samples collected between February 2007 and June 2011 from feral swine USDA's Wildlife Services had removed for wildlife damage-management purposes. The 107 counties sampled represented 28 states across the nation. VDL tested all the samples with the microscopic agglutination test to detect antibodies against six *Leptospira* serovars. A titer of 1:200 or higher was considered positive.



The results showed exposure to *Leptospira* is common in feral swine, and is not limited to certain regions of the country since antibody-positive feral swine were identified in 70% of the states included in this study. Of 2,055 samples tested, 269 or 13.1%, were positive for at least one serovar and 97, or 36%, of these were infected with multiple serovars. Samples tested positive for at least one serovar in 20 of 28 states and 71% of 107 counties.

TRACKING AVIAN INFLUENZA VECTORS

Root JJ, Shriner SA, Bentler KT, Gidlewski T, Mooers NL, Ellis JW, Spraker TR, VanDalen KK, Sullivan HJ, Franklin AB. Extended viral shedding of a low pathogenic avian influenza virus by striped skunks (*Mephitis mephitis*). *PLoS One*. 2014 Jan 29;9(1):e70639. doi: 10.1371/journal.pone.0070639.

Root JJ, Shriner SA, Bentler KT, Gidlewski T, Mooers NL, Spraker TR, VanDalen KK, Sullivan HJ, Franklin AB. Shedding of a low pathogenic avian influenza virus in a common synanthropic mammal—the cottontail rabbit. *PLoS One*. 2014 Aug 11;9(8):e102513. doi: 10.1371/journal.pone.0102513.

CSU VDL Pathologist Terry Spraker collaborated with USDA National Wildlife Center researchers to test the avian influenza viral shedding capability of two mammals common at the intersection of humans and wildlife, and to help clarify their possible role in influenza type A virus ecology.

In the first, striped skunks were experimentally infected with a low pathogenic H4N6 avian influenza virus and monitored for 20 days post infection. All of the exposed skunks shed large quantities of viral RNA, as detected by real-time PCR and confirmed for live virus with virus isolation, from nasal washes and oral swabs. Some evidence of potential fecal shedding was also noted. Upon necropsy following the monitoring period, viral RNA was detected in the nasal turbinates of one individual. All treatment animals yielded evidence of a serological response by that time.

The second study similarly nasally inoculated fourteen cottontail rabbits with a low pathogenic AIV. All inoculated cottontails shed relatively large



quantities of viral RNA both nasally and orally. However, oral shedding tended to decline more quickly than did nasal shedding. No animals showed any obvious signs of disease throughout the study. Evidence of a serological response was found in convalescent sera in all infected rabbits at 22 days post-infection.

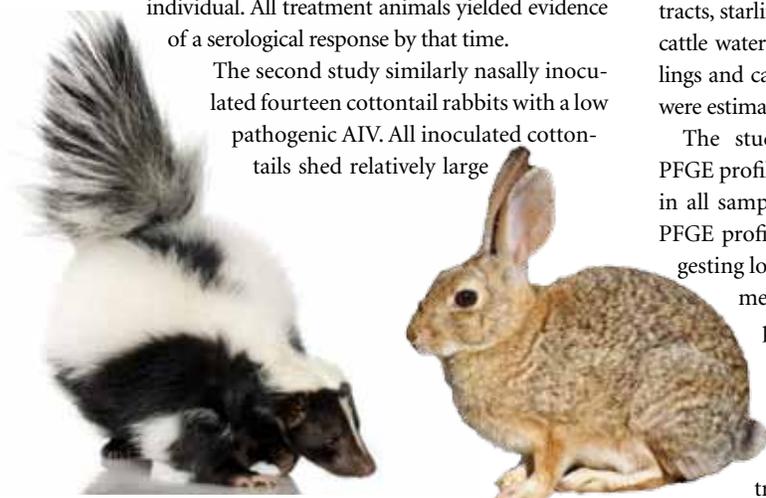
Both studies demonstrate these two peridomestic species obviously shed avian influenza extensively via the nasal and oral routes and thus could easily transport AI short distances. Considering their peridomestic nature, along with the duration of shedding, their presence on poultry and waterfowl operations could influence influenza A epidemiology by introducing virus to a naive poultry flock or acting as a trafficking mechanism between infected and naive populations.

STARLING/CATTLE SALMONELLA INTERACTION

Carlson JC, Hyatt DR, Bentler K, Mangan AM, Russell M, Piaggio AJ, Linz GM. Molecular characterization of *Salmonella enterica* isolates associated with starling-livestock interactions. *Vet Microbiol*. 2015 Aug 31;179(1-2):109-18. doi: 10.1016/j.vetmic.2015.03.015.

VDL Bacteriology Section Head Doreene Hyatt participated in this study using pulsed-field gel electrophoresis to characterize XbaI-digested genomic DNA from 182 *Salmonella enterica* isolates collected from a single 50,000-head Texas cattle feedlot between 2009 and 2012. The analysis screened five different sero-types recovered from five different sample types: starling gastrointestinal tracts, starling external wash, cattle feces, cattle feed and cattle water troughs isolated from both European starlings and cattle within this CAFO. Starling populations were estimated to be greater than 10,000 birds per day.

The study showed indistinguishable *S. enterica* PFGE profiles were recovered from isolates originating in all sample types. It also isolated indistinguishable PFGE profiles across all years of data collection, suggesting long-term environmental persistence may be mediated by starling visits to a CAFO. Samples collected in 2012 were also subjected to antimicrobial susceptibility testing, and results suggested resistant *S. enterica* is transmitted between cattle and starlings and that shared feed sources are likely contributing to infections. ▲



Source: Flickr/Frank Alcazar. Some rights reserved. Used under CC BY-NC-ND 2.0.

Guardians of Public Health

Robust Biosecurity is Basic Business Here

The White House's late-October release of a report critical of the nation's infectious-disease research labs, coupled with investigative reports by *USA Today* highlighting some federal-lab safety lapses, have once again turned focus on labs like CSU's VDL that handle pathogens like avian influenza, plague, Tularemia and anthrax.

Unlike commercial diagnostic labs, our public mission includes participation in the CDC Laboratory Response Network and the USDA National Animal Health Laboratory Network. As a consequence, our facilities include a government-inspected Biosafety Level 3 laboratory, where we test for foreign, emerging and zoonotic diseases that require increased laboratory biosafety. What steps do we follow every day to ensure that biosecurity?

- BSL-3 laboratory personnel receive intense specific training in handling these foreign animal and zoonotic disease agents, including the use of personal protective equipment (PPE).
- Our laboratory is evaluated annually by the CDC and USDA to ensure our compliance with federal Select Agent regulations.
- BSL-3 labs have architectural and mechanical built-

— Barbara Powers, DVM, PhD, DACVP, CSU VDL Director



in safety systems to almost completely eliminate the risk of disease transmission to laboratory workers and escape of the agent from the laboratory.

- Floors, walls, ceilings and windows are all sealed to prevent pathogen escape.
- Specialized air filtration systems.
- Round-the-clock monitoring systems.
- Back-up generators to ensure safety systems continue to operate in the event of a power outage.
- Reporting to CDC and USDA of all select agent isolation and documented safe disposal or storage of agents. ▲

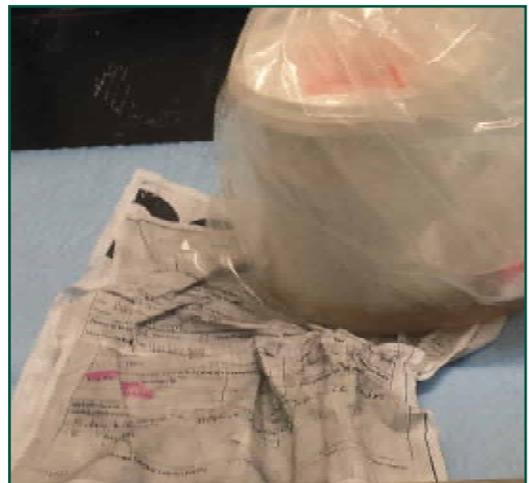
Diagnostic Sample Quality Assurance

Tips on Trouble-Free Biopsy Submission

Help the lab ensure meaningful results on your biopsies by reviewing these friendly reminders on safely and securely packaging formalin-fixed tissues.

- **Label your formalin containers immediately.** A small number of submissions do get separated from their submission forms. Cross-labeling ensures delays don't result if that separation happens.
- **Don't put large samples in narrow-mouthed containers.** Formalin fixation causes samples to become less flexible, making it difficult to remove them from narrow-mouthed jars after they arrive here.
- **Seal well.** Place the lid on the formalin jar and then use wax sealer to cover the *entire* top of the jar. The wax helps seal the jar and prevents formalin leakage.
- **Pack for leaks.** Place absorbent packing material around the formalin jar. The shipping material will help absorb any formalin that may leak in transit.
- **Separate paperwork from formalin jars.** Place paperwork at least outside the securely enclosed formalin jar; ideally, in an individual plastic bag. We often receive unreadable formalin-drenched paperwork.▲

— Nancy Ault, CSU VDL Office Manager; and Christie Mayo, DVM, PhD, CSU VDL Virology Section Head



Preserve the integrity of your results by ensuring leaky formalin containers don't make your submission paperwork unreadable. Here are some tips from the VDL.

Get to Know the Laboratory

New Members Join the Lab Team

Jenna Oxenhandler, new poultry program specialist, was raised in Colorado, where she raised animals her whole life and developed a passion for all things poultry. She studied animal sciences and ag business at CSU, with a focus in meat science and food-safety microbiology. She is finishing a master's degree in agricultural extension education.



Zachary Desmond, new necropsy lab coordinator, grew up in Woodinville, Wash. His interests in biology and astronomy brought him to CSU in 2008, where he worked at three different labs while pursuing a 2013 bachelor's degree in biological science. His most recent position was at the Infectious Disease Research Center for the past two years.



Lauren Harris, from Goshen, N.Y., gained her first research experience studying seed-germination molecular mechanisms while majoring in cell and molecular biology/biochemistry at Maine's Colby College. Following a stint studying hematopoietic stem cells at Harvard's Beth Israel Deaconess Medical Center, she pursued her long-term goal of becoming a veterinarian at Pennsylvania, where she developed interests in global health working as the student One Health Chair and helping farmers in Haiti improve animal health. Her current focus on anatomic pathology in her VDL residency combines interests in molecular biology and empirical research with a passion for global one health, oncology and diagnostic medicine.



Lori Bowker grew up in northern Minnesota with a longstanding interest in all things medical. After moving to Colorado, she began transcribing medical reports for area hospitals, eventually moving on to transcribing pathology reports. She finds her current position transcribing



pathology reports at CSU VDL quite similar to, yet sometimes quite different from, people pathology. She lives in Windsor with husband Tom, three kids, two cats and a dog.

Alex Byas was born and raised in Macon, Ga., earned her bachelor's degree in avian biology and Spanish and her DVM at University of Georgia, and discovered research pathology while working in the Southeastern Cooperative Wildlife Disease Study surveilling avian influenza and studying Newcastle-disease pathogenesis. She looks forward to opportunities afforded by her anatomic pathology residency and PhD program here to continue infectious-disease research as well as oncological pathology.



Anna Farge, born and raised in rural Iowa, pursued some undergraduate study abroad in Tanzania, spending time with the Maasai tribe and learning food-security basics and international public health. The experience inspired her to pursue the MPH/DVM program here, focusing on antimicrobial resistance, biosecurity, zoonoses and infection control, including mapping Brazilian rabies outbreak interventions, collaborating with Kenyan veterinarians and studying food safety in Chile. Her PhD/Microbiology residency with the VDL will give her opportunity to continue studying infectious-disease microbiology, international food security, wildlife-disease epidemiology and disease ecology.



Caitlyn Martinez, new clinical pathology resident and PhD candidate, grew up in Tucumcari, N.M., always wanting to be a veterinarian. Following an animal-science bachelor's degree from Kansas State, she earned her DVM here, where she discovered an interest in clinical pathology. Some of her recent research involved malaria, and her primary research interests are in autoimmune disease and infectious disease. ▲



Lab Updates

VDL Director Honored for Service to National Diagnostic Lab Organization

VDL Director Barb Powers was named the 2015 recipient of the Distinguished Service Award from the American Association of Veterinary Laboratory Diagnosticians at the group's annual meeting in late October.

AAVLD grants the Distinguished Service Award to persons who have generously volunteered their time, energy and professionalism to substantially enrich and advance AAVLD and the field of diagnostic veterinary medicine. Powers, a long-time member of AAVLD, served on the AAVLD accreditation committee from 1998 until 2010, was Southwest

representative on AAVLD's executive board from 2001 to 2006, vice president of AAVLD in 2004 to 2005, president-elect from 2005 to 2006 and president from 2006 to 2007. She was immediate past president and chair of the awards committee and nominations committee from 2007 to 2008. She was also chair of AAVLD's foundation committee from 2002 until 2006.

Powers has been the AAVLD liaison to the Government Coordinating Council for Food and Agriculture since 2006 and the liaison to the Animal Agriculture Coalition since 2006. She was a member of the steering committee of the National Animal Health Laboratory Network in 2006-2008 and chair of that committee in 2008, after which she helped form and has been co-chair of a joint AAVLD/



USAHA Committee on the National Animal Health Laboratory Network. She also co-chairs the AAVLD Government Relations committee since 2006.

"I have personally learned a lot about behind-the-scenes legislative processes through my involvement with her," says AAVLD past president Catherine Barr. "The potential political significance of some AAVLD activities has given me a much better view of the global picture in which we operate."

Director of CSU's VDL since 1996, Powers is a graduate of Purdue, where she earned her bachelor's, DVM and master's degrees. She completed her PhD at CSU.

A Diplomate of the American College of Veterinary Pathologists in anatomic pathology, research interests and areas of specialty include surgical pathology, oncology, radiation pathology, orthopedic pathology and equine endometrial pathology.

Powers was also honored by AAVLD in 2011 with its E.P. Pope, the organization's highest award. She has been active in the Colorado Veterinary Medical Association, serving as president in 2003 and 2004 and currently serving as chair of the Commission on Advocacy and Outreach. She received the veterinarian of the year award from CVMA in 2005, has been an author on over 200 publications and mentor of many graduate students. In 2014 she received the Oliver Pennock Distinguished service Award from CSU. ▲

CSU RESIDENTS COMPLETE ACVP BOARDS AND EARN RECOGNITION AWARDS

Congratulations to these CSU VDL residents and alumni for successful completion of American College of Veterinary Pathologist board exams

New diplomates

- Dan Regan
- Elijah Edmondson
- Laura Brandt

Award winners

- Emily Rout: First place, ACVP young investigator award (poster) in the natural disease section

- Dan Regan: 2015 Charles Louis Davis, DVM Foundation Student Scholarship Award

- Elijah Edmondson: ACVP/ASIP Trainee Travel Award Charles Capen Best Manuscript Award

CSU RESIDENT RECOGNIZED NATIONALLY FOR CONTRIBUTION TO BOVINE MEDICINE

CSU VDL Resident Greta Krafsur has won one of two W.D. Farr Scholarships for the 2015-16 school year from the National Cattlemen's Foundation.

The \$12,000 scholarship recognizes outstanding students who plan to pursue careers in meat science and animal agriculture. Krafsur will receive the scholarship in January at the 2016 Cattle Industry Convention and National Cattlemen's Beef Association Trade Show in San Diego.

Krafsur, from Estelline, S.D., joined the VDL in 2013 and is using her pathology residency to explain the development of bovine high-mountain disease, known as brisket disease. She hopes to identify the biomarkers associated with the phenotype that can be used to predict disease risk,

with the goal of improving selective breeding, preconditioning and finishing regimens.

She received her master of science degree from the University of Tennessee and her doctor of veterinary medicine from CSU. She plans to continue the family tradition and start her own cow/calf herd.

The W.D. Farr Scholarships, established in 2007, recognize superior achievement in academics and leadership, and allow graduate students to further their study in fields that benefit the cattle and beef industry. Farr was the first president of the National Cattlemen's Foundation, and served as president of the American National Cattlemen's Association, which would later become



the NCBA. Before his death in 2007, he was known for his dedication to improving agriculture, livestock and water development, which has resulted in significant changes in farming methods that have influenced the practices of U.S. ranchers and farmers.

CSU VDL ON THE ROAD: UPCOMING CONFERENCES, SYMPOSIA AND APPEARANCES

Rocky Ford Branch Director Gene Niles will be on hand at the **Academy of Veterinary Consultants Spring 2016 Conference**, March 31 to April 2 in Irving, Texas. He recently attended the organization's Winter 2015 Conference in Denver.

VDL Director Barb Powers and **Case Coordinator Charlie Davis** attended the **Colorado Cattlemen's Association** mid-winter conference, Jan. 19 in Denver.

VDL Pathologist Sushan Han attended the American Association of Zoo Veterinarians **47th Annual Conference**, Sept. 26 through Oct. 2, 2015, in Portland, where she presented a case of osteolytic bone disease in a marmoset and also attended the **AAZV 21st Zoo & Wildlife Pathology Workshop** focused on collaborations between anatomic pathology and diagnostic imaging.

Pathologist EJ Ehrhart attended the **Comparative Ocular Pathology Society annual meeting**, Sept. 10 and 11 in Athens, Ga., as well as the American College of Veterinary Pathologists, American Society for Veterinary Clinical Pathology, and Society of Toxicologic Pathology **Combined Annual Meeting**, Oct. 17 to 21, 2015, in Minneapolis, along with **Pathologists Paula Schaffer** and **Chad Frank**. Both **Schaffer** and **Ehrhart** also attended the Colorado Federation of Animal Welfare Agencies' **2015 Colorado Animal Welfare Conference**, Sept. 18, 2015, in Black Hawk, Colo.

In September, **VDL Case Coordinator Charlie Davis** traveled to **Cherokee Ranch** in Sedalia, Colo., as a part of a Field Investigation Unit case along with **Pathologist Gary Mason** and a team of colleagues from Animal Sciences and Clinical Sciences. **Davis** and **Mason** also visited **Gray Ranch** at Rush, Colo., along with senior student Nigel Miller and **Rocky Ford Branch Director Gene Niles** as part of a Field Investigation Unit case management. In November **Davis** presented to the **State Extension Forum Livestock and Range Group** on the FIU and status of the residue testing availability at the VDL. He also attended a **Weld County Extension sheep management seminar** in Greeley, along with **VDL Residents Greta Krafsur** and **Alex Byas** and **VDL Fellow Paula Schaffer**. **Davis** also represented VDL with an information booth at the **Range Beef Cow Symposium**, Nov. 17 through 19, in Loveland. He also attended a **State Veterinarian's office porcine disease seminar** in Brush, Colo., along with **Schaffer**, **Krafsur**, **Byas** and **VDL Pathologist Chad Frank**.

Powers, **Mason**, **Krafsur**, **VDL Virology Section Head Christie Mayo**, **Avian and Molecular Diagnostics Section Head Kristy Pabilonia**, **Chemistry and Toxicology Section Head Dwayne Hamar**, **Parasitology Section Head Lora Ballweber** and **Resident Anna Farge** attended the 58th annual meeting of the **American Association of Veterinary Laboratory Diagnosticians**, Oct. 22 through 28 in Providence, R.I.

REGULAR COLUMNS

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New residue tests available.
- **ONCOLOGY** p. 3
How to ink liver margins.
- **PARASITOLOGY** p. 5
N. American Trypanosomes
- **SAMPLE Q.A.** p. 12
Biopsy sample packing tips.
- **ON THE ROAD** p. 15
Your chance to meet us.

LAB LINES

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Breakthroughs in next-generation diagnostics help us predict the unpredictable. Here's how.



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VDL pathologist helps identify possible case of cystic fibrosis in zoo orangutan.



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Five ways to apply serology in clinical practice.

FACES OF MYOCARDITIS . p. 8
Unraveling the etiology of this common bovine disease remains complicated.



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New bacterial and fungal ID equipment gives lightening fast results.



VDL IN PRESS. p. 12
New research from the faculty of CSU's VDL.

Update from the Director

**TO CONTACT
 BARB POWERS:**
 Call (970) 297-1281
 or email
 DLAB@colostate.edu

This issue of *LabLines* is full of articles I hope you don't miss:

- Our new molecular technology with use of next-generation sequencing.
- Our new MALDI-TOF bacterial identification system, which speeds identification even while improving accuracy.
- New residue testing for food animals.
- Case reports based on faculty research, and tips on better sample submission.
- A number of new people we would like you to meet. We have also received several awards; I was humbled and honored to



**BARBARA POWERS,
 DVM, PHD, DACVP
 DIRECTOR**

receive the Distinguished Service Award from the American Association of Veterinary Laboratory Diagnosticians. At the lab, we are in a period of careful self-evaluation right now. With our annual report near completion, I can reveal we had a near 10% increase in the number of accessions with a near 6% increase in the number of tests performed in the last fiscal year. In the first six months of this fiscal year, we are approaching over 15% increase in the number of tests. Indeed, business is booming!

At the same time, we recognize—sometimes reluctantly—lying on our immediate horizon are several challenges to meeting our dual goal of serving veterinary practices and animal owners even as we advance the public

mission of the university. We're working hard in a couple of ways to identify those potential vulnerabilities so we can tackle them while they are still surmountable.

For one, we recently finished meeting with our excellent External Advisory Committee. Despite the typical snow that always occurs, nearly everyone attended, and the input we gathered from board members and lab users is invaluable. Secondly, we are embarking on a new marketing plan—a task sometimes tackled only as afterthought in the public sector, but one we have learned, following careful anal-

ysis of our entire business process, that we cannot afford to neglect. We have learned we are markedly under-funded in marketing efforts. We have identified some processes we can improve, from your initial sample submission to the end result and the final billing process.

Now I really need to hear more from you about that experience. Whether good or bad, critical or complimentary, seemingly trivial or business-changing, I hope you will take a moment to look for one of us at various meetings or call or email me and give us honest input. ▲

Barbara E. Powers