

LAB LINES

Diagnostic news and trends from the Colorado State University Veterinary Diagnostic Laboratories
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Advances in Molecular Diagnostics

New Diagnostic Applications to Enlighten Antimicrobial Resistance

The U.S. Centers for Disease Control and Prevention estimate antibiotic-resistant pathogens cause over 2 million infections annually, contribute to 23,000 deaths and add \$20 billion in excess direct U.S. health-care costs, with another \$35 billion a year lost in social costs. One of the hotly contested questions surrounding the issue has been—and continues to be—the contribution of agricultural and veterinary use of antibiotics to the problem. Recent diagnostic innovations put to use in research by CSU's Microbial Ecology Group—a collaboration between our Department of Microbiology, Immunology and Pathology, and Clinical Sciences, Animal Sciences, Computer Sciences and the School of Education—have uncovered some important insights about the source and epidemiology of some resistance in the food chain.

TESTING FREED FROM CULTURE CONSTRAINTS

These new diagnostic innovations based on next generation sequencing (NGS) capture genome-specific information and take advantage of the speed and robust amount of data generated. Culture-independent NGS provides an increased resolution for characterizing pathogens without targeted enrichment or a preconceived idea of what the pathogen may be, making it 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.

For those reasons, NGS lends itself to understanding the often complex and pan-microbial genetic dynamics

of antimicrobial resistance. With its ability to combine many samples in a single sequencing run and obtain high sequence coverage per sample, NGS-based metagenomic sequencing can detect members of the microbial community in very low abundance that may be missed or are too expensive to identify using other methods. Such “shotgun metagenomics,” the study of whole-community DNA extracted directly from samples, allows us to comprehensively sample all genes in all organisms present in a given complex sample.

NEW APPLICATIONS, NEW INSIGHTS

That diagnostic capability freed from the need to culture has led to several discoveries at CSU:

- A study just published in December 2015 used shotgun metagenomics in to provide the first description of the resistome of North American dairy and beef production effluents. Sequencing produced over 1.05 billion sequencing reads across all samples, at an average of 30.8 million reads per sample. The study identified 34 mechanisms of antimicrobial resistance—mostly tetracycline-resistance mechanisms—within 34 soil, manure and wastewater samples from feedlot, ranch and dairy operations. Ranch samples contained significantly fewer mechanisms than dairy and feedlot samples, and the dairy resistome differed significantly from feedlots. Soil, manure and wastewater resistomes differed from one another, suggesting each should be managed differently. Further, by sampling a conventional against organic dairy, a U.S. against a Canadian feedlot and cow/calf ranch against a feedlot, we were

Supporting scientific efforts to mitigate the rising incidence of antimicrobial resistant infections is just one of the public-mission responsibilities of the Colorado State University Veterinary Diagnostic Labs. As a Level 1 laboratory in the National Animal Health Laboratory Network, the labs play a direct role in protecting a safe, stable and nutritious food supply for the nation. See page 13 for details.



See *SHOTGUN*, page 2

ANTIMICROBIAL RESIDUE TESTING AT CSU

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

SHOTGUN

(Continued from page 1)

able to baseline resistomes based on differences in, respectively, antimicrobial use, diet, and intensive vs. extensive systems.

- In another study, a metagenomic approach and shotgun sequencing technology detected pathogenic bacteria in environmental samples collected from the same groups of cattle at different processing steps in the beef-production chain: At feedlot entry and exit, in transport trucks, in slaughter holding pens and at the end of the fabrication system. It found some surprising results. Log read counts classified as pathogens per million reads for *Salmonella enterica*, *Listeria monocytogenes*, *E. coli*, *Staphylococcus aureus*, *Clostridium botulinum* and *perfringens* and *Campylobacter jejuni*, *coli* and *fetus* all decreased over each sequential processing step. In fact, normalized read counts for *S. enterica*, *E. coli*, and *C. botulinum* were greater in the final product than at the feedlots. Although some limitations of shotgun metagenomics makes this approach impractical for regulatory and confirmation purposes, its ability to characterize the microbiome and shifts in pathogen populations during production does shed important light on the potential role of resistome dynamics in the environment and the general products in the chain.
- Recognizing that treating antimicrobial-resistant infections is, in its current state, a purely reaction-

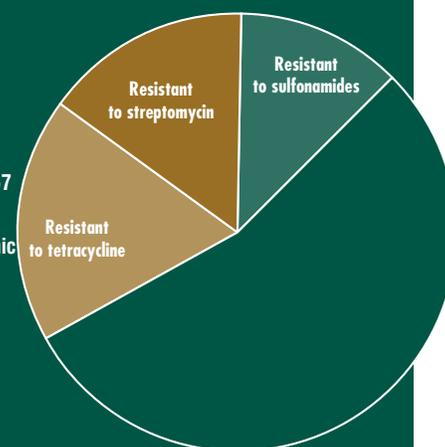
ary therapeutic process, another pilot study identified novel antimicrobial drug resistance markers that could lead to more clinically applicable detection and therapeutic tools. Such assays capable of rapidly identifying phenotypic expressions of resistant infections would not only improve therapeutic efficiency but also enhance surveillance. This study grew multi-drug resistant isolates of *Salmonella typhimurium* containing similar integron profiles in a liquid nutrient broth both with and without an ampicillin/chloramphenicol/streptomycin/sulfisoxazole/tetracycline drug panel. It then subjected the isolates to protein and metabolite extraction, followed by non-targeted proteomic analysis via liquid chromatography coupled with tandem mass spectrometry, ultra-performance liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry. Proteins were annotated using the Uniprot database, and metabolites annotated after screening against several spectral libraries and the Golm database. Results indicate distinct biomarker patterns in both protein and metabolite expression levels between isolates grown with and without the specific antimicrobials. This better understanding of biomolecule expression key to antibiotic resistance should open avenues for future larger studies to develop novel surveillance and diagnostic tools. ▲

CSU VDL Bacteriology Section Head Doreene Hyatt collaborated on a just-published study using conventional PCR to document antimicrobial-resistance patterns in enterohemorrhagic shiga toxin-producing *E. coli* O157 isolated from Colorado dairies. Individual-cow fecal samples collected monthly for a year from three dairies representing 2,750 lactating cows were tested using multiplex PCR assay to identify shiga-toxin producing *E. coli*. The 75 positives recovered were then tested for resistance to 10 gram-negative antimicrobials: amoxicillin-clavulanic acid, ampicillin, ceftiofur, enrofloxacin, florfenicol, streptomycin, sulfisoxazole, tetracycline, trimethoprim-sulfamethoxazole and tulathromycin. The study found only six isolates, or 8%, resistant to at least one of the 10 tested antimicrobial agents, all from a single dairy. The study detected no



significant effects on resistance profiles due to virulence genes, parity or previous antimicrobial treatments within the current lactation period. A follow-up study performing logistic regression analysis on O157 outcomes found humidity a risk factor for shedding enteropathogenic *E. coli* and an increase in temperature and recent antibiotic treatment a positive risk factor associated with enterohaemorrhagic *E. coli* shedding.

E. COLI RESISTANCE PROFILES IN ONE DAIRY



REFERENCE

McConnel CS, Stenkamp-Strahm CM, Rao S, Linke LM, Magnuson RJ, Hyatt DR. Antimicrobial Resistance Profiles in *Escherichia coli* O157 Isolates from Northern Colorado Dairies. *J Food Prot.* 2016 Mar;79(3):484-7.



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Advances in Parasitology

Tracking the Ominous Rise of Anthelmintic-Resistant Parasites

CSU Veterinary Diagnostic Labs' new polymerase chain reaction test for parasites can now successfully differentiate the five major bovine strongyle genera—*Cooperia*, *Haemonchus*, *Ostertagia*, *Trichostrongylus* and *Oesophagostomum*—within just two to three days. Although generalized benchmarking of parasite levels was always possible with fecal egg counts, this new option makes creating baselines for different geographic regions, herds and animal classes within herds more specific to the parasites present.

With that ability to quickly and accurately speciate parasites, researchers now have available a powerful tool for parasite surveillance, as well as for tracking the effect of longterm anthelmintic programs and management systems on individual parasite populations. These improvements will be important in helping keep a tab on the development of resistance to chemical anthelmintics in particular genera.

EVIDENCE OF RESISTANCE

CSU's VDL recently participated in a multi-state study to help assess the possible extent of that emerging resistance. During USDA's National Animal Health Monitoring System's 2007–2008 beef study, producers from 24 states were offered the opportunity to evaluate their animals for internal parasites and overall response to anthelmintics. Fresh fecal samples were collected from 20 animals, or from the entire group if less than 20, and then randomly assigned to either CSU VDL or one of two other participating labs for exam.

Samples in which strongyle egg counts exceeded 30 per gram by flotation were then subjected to pooled PCR analysis for the presence of *Ostertagia*, *Cooperia*, *Haemonchus*, *Oesophagostomum*, and *Trichostrongylus*.

Results from 72 producers in 19 States indicated that in over a third of the operations tested, anthelmintic treatment resulted in 90% or less reduction in fecal egg

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

counts at around two weeks after treatment. The 90% threshold is important because egg-count data are key components of licensing new anthelmintics, and average egg-count reduction values for all trials submitted must be greater than 90%.

All operations exhibiting less than a 90% reduction had used pour-on macrocyclic lactones as the anthelmintic treatment. While some of these reductions could have been the result of improper drug application, PCR analyses of the parasite populations surviving treatment, coupled with follow-up studies at a limited number of sites, indicated they were most likely due to anthelmintic resistance in *Cooperia* spp. and possibly *Haemonchus* spp. The PCR data indicated lack of efficacy is biased toward members of those two genera.

PCR results also argued against another alternate explanation: That some animals were simply missed during anthelmintic treatment. But PCR results confirmed reductions were typically observed in all genera except *Cooperia* and *Haemonchus*—a phenomenon that wouldn't have occurred in missed animals.

It is clear that the efficiencies of treating with macrocyclic lactones today in U.S. commercial operations are not the same as the efficacies generated when the drugs were first licensed. Our results should be a warning to cattle producers: They can no longer assume treatment is synonymous with successful control, they should rotate their anthelmintics, they should consider the given that pour-on formulations offer the best opportunity to encourage resistance, and they must remember that successful long-term and sustainable control can't be obtained by relying on anthelmintic treatment alone. ▲

PARASITE PCR TESTING AT CSU

SAMPLES

- At least 3 grams of feces from each individual as or immediately after being passed.
- Select samples from no more than five individuals for pooled sampling.
- Include fecal egg counts if known.
- Package in clean, enclosable container, like a sealable plastic bag, devoid of air.
- Refrigerate for no more than 24 hours. Ship cold, but protect from freezing and direct contact with ice.
- Ship to arrive within 48 hours.

COST

- \$70 per sample

TURNAROUND

- Two to three days

Zoo Corner

Tularemia in Zoo Primates

CSU's Veterinary Diagnostic Labs have confirmed a noticeable increase in the number of positive tularemia cases in both wild and domestic animals in the past few years. Environmental conditions have favored significant expansion of both wildlife reservoirs and insect vectors, increasing the incidence in domestic animals and posing an important zoonotic risk for people.

Caused by the Gram-negative coccobacillus *Francisella tularensis*, tularemia is endemic in North American wildlife, particularly in south-central United States. Among domestic animals, cats are particularly sensitive, with cases commonly occurring in dogs, pigs, horses and sheep. U.S. human cases also occur every year; 16 human cases were confirmed in Colorado in 2014 and over 20 in 2015.

Localized disease manifests as ulceroglandular or oropharyngeal disease involving the skin or mucous membranes, with lymphadenitis of draining nodes. Oropharyngeal disease is particularly common in cats, which capture and consume infected prey. Localized disease can progress to septicemia with generalized lymphadenitis and severe inflammation and major-organ necrosis.

Tularemia has been well described in both new- and old-world non-human captive primates, in which the disease can be rapidly fatal, often involving multiple animals. CSU's Veterinary Diagnostic Labs have confirmed tularemia in three different species of non-human primates in two south-central facilities in 2015 and 2016. All were housed in outdoor/indoor exhibits with the same species.

SIAMANG

A 24 year-old female Siamang found acutely moribund, severely weak and stumbling received supportive treatment but continued to rapidly decline. Bloodwork and physical exam suggested severe septicemia with advanced organ failure. Necropsy following euthanasia showed dehydration, fibrino-hemorrhagic peritonitis, marked hepatic swelling and segmental hemorrhagic serositis of the small intestine. Histopathology found severe hepatitis and splenitis that was suppurative and necrotizing and effaced up to 30% of the parenchyma. Though Gram and silver stains identified no bacteria, PCR confirmed tularemia. This female's offspring was also found listless and febrile within days of its death. Treated aggressively with doxycycline, the male made a full recovery, and no other primate in the enclosure was affected.

BLACK-CRESTED MANGABEY:

A 28 year-old male black-crested mangabey was examined following four days of clinical lethargy and weak-

— *Sushan Han, DVM, PhD, DACVP, CSU VDL Pathologist*

ness. Exam showed fever, hypoglycemia and leukopenia suggestive of septicemia. The monkey was managed aggressively with antibiotics and supportive therapy, but continued to decline over the next day, remaining hypoglycemic and developing ascites. Necropsy confirmed ascites, mild thoracic effusion and swelling of the liver and spleen with generalized lymphadenopathy. Lymph nodes bulged and hemorrhaged on the cut section, suggestive of active lymphadenitis. Histopathology confirmed fibrino-necrotizing hepatitis, splenitis and severe suppurative, necrotizing lymphadenitis involving most internal lymph nodes and the tonsils. The lesions yielded no microorganisms upon Gram and silver stains, but PCR confirmed tularemia. No additional animals in this group developed clinical signs.

COMMON MARMOSETS

A 7-year-old female common marmoset found acutely depressed, weak and listless with possible neurological deficits was treated with supportive care under the assumption it may have suffered head trauma. Despite treatment, it rapidly deteriorated in 24 hours and died. Five days later, a second 7 year-old female marmoset with no prior clinical signs was found dead in the same enclosure. Gross findings in both females showed marked dehydration, prominent generalized peripheral and internal lymphadenopathy, and moderate hepatosplenomegaly with fine tan-white pinpoint foci throughout the parenchyma. The lungs were diffusely and moderately congested and edematous on the cut section.

Histology demonstrated severe suppurative and necrotizing hepatitis, splenitis and lymphadenitis, interstitial pneumonia and moderate pulmonary edema. One marmoset also had necrosis of the bone marrow. The other had moderate lymphoplasmacytic meningitis. In one marmoset, large numbers of fine Gram-negative coccobacilli were associated with necrotizing lesions, though no organisms were detected in the other marmoset. Fresh liver submitted for PCR showed tularemia in both animals, but no plague, toxoplasmosis or lymphocytic choriomeningitis virus. No additional animals were affected.

SEARCH FOR THE INFECTION SOURCE

Animals in these four cases, and all published cases of natural infection, had access to outdoor enclosures. Tularemia is endemic in several wildlife reservoirs. In the south-central United States, it is commonly reported in prairie dogs and other rodents, including

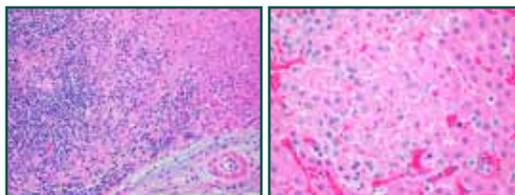
mice. Disease occurs from early April to late October, when vectors are prevalent. The bacteria are generally transmitted via ticks, fleas and deer flies, but they can also be transmitted via inhalation, wound or mucous-membrane contamination or ingestion of bacteria from contaminated water, environmental sources or infected tissues of dead hosts.

Though a source of tularemia was not definitively determined in any of these four cases, it is presumed to be due to exposure to infected rodents or lagomorphs within the enclosures, resulting in transfer of infected ticks and fleas, contamination of water and food sources, and possibly hunting and consumption of infected prey by the primates.

Captive non-human primates of several species are very sensitive to tularemia. Each of these four reported cases progressed to septicemia rapidly, with few non-specific and even presumptive neurological signs, prior to severe morbidity and death. Early recognition of tularemia-like signs and aggressive treatment with effective antibiotics and supportive care can be successful, though septicemic cases, as demonstrated, can progress rapidly despite proper treatment. ▲



A 7 year-old female common marmoset showed marked caseous and necrotizing submandibular and mesenteric lymphadenitis, confirmed by PCR to be positive for Tularemia.



Photomicrographs of a mesenteric lymph node (left) and the liver (right) showed marked necrotizing lymphadenitis and random hepatic necrosis, respectively, consistent with embolic showering by bacteria.

SOURCES

Sammak RL, Rejmanek DD, Roth TM, Christe KL, Chomel BB, Foley JE. Investigation of tularemia outbreak after natural infection of outdoor-housed rhesus macaques (*Macaca mulatta*) with *Francisella tularensis*. *Comp Med*. 2013 Apr;63(2):183-90.

Ferrecchia CE, Colgin LM, Andrews KR, Lewis AD. An outbreak of tularemia in a colony of outdoor-housed rhesus macaques (*Macaca mulatta*). *Comp Med*. 2012 Aug;62(4):316-21.

Guthrie AL, Gailbreath KL, Cienava EA, Bradway DS, Munoz Gutierrez JF. Septic tularemia in 2 cottontop tamarins (*Saguinus oedipus*). *Comp Med*. 2012 Jun;62(3):225-8.

Mätz-Rensing K1, Floto A, Schrod A, Becker T, Finke EJ, et al. Epizootic of tularemia in an outdoor housed group of cynomolgus monkeys (*Macaca fascicularis*). *Vet Pathol*. 2007 May;44(3):327-34.

Chemistry and Toxicology

Old Stored Toxicants Can Still Kill

The VDL received a call from a practitioner reporting about 20 cattle found dead in an area of old farm buildings. The cattle had broken through a gate and entered one of the buildings; inside was found some green material. When one thinks of green colored material that might be toxic, one first suspects products that are currently available. The most common substance that comes to mind is some type of rat bait, likely an anticoagulant.

A sample of the green material and tissue from one of the dead animals was submitted to the laboratory for testing. The green material was 10.1% arsenic and 11.3% copper, by dry weight. The arsenic concentration by dryweight of the liver was 290 ppm; of the kidney, 340 ppm.

Equipped with that information, our consultation with the veterinarian brought to mind a product available many years ago and used in agriculture as a pesticide: Paris Green. Finding high concentrations of both copper and arsenic confirmed the toxicity was caused by old insecticide and rodenticide, whose chemi-

— Dwayne Hamar, PhD, CSU VDL Chemistry and Toxicology Section Head

cal name is copper(II) acetoarsenite. But it also reminds us that when dealing with an old building, one needs to consider what was available in years past, not just today. ▲



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Serology

Get the Most from Your Bovine Serology

The most common reason the CSU Veterinary Diagnostic Lab sees clinicians submitting bovine serology is to deduce the etiologic agent for either bovine respiratory disease or bovine abortion. Serological assays—which by definition involve not only detection of specific changes in the serum, such as antibodies to the pathogen produced by the host, but also antigens of the infecting agent itself or its components—have commonly been used to diagnose viral infections. However, today's ubiquitous exposure of cattle in the United States, both naturally and by vaccination, has made serology difficult to interpret.

Here are a few ideas to help you get more out of this traditional diagnostic tool. Keeping these ideas in mind will help maximize your use of diagnostic testing, allowing for the time to make a reasonable diagnosis and easing the frustration of limited information you receive from a single titer.

Test selection is paramount. If a practitioner is going to embark on the journey of using serology to help diagnose disease, it's prudent to make appropriate selections of animals based on diseases affecting the herd and their timing.

It is imperative to remember the type of test performed depends on the herd history. For example, if type II BVDV was isolated from a fetus or calf in the herd, type II BVDV serum-neutralization tests might be indicated. An animal infected with type II BVDV generally will have lower antibody titers to type I BVDV.

In regard to timing, screening of young, unvaccinated cattle between 6 and 12 months of age may be useful in determining if viral pathogens have recently been or are currently circulating in a herd. Serological assays are also available to help differentiate exposure.

Paired sera still matter. In individual cattle thought to have acute exposure in a herd, there's still no comparison to the traditional uses of serological assays for diagnosing viral infections through paired acute and convalescent sera. In cases of acute illness, paired acute and convalescent serum samples are more likely to be useful information than a single sample. A single SN

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

titer is unlikely to yield definitive information since it does not distinguish between current infection, previous exposure or vaccination. If a virus is involved in the disease, a four-fold increase in titer will be observed over time, which can be benchmarked against.

It's important to remember that because the serum neutralization assay, the most commonly used method for detecting viral antibodies, measures the ability of antibodies in test serum to neutralize a reference viral isolate, some within-lab titer variations may exist over time. For that reason, it is important to submit those paired acute and convalescent sera simultaneously. Titers to different pathogens can differ substantially depending on the antigenic exposure.

Look for additional comparisons. Even paired acute and convalescent serum samples from an aborting cow rarely will show a change in titer, because cows infected with BVDV and BHV-1 abort weeks to months after infection. Therefore, it may be more useful to compare SN titers from cows with healthy calves to the titers of cows that have aborted. Fetal serum samples can be helpful in some cases. If the fetus was infected during the last half of gestation, it may have made antibodies to the infecting virus. ▲

BOVINE SEROLOGY QUICK TIPS

- Consider the presenting complaint: Abortion, respiratory disease, duration of illness
- Record type and age of cattle—beef, dairy, cow/calf, feedlot, calves, lactating cows
- Include number of animals in the herd, ill and dead
- Consider vaccinations—brand of vaccine, modified live or killed, age at vaccination
- Note date of last vaccination and product used
- Account for colostrum intake: Serology tests do not distinguish between passive immunity via maternal antibodies and active immunity induced by infection or vaccination

Guardians of Public Health

National Animal Health Lab Network More Critical than Ever

The Veterinary Diagnostic Laboratories at CSU are a Level 1 laboratory in the USDA's National Animal Health Laboratory Network. NAHLN-member labs 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. Our full accreditation by the American Association of Veterinary Laboratory Diagnosticians allows admittance to the NAHLN without additional requirements related to documentation of a quality management system.

NAHLN plays a direct role in protecting a safe, stable and nutritious food supply. It will play an essential role in responding to a biological attack affecting animals or people. An example of the critical surveillance role NAHLN plays was evident during the 2015 highly pathogenic avian influenza outbreak. NAHLN laboratories operated 24 hours a day, seven days a week to test poultry samples. Those quick, reliable tests permitted rapid depopulation of infected flocks, surveillance testing to halt spread of the virus and testing to permit repopulation of farms and resumption of trade.

NAHLN was developed in response to the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. During the past 12 years the NAHLN, composed of federal, university and state veterinary diagnostic labs, has established the framework of a surveillance and emergency response system that provides critical and ongoing resources for laboratory testing, surveillance and information management, including data analysis and sharing, quality assurance with an auditing system and the development and validation of new tests.

Today, it requires increased funding to improve compliance with 2012's Homeland Security Presidential Directive, which required the department to "develop nationwide laboratory networks for food, veterinary, plant health and water quality that integrate existing Federal and State laboratory resources, are interconnected, and utilize standardized diagnostic protocols and procedures," according to the directive.

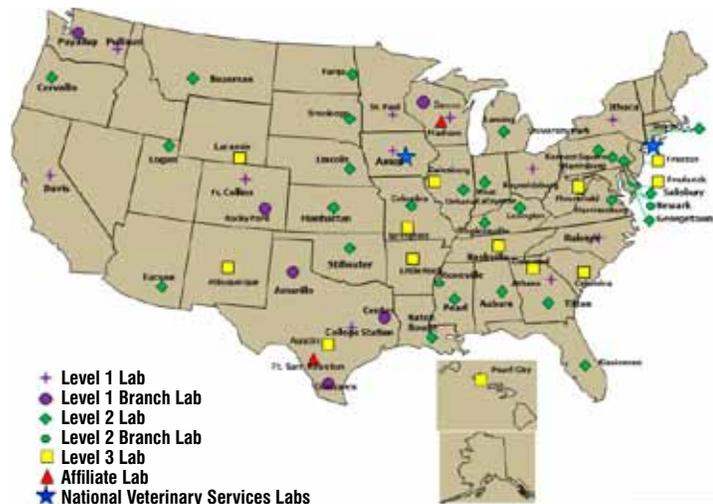
That improved compliance with the directive will

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

require expanding surveillance and surge capacity of the NAHLN by increasing the number and level of participating state laboratories. It will require additional development of the infrastructure for electronic transmission of data between sample collectors, laboratories and state and federal databases. It will also call for increasing efficiency and effectiveness of lab personnel training nationwide.

NAHLN is essential to the health of U.S. animal agriculture, bioterrorism surveillance and the U.S. economy. USDA estimates the cash receipts of the U.S. animal industries at \$185.68 billion; therefore, even a fully funded level of \$30 million would represent only a 0.016 percent federal investment for disease surveillance to protect essential agriculture. ▲

NAHLN LAB DESIGNATIONS



CSU VDL in the Field: Case Studies

The Many and Varied Presentations of Loco Weed Toxicosis

Over the last two years, CSU VDL's Rocky Ford laboratory has been involved with several cases involving locoweed toxicosis:

■ In summer 2015, we became involved with a case of suspected locoism in which one horse, while being checked, acted very nervous. When approached, it reared up, fell backward, struggled to get up and died within minutes. A second horse, pastured in the same remote location, was not approached and considered normal by the owner; however, it later died in a similar manner while being trailered. Neither horse was necropsied, but the southeastern Colorado pasture they were in is known for heavy locoweed contamination.

■ At the same time, a rancher in the same area reported a group of stocker calves exhibiting a variety of clinical signs ultimately associated with locoweed:

- Several died from right heart failure. Cattle grazing pastures above 6,500 feet containing locoweed frequently exhibit right heart failure. The calves in this case were at an altitude of 4,000 feet.
- Many calves performed poorly, with progressively worsening dull, rough hair coats.
- Compared to previous years, they experienced an increase in the number of foot problems, joint abscess and miscellaneous injection-site abscesses.
- The number of total pneumonias and non-responsive pneumonias were increased. Although death loss was higher than previous years, none of the calves tested were diagnosed with BVD. No PIs were found.

Necropsies along with liver trace-mineral profiles were performed on the majority of the animals that died, and no common underlying cause was identified, other than ingestion of locoweeds. Three samples of woolly loco were taken from different paddocks on the ranch, and a sample of 2014 hay was analyzed for swainsonine, the agent in locoweed responsible for locoism. Swainsonine interferes with

— Gene Niles, DVM, Director, CSU VDL Rocky Ford Branch; and Charlie Davis, DVM, CSU VDL Lab Coordinator



Non-specific clinical signs of locoweed toxicity in cattle can include (left) poor-doing calves with dull, rough haircoats, indicative of immunosuppressive effects or reduced reproductive performance of dams, and (right) submandibular/brisket edema related to swainsonine's effect on pulmonary vascular resistance and hypertension.

glycoprotein metabolism in tissues throughout the body. The samples contained 0.18%, 0.21%, 0.27% and 0.15% swainsonine, respectively. These values are 3.5 to more than five times above the recognized toxic level of 0.05%.

■ Over the last two years, we have had several eastern and southeastern Colorado cattle herds with poor reproductive performance, abortions and the birth of small, weak calves that fail to thrive in which we were unable to determine an infectious or nutritional cause. In one of these herds, the cows exhibited neurological signs of locoism the following summer, leading to rough-coated, poor-doing calves this spring and a second round of poor conception rates, abortions, hydrops amnii, weak calves that fail to nurse and aborted calves with joint laxity and very high liver selenium values. In two other herds, degenerative vacuolation compatible with swainsonine toxicosis was documented in fetal tissues. Congenital alpha-mannosidases deficiency was not ruled out in either herd—both of which were black-headed—but neither had a history of storage disease.

VARIED CLINICAL SIGNS IN CATTLE AND HORSES

All these cases demonstrate some of the varied clinical presentations of locoweed toxicity. Five clinical syndromes are associated with locoweed toxicosis. Four occur in North America which include locoism,

selenosis, photosensitization and nitrotoxicosis. The fifth, thiamine-related neurotoxicosis, affects sheep in Morocco.¹

Clinical signs of locoism in horses include depression, apparent blindness, blank stare, stiff-legged exaggerated leg movements, nibbling lip movement and difficulty eating. When startled, horses may rear up and fall over backward. Belligerence and violent behavior may follow. Surviving horses may have difficulty seeing and hearing along with incoordination due to an exaggerated gait making them unreliable for future use.¹

Neurological signs seen with cattle, sheep and goats are similar to horses but progressively less violent. Nervousness and belligerence, weakness, depression, difficulty eating, progressive weight loss and rough hair coats occur. Cattle may have exaggerated gaits, isolate themselves and jump for no apparent reason. Sheep become ataxic and high-headed and tend to isolate themselves. Goats may have temporary hind-limb paralysis that can become permanent.

Swainsonine suppresses the immune system, leading to increased respiratory disease and mortality in feedlot calves. Days on feed were increased by more than 60 days in one study.³

Ingestion of swainsonine causes poor reproductive performance in all livestock. An increase in non-pregnant animals, ovarian dysfunction, lengthened estrus cycles, increased calving intervals, abortions, musculoskeletal defects, birth of small weak offspring that fail to suck and loss of mothering ability and bonding instinct are seen with locoism. Increased numbers of hydrops amnii cases may occur.^{3,4} Swainsonine has an effect on all stages of female reproduction with a significant drop in serum alpha-mannosidase and increase in serum swainsonine levels within 24 hours of exposure to locoweed. Lengthened estrus cycles and reduced conception rates have been observed in ewes and cows fed 15% to 20% locoweeds in their diets for 20 and 30 days, respectively.³ Males have poor libido, decreased sperm motility and increased morphological defects.

It is recommended that breeding females should not be allowed to graze locoweed for more than two to three weeks at a time, and pregnant animals should not be allowed to graze locoweed at any time.³ Breeding males should not graze locoweed pastures during



Cattle grazing high-altitude pastures containing locoweed frequently exhibit right heart failure (top). Other pathologies include (above) congested liver and ascites, as well as (right) liver abscesses.



breeding season or for 90 days pre-breeding. The reproductive effects on both female and male animals are self-limiting and return to normal within 60 to 70 days after removal from locoweed.^{3,4}

Swainsonine is found in all parts of the plant with the highest amount in the seeds and pods and least in the foliage. Flowers contain intermediate levels of swainsonine. Although 0.05% swainsonine is considered the toxic dose with routine lengths of exposure, swainsonine is cumulative and concentrations of as little as 0.001% can cause disease with prolonged consumption of the plant. Clinical signs occur when the threshold dose reaches 0.3 mg/Kg of body weight. At this dose, all of the available alpha-mannosidase is inhibited and larger doses do not cause additional effects.^{1,2} ▲

IN THE NEXT ISSUE OF LAB LINES:

High blood and tissue selenium values and other clinical syndromes that occur with locoweed toxicosis.

REFERENCES

- 1 Borrows GE, Tyril RJ. editors. Toxic plants of North America. 2nd Edition. Iowa: Wiley-Blackwell 2013.
- 2 James Lynn F, Panter Kip E, Stegelmeier Bryan L, et al, Astragalus and oxytropis poison livestock with different toxins, In: Toxicology locoweed research: updates and highlights 1999 Available at: http://aces.nmsu.edu/pubs/research/livestock_range/RR730/toxicology.pdf
- 3 Panter KP, James LF, Gardner DR ,et al, Reproductive losses to poisonous plants: influence of management strategies, J Range Manage 2002 55:301-308
- 4 Ralphs Michael H, Stegelmeier Brian L, Locoweed toxicity, ecology, control and management in International journal of poisonous plant research: Vol 1, fall 2001; 47-58 Available at: <http://www.ars.usda.gov/pandp/people/publication.htm?personid=5392>.
5. Stegelmeier Bryan L. Poisonous plants that contaminate hay and forages in the western United States. Proceedings, 2013 Western States Alfalfa and Forage Symposium, Reno NV. UC cooperative Extension, Plant Sciences Department, University of California, Davis, CA. 2013. Available at: http://alfalfa.ucdavis.edu/+symposium/proceeding/2013/13was-2_29_stegelmeier_poisonousplants.pdf.

Innovations in Histopathology

Chronic Wasting Disease Diagnostic Discoveries from Ground Zero

Here at “ground zero” of the outbreak of Chronic Wasting Disease, a geographically expanding transmissible spongiform encephalopathy occurring naturally in captive and free-ranging cervids, CSU’s Veterinary Diagnostic Laboratory collaborates with researchers from various governmental organizations and CSU faculty across the globe to explore many aspects of such unusual and enigmatic prion diseases. One of the groundbreaking aspects of this work has been development and refinement of an extremely sensitive, reproducible antemortem prion diagnostic test: An immunohistochemistry staining technique of recto-anal mucosa-associated lymphoid tissue capable of identifying the misfolded variant of native prion protein, termed “PrP^{CWD},” which leads to neurodegeneration and ultimately death. Because PrP^{CWD} accumulates in tissues of the lymphatic system early in the infection process, particularly in lymphoid follicles, it can be detected in live animals.

Using this test, several important findings about the disease have been published:

- Biopsy of rectal mucosa-associated lymphoid tissue provides a useful live-animal test for chronic wasting disease in mule deer, white-tailed deer and Rocky Mountain elk. It is difficult and expensive to complete these tests on free-ranging animals, but wildlife health managers will benefit from methods that can accommodate test results of varying qual-

— Terry Spraker, DVM/PhD/DACVP, CSU VDL Pathologist

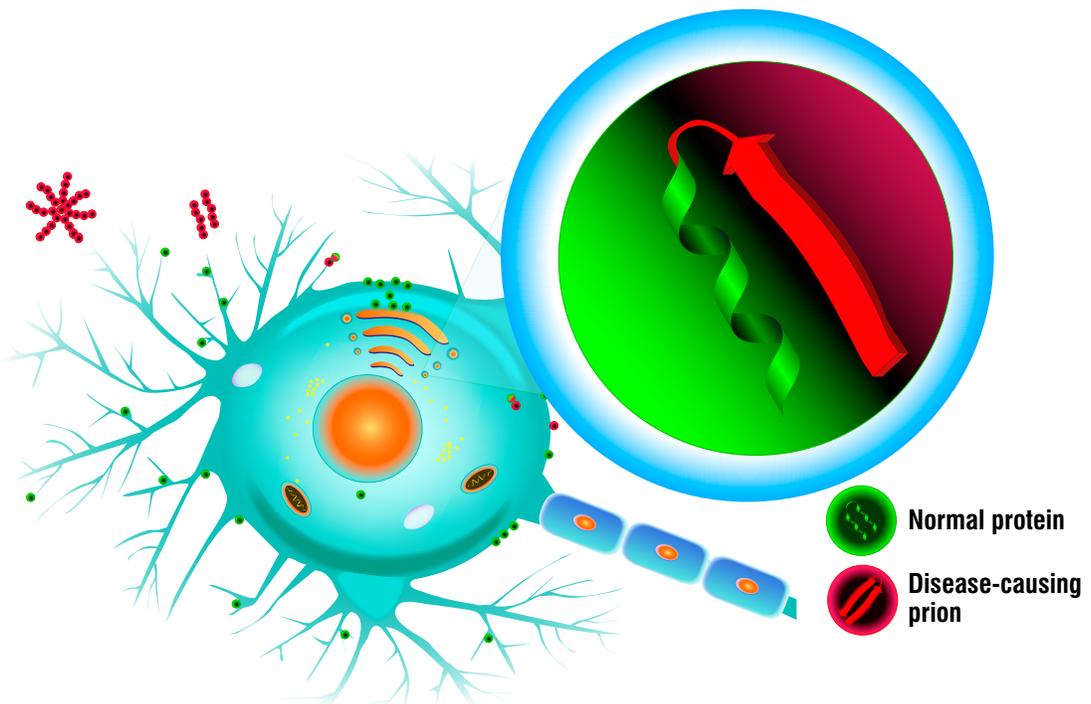
ity. To this end, researchers with the CSU Department of Wildlife developed a hierarchical Bayesian model to estimate the probability that an individual is infected based on test results. Using data on 210 adult female mule deer, they demonstrated at least five follicles were needed in a biopsy to assure a 95% accurate test.

- Using the progressive accumulation of the abnormal conformer of PrP^{CWD} and spongiform degeneration in a single section of brain stem in Rocky Mountain elk, we were able to create a formula to generate an overall obex score. This scoring technique using a single section of obex may prove useful in future work for estimating the presence and abundance of PrP^{CWD} in elk peripheral tissues and the nervous system.

- Although no one has yet documented natural cross-species CWD transmission, we do know infectious prion material can be passed in the feces of crows. Another study demonstrated CWD-infected elk brain material could similarly pass through the gastrointestinal tract of coyotes and be infectious for at least three days in a cervidized transgenic mouse model. Coyotes and other common scavengers from CWD-enzootic areas may play an indirect role in disease transmission. ▲

REFERENCES

- Nichols TA, Fischer JW, Spraker TR, Kong Q, VerCauteren KC. CWD prions remain infectious after passage through the digestive system of coyotes (*Canis latrans*). *Prion*. 2015 Sep 3;9(5):367-75.
- Geremia C, Hoeting JA, Wolfe LL, Galloway NL, Antolin MF, Spraker TR, Miller MW, Hobbs NT. Age And Repeated Biopsy Influence Antemortem Prp(CWD) Testing In Mule Deer (*Odocoileus Hemionus*) In Colorado, USA. *J Wildl Dis*. 2015 Oct;51(4):801-10.
- Spraker TR, Gidlewski T, Powers JG, Nichols T, Balachandran A, Cummings B, Wild MA, VerCauteren K, O'Rourke KI. Progressive accumulation of the abnormal conformer of the prion protein and spongiform encephalopathy in the obex of nonsymptomatic and symptomatic Rocky Mountain elk (*Cervus elaphus nelsoni*) with chronic wasting disease. *J Vet Diagn Invest*. 2015 Jul;27(4):431-41.





Young women from a group of about 80 high-school science students from around Fort Collins line up to view Mycobacterium tuberculosis during March's World TB Day, sponsored by the CSU Department of Microbiology, Immunology and Pathology.
Photo: John Eisele/CSU Photography

Lab Updates

VDL Members Promote 'Girls & Science'

Two CSU VDL faculty members participated at the Denver Museum of Nature & Science's Girls & Science event in March, designed to help young women meet successful women scientists and ignite a passion for science.

Five faculty members and eight students from CSU participated in this effort. In this new spin on the traditional career fair, attendees got the chance to explore "Science Clubhouses" throughout the museum, where girls met role-model women scientists and participated in hands-on activities to experience the many diverse opportunities in science,

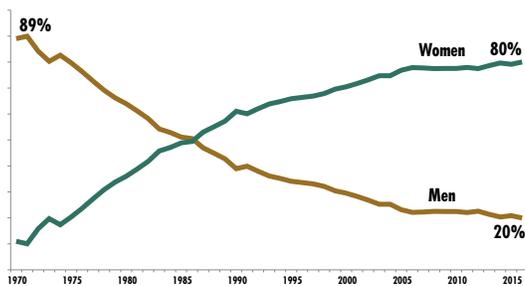
— Deanna Daily, DVM, DACVP, PhD, CSU VDL Pathologist; and Christie Mayo, DVM, PhD, CSU VDL Virology Section Head

technology, engineering, art and math. The full-day event this year nearly doubled the number of attendees of last year's inaugural year, with a reported total attendance of 11,569.

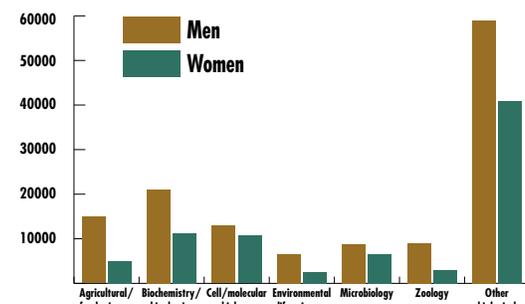
CSU participants represented areas of expertise in veterinary medicine, human medicine, geology, archaeology, entomology, genetics and even scientific illustration.

Outreach efforts like the Girls & Science program are important because women remain under-represented in college majors, graduate school programs and the professoriate in mathematically intensive fields like geoscience, engineering, economics, computer science and the physical sciences. Even in veterinary science, where women enrollees now outnumber men by 80% to 20%, professional women still earn 18% to 21% less than men. ▲

ENROLLMENT IN THE US VETERINARY MEDICAL COLLEGES



DOCTORATES GRANTED (YEAR 2013)



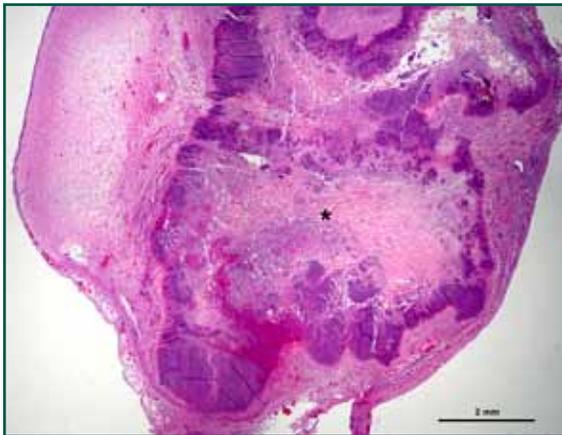
CSU VDL In Press

A Roundup of VDL Faculty Research

BVD IMMUNOSUPPRESSION

Vilander AC, Niles GA, Frank CB. Cerebral Candidal Abscess and Bovine Viral Diarrhoea Virus Infection in an Aborted Bovine Fetus. *J Comp Pathol.* 2016 Feb-Apr;154(2-3):161-4. doi: 10.1016/j.jcpa.2016.01.005.

VDL Rocky Ford Branch Director Gene Niles and Pathologist Chad Frank collaborated with CSU Veterinary Resident Allison Vilander in this study, only the third ever to report an infection of the brain in an animal by the opportunistic fungi *Candida*.



The case study of a 250-day-gestated calf submitted without placenta for necropsy showed about 40% of the cerebrum was effaced by a soft, fluctuant, mottled red to white exudative mass. GMS stain revealed several fungal pseudohyphae within the areas of necrosis and inflammation consistent with *Candida* spp. organisms. Fungal culture revealed heavy *Candida* growth, confirmed by our fungal polymerase chain reaction. *C. etchellsii*, which was identified, has never been associated with bovine abortion and has never been reported to be pathogenic in any species.

The exact route of exposure to *C. etchellsii* in this case is unknown. However, invasive candidiasis occurs secondary to immunocompromising conditions, and BVDV capture ELISA and FA testing on the calf's brain,

liver and lung tissue were all positive, consistent with persistent infection.

BVDV is a pestivirus that causes persistent infection in calves infected between day 40 and day 120 of gestation or that are born to a persistently infected mother. It has also been associated with some cases of bovine mycotic abortion. Since *C. etchellsii* has not previously been considered a pathogenic fungus, it is unlikely fetal invasion would have occurred in the absence of the coinfection or immunosuppression.

RETINAL LYMPHOMA IN DOGS AND CATS

Malmberg JL, Garcia T, Dubielzig RR, Ehrhart EJ. Canine and feline retinal lymphoma: a retrospective review of 12 cases. *Vet Ophthalmol.* 2016 Feb 12. doi: 10.1111/vop.12356.

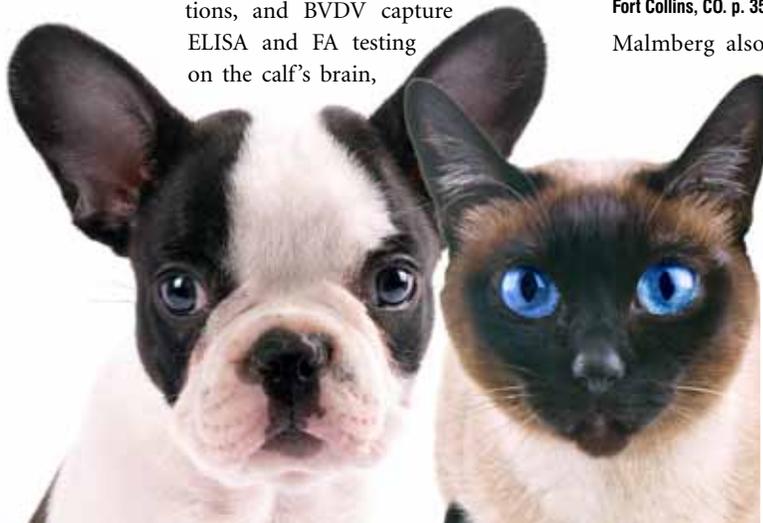
VDL Resident Jennifer Malmberg and VDL Pathologist EJ Ehrhart collaborated on this study that queried the databases of CSU's Veterinary Diagnostic Lab and Wisconsin's Comparative Ocular Pathology Lab to identify cases between 1996 and 2013 of retinal lymphoma in dogs and cats, an uncommon and previously uncharacterized entity of adult dogs and cats.

Eight canine and six feline cases were ultimately incorporated into this retrospective study, based on explicit inclusion criteria. Their findings demonstrate the typical morphologic and immunophenotypic features parallel the malignant counterpart in humans. While the characteristic dissemination pattern of retinal lymphoma in people is largely confined to the central nervous system, the frequency and extent of neoplastic involvement of extraocular tissues in the dog and cat remains unclear. Early enucleation and clinical staging may improve the prognosis.

UNIQUE VIRAL SPECIES IN TWO FELINE HOSTS

Malmberg JL, Lee J, Templin-Hladky S, Troyer RM, VandeWoude S. Evidence for frequent lentiviral transmission from bobcats to mountain lions in California and Florida: Implications for emergence of lentiviral epidemics. *Proceedings of the 17th Annual Research Day; 2016 Jan 30; Fort Collins, CO.* p. 35.

Malmberg also collaborated with colleagues from CSU's Department of Microbiology, Immunology and Pathology to document frequent natural cross-species transmission of a subtype of feline immunodeficiency virus (FIV) between bobcats and mountain lions in California and Florida. In this study they investigated host selection pressures, estimated within-host viral



fitness, and examined phylogenetic relationships of this FIV subtype in free-ranging bobcats and mountain lions. By identifying ongoing selection pressures and low viral fitness in the mountain lion, they demonstrated that infections in mountain lions are largely dependent on contact with infected sympatric bobcats. The results provide empirical evidence that the virus is host adapted to the bobcat, less fit in the mountain lion, and therefore under intense selection pressure to adapt and emerge in the novel mountain lion host.

NECROPSY OF THE HORSE

Frank C, Madden DJ, Duncan C. *Field Necropsy of the Horse*. *Vet Clin North Am Equine Pract.* 2015 Aug;31(2):233-45. doi: 10.1016/j.cveq.2015.04.002.

CSU VDL Pathologist Colleen Duncan co-edited the first issue of *Veterinary Clinics of North America: Equine Practice* that has been entirely devoted to pathology and diagnostics. While some equine pathology subjects had been covered in individual issues, this single issue devoted to the broad category of diagnostic pathology was intended as a useful single resource for equine practitioners. The need for such an issue highlights both the continuous stream of new information in the field of diagnostics and the emergence and evolution of equine diseases that can provide a diagnostic challenge for veterinarians.

In a contribution to the issue, Duncan and VDL Pathologist Chad Frank detail a generalized overview of the equine necropsy that can be used by veterinarians in the field. Use of a systematic process enables the practitioner to develop a familiarity with



normal anatomic positioning and tissue appearance such that abnormalities are quickly identified, Necropsy is an invaluable diagnostic tool that can be used not only to determine cause of death, but also answer a range of clinical questions, provide legal documentation, and serve as a source of education to the client and practitioner. By following the systematic steps outlined in this article the practitioner can confidently perform a thorough field necropsy to enhance the chances of achieving a diagnosis and to gain a better understanding of the case. ▲



VDL Molecular Diagnostics and Avian Diagnostics Section Head Kristy Pabilonia (center, back row) developed and conducted a training course on avian influenza virus diagnostic testing, March 20 through 24, at Jordan University of Science and Technology in Irbid, Jordan. Participants from Jordan, Egypt, Algeria and Morocco consisted of veterinarians, laboratory veterinarians, public health vets and virologists, all involved in the diagnosis of avian influenza virus. The course was coordinated by CRDF Global, an independent nonprofit organization that promotes international scientific and technical collaboration through grants, technical resources, training and services.



VDL Virology Section Head Christie Mayo participated in a computational biology workshop at the CSU campus in Todos Santos in April, the first computational biology and genomics workshop for regional researchers in Todos Santos. Participants attended from Biotech del Norte, Centro de Investigaciones Biológicas del Noroeste, Centro de Investigación Científica y de Educación Superior de Ensenada, Centro Interdisciplinario de Ciencias Marinas, Dalhousie University, University of California at Irvine and Universidad Autónoma de Baja California Sur.

Lab Updates

VDL in the Field

CSU VDL personnel stay in touch with Colorado animal industries by make periodic tours of operations. Recent tours included:

- Circle Ranches, Ed Hansen and Sons, Livermore, Colo., in March. Cow/Calf operation toured during calving. Attending were Terry Spraker, Anna Fagre and Lora Ballweber, technicians Denise Bolte, Mike Russell, building monitor Michelle Miller, IT tech, Carrie Schmer and transcriptionist, Lori Bowker.
- Spence and Connie Rule and Family Livestock Operation, Brush, Colo., in March. Participants included Dwayne Hamar, Terry Spraker, transcriptionist, Julie Wright, Michelle Miller, building monitor, Jessica Alex, sample receiving, Mike Russell and Denise Bolte, bacteriology technicians.



Lab Updates

Next-Gen Equine Neurology Study

Three researchers from CSU's Veterinary Diagnostic Labs have received a Young Investigator Grant to pursue improvements in diagnosing central nervous system dysfunction in horses. They will apply next-generation sequencing, a technology currently used in basic research that could become a powerful diagnostic tool, to refine CNS diagnostics in equines.

A significant number of equine neurologic cases presenting with CNS dysfunction consistent with encephalitis remain undiagnosed, despite clinical laboratory testing. Conventional diagnostic methods require the clinician to have prior knowledge of what they are testing for. For instance, current PCR testing for viral agents causing encephalitis requires you to know what to test for in the first place, in order to select the appropriate test primers specific to the virus.

In contrast, next-generation sequencing has the potential to find any infectious agent with nucleic acids, including viruses, bacteria, and fungi, without the clinician or researcher having to anticipate what to test for. It has successfully been used to diagnose human illness when other conventional techniques have failed.

The CSU researchers hope this method will allow them to detect any unknown pathogen that could be causing these undiagnosed encephalitis cases. It is also possible the method will detect viral, bacterial or fungal agents already known to cause disease in horses, but are not being detected by current testing methods.

Coordinated by CSU's Center for Companion Animal Studies, the Young Investigator Grant program distributes money donated by corporate sponsors to support companion-animal research that involves veterinary students.

Tissues from neurologic horses that come through the VDL's necropsy service or through equine service at the Veterinary Teaching Hospital will be eligible for this study. If you are interested in knowing more about this study or have any questions, please contact the Young Investigator Award grant recipients:

- Veterinary student Teresa Garcia at Teresamg@rams.colostate.edu
- VDL Pathologist Chad Frank at chad.frank@colostate.edu
- VDL Virology Section Head Christie Mayo at Christie.Mayo@colostate.edu



NEW STAFF MEMBER

Katherine Wadsworth, originally from Saint Louis, moved to Grand Junction to join VDL's Western Slope Lab as a new lab technician. She recently graduated with a bachelor's degree in cellular and molecular biology from Tulane University in New Orleans. She gained lab experience working for Louisiana State University's Health Sciences Center. Outside of work, she enjoys live music, painting and hiking.

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

Parasitology Section Head **Lora Ballweber** will attend the **American Association of Veterinary Parasitologists** 61st annual meeting Aug. 5 through 9 in San Antonio.

VDL Director **Barb Powers**, Avian Diagnostics and BSL3 Operations Section Head **Kristy Pabilonia**, Virology Section Head **Christie Mayo** and Chemistry and Toxicology Section Head **Dwayne Hamar** are scheduled to attend this year's 59th annual meeting of the **American Association of Veterinary Laboratory Diagnosticians**, Oct. 13 to 19 in Greensboro, N.C.

Pabilonia will also be at the **National Poultry Improvement Plan Biennial Conference**, Aug. 30 to Sept 1 in Seattle.

VDL Pathologists **Chad Frank** and **Paula Schaffer** will be in attendance at the annual meeting of the **American College of Veterinary Pathologists**, Dec. 3 through 7 in New Orleans. **Schaffer** attended the 47th annual conference of the **International Association for Aquatic Animal Medicine** in May in Virginia Beach.

Rocky Ford Branch Director **Gene Niles** will present on plants poisonous to horses and cattle at a **Las Animas County Extension** meeting in July, will attend the **Academy of Veterinary Consultants** summer meeting, Aug. 4 to 6 in Kansas City, and the group's winter meeting Dec. 1 to 3 in Denver, and will present

on poliоencephalomalacia and bovine abortion cases seen at the Rocky Ford lab at this year's annual **Colorado Veterinary Medical Association** convention, Sept. 25 in Loveland.

Powers, **Pabilonia** and **Mayo** will also be on hand at this year's annual **Colorado Veterinary Medical Association** convention, Sept. 25 in Loveland.

VDL Pathologist **Colleen Duncan** will return this fall to Alaska for an **adult fur seal capture trip** to continue field research. She will also travel to Alaska in August to attend a meeting of the science panel of the **North Pacific Research Board**, to which she was appointed in May.

VDL Pathologist **Sushan Han** will present on emerging diseases in zoo and wildlife pathology during a workshop at the **American Association of Zoo Veterinarians** Annual Conference, July 16 through 22, in Atlanta.

VDL Pathologist **Terry Spraker** will attend the **12th European Multicolloquium of Parasitology**, July 20 to 24, in Turku, Finland. He will also participate in a workshop on chronic wasting disease in reindeer and moose beginning in the end of July, in Trondheim, Norway. Spraker is also scheduled to attend the **12th Conference of the European Wildlife Disease Association**, Aug. 27 to 31 in Berlin.



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Update from the Director

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

This Spring certainly went by fast, and summer has fully arrived.



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

We hope you enjoy this fact-filled issue of *LabLines* with pertinent current information and ongoing laboratory activities. We have had a very busy year, with an increase in over 10% of laboratory use in all sections.

In January, we had an excellent meeting of our External Advisory Committee, and in July our new residents have arrived.

Meanwhile, we are having a number of faculty changes. We are sad to see them go:

- Doreene Hyatt, our Bacteriology Section Head, has left the laboratory to concentrate on undergraduate teaching in the Department of Microbiology, Immunology and Pathology
- Lora Ballweber, the Section Head of Parasitology, has done the same to concentrate on DVM education.
- Don Kitchen, Director of the Western Slope Diagnostic Laboratory, has retired after many years of excellent service.

Searches are under way for the replacements for Dr. Hyatt and Dr. Kitchen, while Dr. Ashley McGrew will be stepping in as the new Section Head of Parasitology.

More information on the new faculty members will be in the next issue.

On page 7, we remind everyone of the importance of the need for the continued funding of the National Animal Health Laboratory Network. We were proud to be recently designated as a Level 1 Laboratory. NAHLN plays a direct role in protecting a safe, stable and nutritious food supply. We work with congress and USDA to try to expand the funding to the needed \$30 million per year for the entire country.

We look forward to seeing many of you at various state and national meetings this coming year. We always welcome the chance to meet with customers of the VDL, our colleagues and other stakeholders in the field. Please take a look at our "VDL On the Road" section on page 15, which lists the conferences, meetings, symposia and other appearances we are schedule to attend. If you find you'll be sharing attendance at one and would like to share some thoughts on our performance, please drop us a line and let's take a few minutes to talk.

Sincerely,

Barbara E. Powers