

LAB LINES

Diagnostic news and trends from the Colorado State University Veterinary Diagnostic Laboratories
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CSU VDL in the Field: Disease Updates

Avian Influenza Situation Update

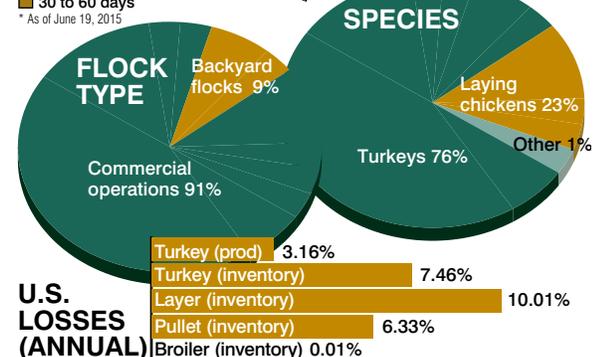
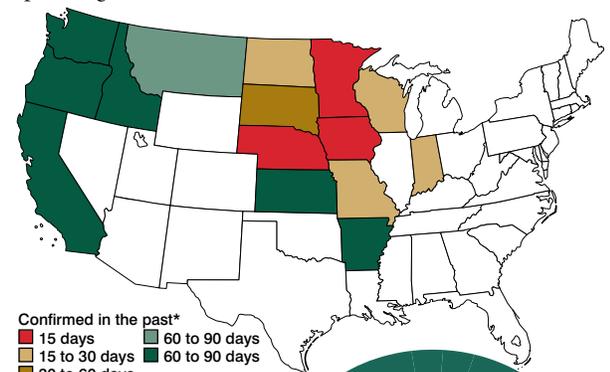
As of June 19, highly pathogenic avian influenza viruses had been detected in commercial and backyard poultry flocks in 15 states, involving 223 premises and affecting more than 48 million birds, resulting in death due to infection or depopulation as part of the control effort. The threat has struck close to home. On March 26, federal officials announced a wild Canada goose in Laramie County, Wyo., tested positive. Because avian influenza viruses are carried globally in wild migratory waterfowl and shorebirds, they pose a risk for transmitting the viruses from these birds to domestic birds through direct and indirect contact. Avian influenza can also be transmitted between domestic bird flocks.

CSU Veterinary Diagnostic Laboratory's Avian Health program team is closely monitoring the outbreaks and conducting surveillance to help protect the commercial poultry flocks in the state and the backyard chicken flocks now allowed in many municipalities and counties along the Front Range, including Fort Collins, Denver and Colorado Springs. In 2013-14, VDL routinely tested 7,721 submitted birds. None came back positive. The CSU VDL also tested more than 2,000 wild bird samples over the past few months. The outbreak response will drive testing numbers even higher this year.

No reported human infections have resulted from the current outbreak of HPAI in the United States. The Centers for Disease Control and Prevention say the viruses circulating in the country pose low risk to people. However, the outbreak is resulting in significant bird and economic losses to both large and small producers. Veterinary health officials are concerned the disease will resurface this fall,

— Jeff Dodge, CSU Public Relations Coordinator, and Kristy Pabilonia, DVM, DACVM, CSU VDL Avian and Molecular Diagnostics Section Head

with the cooler weather and increased migration of wild birds. CSU VDL will stay heavily involved, and all flock owners should work to help prevent avian influenza from spreading further.



See page 3 for more on how CSU's Veterinary Diagnostic Labs work with the state department of agriculture to monitor for outbreaks

NEED HPAI HELP?

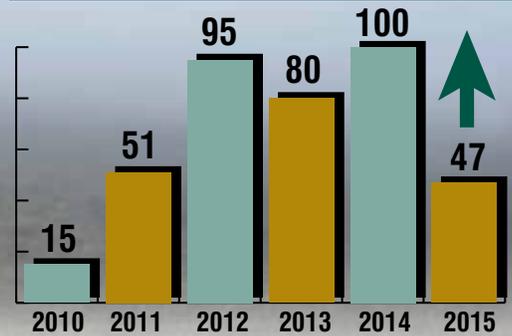
Veterinarians: Call (970) 297-4008 or (970) 297-4109 to see if your sick poultry case qualifies for free avian influenza virus testing

Need to report multiple sick birds or unusual bird deaths to state officials? Contact the State Veterinarian's Office at (303) 869-9130 or USDA at (303) 231-5385.



Guardians of Public Health

Legal cases handled by CSU VDL



CSU Diagnostic Lab Pathologists Step Up In Forensic Cases

— Paula Schaffer, DVM, MS, CSU VDL Postdoctoral Fellow

Forensic cases represent a small but rising fraction of necropsies performed at CSU's Veterinary Diagnostic Laboratory. Usually these cases involve an unexplained or unexpected death considered suspicious by an owner or client. For example, a client may present a pet that was found dead unexpectedly in the back yard or a pet that dies at the groomer or boarding facility. Many of these cases are natural deaths, and in these cases a post mortem exam performed by a veterinary pathologist can help provide peace of mind for the client. In other cases, however, there might be direct evidence of abuse or neglect. Often, these cases are handled through law enforcement or animal control. Veterinary pathologists involved in these cases not only help provide justice for the animals involved, but also serve an important part of the VDL's role in protecting the health and safety of our community.

While some legal cases may result from a simple lack of husbandry knowledge, others represent premeditated violent acts. Perpetrators of animal abuse and neglect can represent an ongoing hazard to animals and may also be more likely to carry out abuse and neglect against human beings. Many studies demonstrate links between

animal abuse and violence against humans. For example, one study based in Chicago found 65% of individuals arrested for an animal-related crime had at some point previously been arrested for battery against a human.¹ Interviews of 36 convicted multiple murderers revealed that 46% admitted committing acts of animal torture as adolescents.² Between 71% and 83% of women entering domestic-violence shelters reported their partners also abused or killed the family pet.³

Legal necropsies document cases of suspected animal abuse or neglect. As a result, VDL pathologists take these cases very seriously and spend extra time to fully document each case, collect evidence, and report findings objectively and professionally, both in written formats and on the witness stand.

If you have any questions regarding animal abuse or neglect, please don't hesitate to contact the laboratory or your local animal control office or humane society. Additional resources include the Colorado Link Project and Dumb Friends League. ▲

REFERENCES

- 1 Degenhardt B. 2005. *Statistical Summary of Offenders Charged with Crimes against Companion Animals July 2001-July 2005. Report from the Chicago Police Department.*
- 2 Cohen W. 1996. *Congressional Register*, 142(141), Oct. 3.
- 3 Walton-Moss, et al. 2005. *Risk factors for intimate partner violence and associated injury among urban women. J Comm Health*, 30(5), 377-389.
- 4 DeViney E, Dickert J, Lockwood R. 1983. *The care of pets within child abusing families. Int J Study Anim Probl* 4(4): 321-329.

ADDITIONAL RESOURCES

- Colorado Link Project
ColoradoLinkProject.com
- Colorado Humane Society
ColoradoHumane.org
- The Dumb Friends League
www.ddff.org
- Colorado Veterinary Medical Association
www.colovma.org/?57
- Local animal control office

DEFINITIONS

ANIMAL CRUELTY. A person commits cruelty to animals if he knowingly, recklessly or with criminal negligence overdrives, overloads, overworks, torments, deprives of necessary sustenance, unnecessarily or cruelly beats, allows to be housed in a manner that results in chronic or repeated serious physical harm, carries or confines in or upon any vehicles in a cruel or reckless manner, engages in a sexual act with an animal or otherwise

mistreats or neglects any animal. Causing or procuring the previous also constitutes cruelty, as does having charge or custody of any animal and abandoning it or failing to provide it with proper food, drink or protection from the weather consistent with the species, breed and type of animal involved.
ANIMAL MISTREATMENT. Every act or omission that causes or unreasonably permits continued unnecessary or unjustifiable pain or suffering.

NEGLECT. Failure to provide food, water, protection from the elements, or other care generally considered to be normal, usual and accepted for an animal's health and well-being consistent with the species, breed and type of animal.
AGGRAVATED CRUELTY. A person commits aggravated cruelty to animals if he knowingly tortures, needlessly mutilates or needlessly kills an animal.

Reportable Disease Update

VDL Responds to Avian Influenza

Kristy Pabilonia, section head for CSU Veterinary Diagnostic Labs Avian and Molecular Diagnostics, coordinates the Colorado Avian Disease Surveillance Program. Along with her, Avian Health field team members Sarah Millonig, Jerusha Nichols and Kyran Cadmus, with lab technicians Christina Weller, Kathi Wilson and Leah Powers all work together toward the goal of early detection of an outbreak of avian influenza virus in Colorado.

The program is a joint effort with the Colorado Department of Agriculture, through funding from USDA. It's goal is to monitor infectious diseases, including avian influenza viruses, for the Colorado poultry industry and respond quickly to outbreaks that pose a threat to the industry or public health. In addition to routinely testing thousands of commercial birds every year, the program randomly tests backyard birds at events such as county fairs, bird shows and swaps.

CSU VDL RESPONSE

As part of the National Animal Health Laboratory Network, the VDL uses state-of-the-art equipment to test for significant foreign animal diseases such as avian influenza, including 7,721 domestic bird tests in 2013-14. The service is available to all bird owners, whether commercial producers or backyard enthusiasts. Those who have sick birds or birds that have died from unknown causes can contact the Colorado Avian Health Call Line at CSU at 970-297-4008. Veterinarians are also encouraged to submit samples or dead birds for free avian influenza testing at Fort Collins' VDL. Call them at (970) 297-4008 or (970) 297-1281, or visit dlab.colostate.edu. ▲

TIPS FOR BACKYARD FLOCK OWNERS



Keep backyard chickens in a fully fenced enclosure with netting on top. Feed should be kept in the coop, not in the open, to avoid attracting wild birds.



Open ponds or water pools are an invitation for wild waterfowl to land and possibly infect domestic flocks. Game birds and migratory waterfowl should have no contact with your flock.



Use a dedicated pair of shoes and clothing to wear only when in contact with the flock. Scrub and disinfect boots and hands before entering coop. Clean cages and change food and water daily.



Don't share equipment and supplies between flocks. Require visitors to follow all procedures. Isolate birds coming from exhibitions for at least two weeks before reintroducing to the flock.

Have sick birds or birds that have died from unknown causes? Contact CSU's Colorado Avian Health Call Line at (970) 297-4008.

Canine Virology

What about Canine Influenza?

In late spring, veterinarians in the Chicago area noticed an increase in the number of dogs coming into their clinics with respiratory illness. The disease, which sickened more than 1,000 dogs, was first thought to be canine influenza virus H3N8, which was first isolated in Florida in 2004 and has since become endemic in areas of Colorado. Subsequent testing, however, identified the Chicago cause as a virus closely related to Asian strains of influenza A H3N2 virus, currently in wide circulation in Chinese and South Korean dog populations since at least 2006, and never before seen in the United States.

The CSU VDL has not detected a positive case of canine influenza in the past year. Influenza can be detected by PCR or by identifying antibodies in the later stages of a clinically ill dog. PCR samples should be taken within four days of clinical onset, using a nasal swab. Place swabs in a sterile tube in 1 ml sterile saline. Do not use bacteria transport media. Refrigerate and send them overnight on freezer packs. Serology testing is also available to test for exposure.

A commercial vaccine for H3N8 canine influenza is available, but the efficacy of the vaccine is unknown against H3N2. ▲



Virology Quality Assurance

How to Ensure Frustration-Free Equine Infectious Anemia Testing

VDLs Fort Collins, Rocky Ford, and Western Slope laboratories all perform diagnostic testing for Equine Infectious Anemia. We often receive the federal form VS Form 10-11 accompanying samples with inadequate information. That missing information encumbers our staff members with contacting veterinarians, leading to delay. It is imperative for the form to be completed by the accredited veterinarian responsible for each submission, whether electronic or non-electronic. Here's how to help ensure you receive timely results.

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

NON-ELECTRONIC FORMS

- Only the USDA/APHIS “Equine Infectious Anemia Laboratory Test” (VS Form 10-11) can be accepted.
- An individual form with complete description must accompany each animal's sample.
- We cannot run diagnostic testing (AGID or ELISA) until the completed and signed form is submitted.
- For incomplete forms, the veterinarian will either have to come to the laboratory to complete the form or the form will have to be mailed back to the veterinarian for his/her signature.
- Failure to submit the completed form will delay receipt of results!

ELECTRONIC FORMS (USDA-APHIS OR GLOBAL VET LINK)

- Obtain level 1 e-authentication by applying for a user ID and a password at www.eauth.usda.gov.
- Ensure electronic forms include information pertaining to veterinarian's name and address a list of animals with owner addresses and digital photos of the animals if desired.
- Send serum samples to us with a complete accession form, including veterinarian, owner and animal information. We also prefer you include a printed copy of the completed online form indicating you are requesting the EIA AGID or ELISA.
- Electronic forms must submitted to the respective on-line website before our technicians can release any results to you. If the forms are not submitted on-line by the time test results are entered by the lab, it is the veterinarian's responsibility to follow up and call the laboratory. Failure to do so will result in delayed receipt of results.
- EIA testing requires submission of 1 ml serum. Fees are \$8 for AGID and \$13 for ELISA. ▲



Annual Diagnostic Summaries

Annual Diagnostic Stats Snapshot

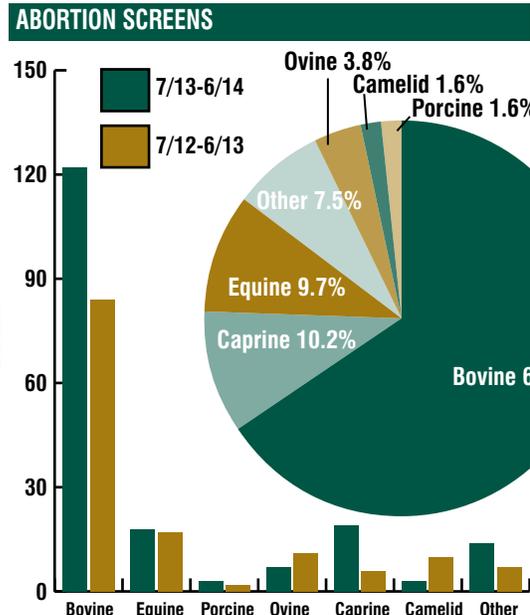
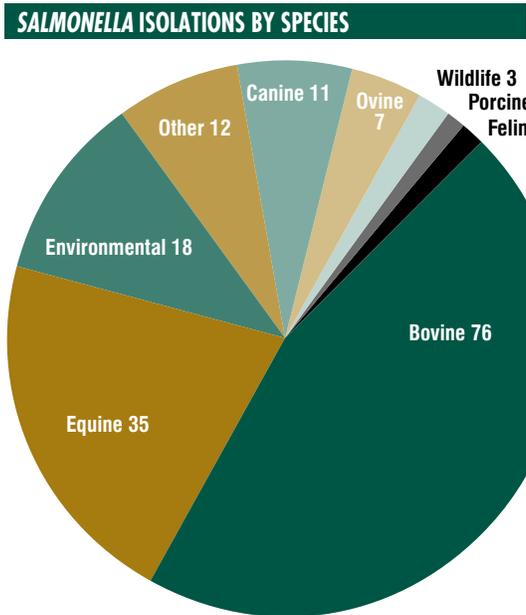
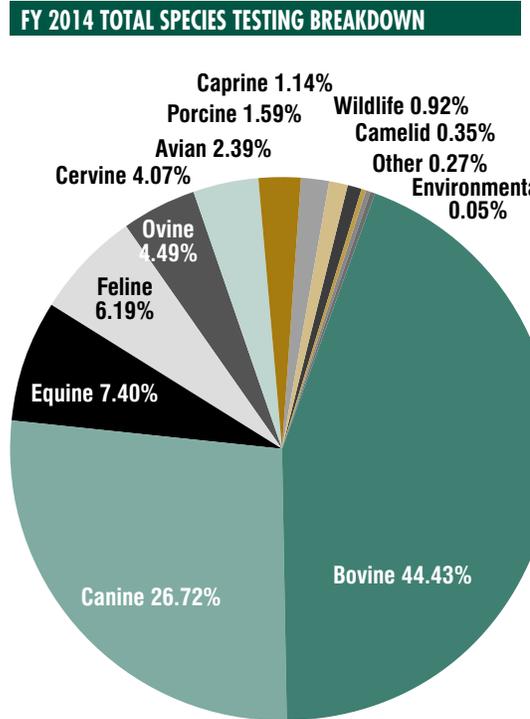
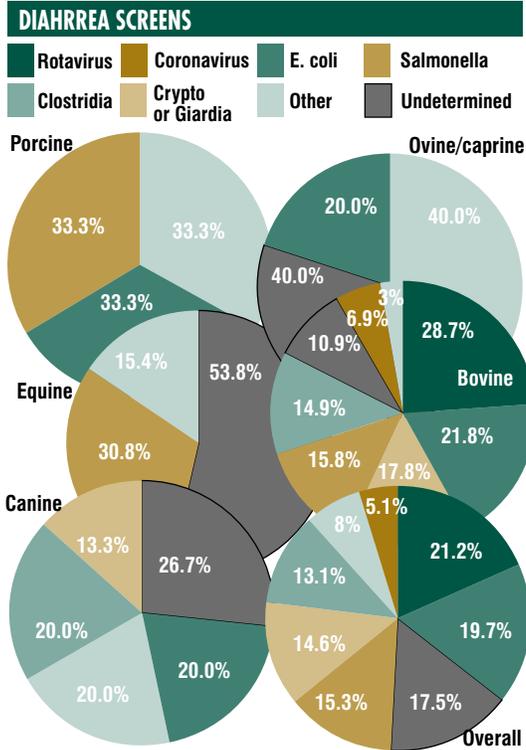
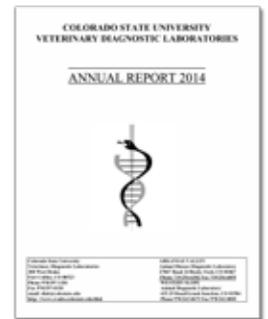
In addition to its mission to provide timely, accurate, and pertinent diagnostic services to veterinarians and animal interests, CSU's Veterinary Diagnostic Labs also pursues the wider mission of contributing to research and better understanding of disease identification,

— Barb Powers, DVM, PhD, DACVP, CSUVDL Director

investigation and prevention. In this issue, our annual report helps fill in the historical diagnostic picture for some common conditions and pathogens.

WANT EVEN MORE?

Review our entire 56-page 2014 Annual Report, under the "Regulations & Resources" tab at www.dlab.colostate.edu





CSU VDL in the Field: Case Study

Curly Calf Syndrome

The owner of a newborn Charolais calf noticed deformed rear legs on the animal. Because the calf came from a herd of 800 cattle from which three other calves had been born within the year with rear leg deformities, the owner was concerned about whether the condition could be genetic or congenital. The calf was euthanized and submitted for necropsy and workup.

NECROPSY FINDINGS

The front legs of the animal were grossly normal, as was the rest of the skeleton. However, the rear legs were deformed, and the pelvis was very narrow and angled toward the tail. The acetabulum, the femur and the knee appeared to be normal. The metatarsal bone and phalanges tended to bow upward, and the animal would have walked on the pastern joints. The muscles of the legs were bilaterally atrophied, and the sciatic nerve was difficult to locate and appeared small.

Neither the teeth nor the ribs had abnormalities, and no abnormalities were observed in the internal organs. The head and mouth were free of malformations.

HISTOPATHOLOGY

Lab pathologists processed and examined multiple transverse and longitudinal sections of thoracic spinal cord. One was taken from the anterior thoracic cord at approximately T-2 and the other at approximately T-12. The significant lesion was bilateral myelodysplasia characterized by the lack of development of the dorsal horns in both thoracic spinal cord sections. The ventral horn was normal; however, the dorsal horn

— Don Kitchen, DVM, PhD, ACVP, Director, CSU VDL Western Slope Branch, and Chad Frank, DVM, MS, DACVP, CSU VDL Pathologist

contained few nuclei and other components of the gray matter. The other areas within the spinal cord including the central canal were histologically normal. Despite the gross atrophy of the rear-leg muscle groups, the skeletal muscle was considered histologically normal.

DIAGNOSTIC INTERPRETATION

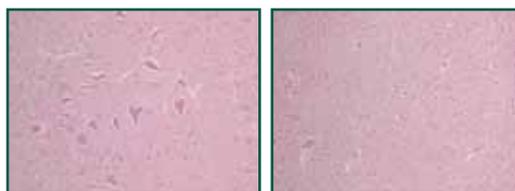
The differential diagnoses for this case included osteogenesis imperfecta, other less defined skeletal dysplasias and arthrogryposis multiplex, or curly calf syndrome. The lesions were diagnostic for arthrogryposis.

The causes of arthrogryposis are often not clear, although the underlying cause is generally agreed to be decreased limb movement in utero. Both myogenic and neurogenic disorders can result in decreased limb movement, but denervation is by far the most common cause. In this case abnormalities were observed in the spinal cord; however, they were not confirmed in the skeletal muscles or peripheral nerves. Curly calf syndrome can be an inherited genetic defect. It is recessive lethal. It can also be caused by damage to the fetal central nervous system in utero. The bluetongue virus as well as ingestion of poisonous plants by the dam (lupine poisoning) can cause this syndrome. The fact that several other animals in this herd have been affected by this syndrome suggests a genetic cause.

REFERENCES:

- 1 Allison Van Eenennaam, UC Davis Curly calf syndrome (arthrogryposis multiplex), California Beef Cattle Improvement Association conference, May 2009, Sacramento California.
- 2 John Van Vleet and Beth Valentine, Arthrogryposis, Pathology of Domestic Animals, Volume 1 2007:204-206.
- 3 Stephen Weisbrode, arthrogryposis, Pathologic Basis of Veterinary Disease, 4th edition, 2007, 1094-1095.

Tests for this syndrome are available. They require submission of whole blood or semen and are commercially available. Genetic testing is also available in Angus cattle; Jonathan Beever from the University of Illinois and David Steffen at the University of Nebraska have both worked extensively with this syndrome in the Angus breed. The available genetic test for Angus cattle identifies animals as carriers or non-carriers for the arthrogyposis mutation. ▲



Normal ventral horn from the thoracic spinal cord extracted at T-12 (left), compared to myelodysplasia characteristic of arthrogyposis (right).

Reportable Disease Update

Vesicular Stomatitis Virus Updates

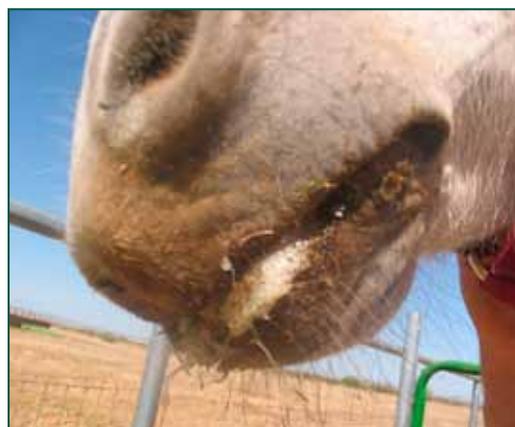
As spring and summer bring warmer weather, index cases of Vesicular Stomatitis (VS) have been reported in the southwestern United States, affecting Arizona, New Mexico, Utah, Texas and now Colorado. As of July 3, the first cases have been reported in this state. Vesicular Stomatitis is a viral disease affecting horses, cattle, pigs, sheep and goats. Two vesiculoviruses of particular importance in the United States are serotypes New Jersey and Indiana. The viruses are morphologically similar but generate distinct neutralizing antibodies.

Transmission can occur via infected insect bite, but animal-to-animal contact can also be important for within-herd spread. VS is considered endemic in Colorado, but epidemic cycles occur at irregular intervals. Increased transmission within particular years—2004, 2005, 2012, 2014—is thought to be associated with wet springs coupled with warm summers. The combination of these variables may provide the perfect situation for increased breeding habitat and induced larval hatching of suspected vector species, such as Simuliidae, or black flies, and Culicoides, or gnats. Climatic and environmental factors affecting VS outbreaks have been studied, but definitive associations have not been found; therefore, further investigations are warranted in order to understand definitive drivers of transmission. We do know transportation of infected animals has been demonstrated to spread VS from one location to another.

During the course of infection, clinical signs follow a typical viral incubation period of three to seven days. Following an initial febrile period, ptialism and vesicles or ulcers in and around the mouth or feet occur. In particular, lesions of the oral mucosa present as raised, blanched vesicles that are very short-lived, leaving ulcerations and erosions when ruptured. The lesions in cattle appear similar to those caused by foot and mouth disease.

Historically, VS has been investigated and managed as a foreign animal disease impacting US agriculture through reporting requirements for trade. In January 2015, it was delisted from the World Organization for Animal Health list of notifiable terrestrial disease;

— *Christie Mayo, DVM, PhD, CSU VDL Virology Section Head; Kristy Pabilonia, DVM, DACVM, CSU VDL Avian and Molecular Diagnostics Section Head; and Barbara Powers, DVM, PhD, DACVP, CSU VDL Director*



however, it remains on the proposed US national list of reportable animal diseases, as well as on the reportable diseases list of each state. The 2015 national-level VSV after-action review recommendations recommended the National Veterinary Service Laboratories finalize and deploy validate real-time polymerase chain reaction testing to be used in equidae to respond to an outbreak. The CSU VDL has passed proficiency testing on this assay and is ready to respond to an outbreak.

Despite these recent changes, it is important to continue reporting identification of clinical animals to the Office of the State Veterinarian. Criteria for interstate and international movement of nonclinical animals remain the same; veterinarians should always review guidelines and requirements of each destination state (state veterinarian's office) or country (national center for import/export) before movement. For clinical animals, call your veterinarian and have them work with the state veterinarian's office in order to obtain samples for diagnostic testing and determine appropriate quarantine and control measures. ▲

VESICULAR STOMATITIS TESTING AT CSU

CSU VDL is now proficiency tested and approved by USDA to conduct Polymerase chain reaction testing on equine clinical suspects. PCR and complement fixation assay are recommended for diagnosis.

■ PCR

- Submit swab in TBTB or sterile saline
- \$35/sample

■ Serum neutralization assay

- Ruminant or equine serum
- \$18/sample

■ Complement fixation assay

- Equine serum
- \$40/sample

REFERENCES

- 1 Vesicular Stomatitis. Chapter 2.1.19. OIE Terrestrial Manual.
- 2 McCluskey, B. J., A. M. Peizel-McCluskey, L. Creekmore, and J. Schiltz. 2013. Vesicular stomatitis outbreak in the southwestern United States, 2012. *Journal of veterinary diagnostic investigation: official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc* 25: 608-613.
- 3 Mead, D. G., F. B. Ramberg, D. G. Besselsen, and C. J. Mare. 2000. Transmission of vesicular stomatitis virus from infected to noninfected black flies co-feeding on nonviremic deer mice. *Science* 287: 485-487.



Innovative PCR Applications

Pooled Ear Notch PCR Testing to Detect BVD Virus

For further information call the Rocky Ford Laboratory at (719) 254-6382 or the Western Slope Laboratory at (970) 243-0673.

The Rocky Ford Branch of the CSU VDL has tested nearly 600,000 ear notch samples since it initiated Polymerase Chain Reaction testing for bovine viral diarrhea virus on pooled ear notch samples in 2006. When pools test positive by PCR, the individual ear notches within the pool are tested by BVDV antigen capture ELISA (ACE) to identify the positive animal or animals within the pool.

During the period from Jan. 1, 2011, to Dec. 31, 2014, the Rocky Ford laboratory analyzed 202,815 ear notch samples for the presence of BVD virus. During this time period, 4,834 pools averaging 42 ear notches (range 12 to 52) per pool were analyzed for BVDV. The number of pools that tested positive for BVDV by PCR was 220, or 4.6%. Of the individual ear notches from each positive pool then tested by follow-up BVD ACE, 140, or 64%, of the pools contained at least one ACE positive ear notch sample. There were 80 PCR-positive pools in which no positive animals were detected. Of the false positive pools, 53, or 66%, came from dairy herds.

There were a total of 226 ACE positive animals within the 140 PCR positive pools. Dairy animals accounted for 142, or 62%; 86, or 38%, were beef cattle.

TRACKING PERSISTENT INFECTION

Although confirmation of BVD persistent infection (PI) status requires a second positive test at least three weeks after the initial test, we only received samples from 90 of the 226 ACE-positive animals, or 39.8%, for confirmatory testing during this time span. Out of those 90, 71, or 79%, were confirmed to be persis-

— Gene Niles, DVM, Director, CSU VDL Rocky Ford Branch

tently infected. Fifty-two of the confirmed PIs were beef cattle and 19 were dairy calves, and all 19 dairy PIs were from a single dairy—the only dairy that submitted samples for confirmation. Of the beef animals, 52 out of 71, or 73%, were confirmed PIs.

BVDV has been detected in skin samples from cattle with transient BVD infections for up to three months after the initial positive test.¹ To eliminate this possibility, serum samples or whole blood should be submitted for confirmation purposes.

Of the 202,815 animals tested over this four-year period, only 71, or 0.035%, were confirmed to be persistently infected with BVDV. The percentage of confirmed PI beef cattle was 0.03%, while 0.06% of the dairy calves tested were classified as PIs.

These data included registered and commercial cattle producers, stocker operations, feedlots, cattle used in research and dairy heifer replacement ranches. A search of the current computer system implemented in 2010 found that many seedstock producers and dairy clients have tested their calf crops each year for the last six years. During this time the laboratory has not received any reports of incorrectly classifying an animal as to its PI status.

UNDERUTILIZED COST-EFFECTIVE SCREENING?

This type of PCR analysis of pooled ear notch samples has proven to be a very sensitive and cost efficient method to screen large populations of cattle for the presence of BVDV. When coupled with BVD ACE tech-

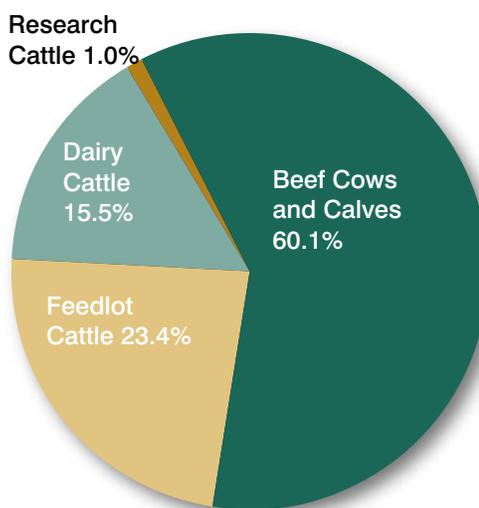
¹ J Cornish TE, van Olphen AL, Cavender JL, Edwards JM, Jaeger PT, Vieyra LL, Woodard LF, Miller DR, O'Toole D. Comparison of ear notch immunohistochemistry, ear notch antigen-capture ELISA, and buffy coat virus isolation for detection of calves persistently infected with bovine viral diarrhea virus. *J Vet Diagn Invest.* 2005 Mar;17(2):110-7.

niques, persistently infected animals can be reliably detected and removed from the herd by either isolation and slaughter or euthanasia. Unfortunately, only 40% of the cattle that initially tested ACE positive over this four-year period were retested to confirm their true PI status. It's commonly believed that the majority of the calves that initially test positive are sold instead of being retested.

Twenty-seven% of the beef cattle that initially tested positive by ACE were negative when retested, indicating they were either transiently infected, had been recently vaccinated with a modified live BVD vaccine, or the ear notch sample was contaminated with BVDV. Some false positive results have been traced back to cross-contamination when the person administering MLV BVD vaccine during processing also handles the ear notch sample.

Both the Rocky Ford and Western Slope laboratories offer pooled BVD testing. Submit ear notches in 10 ml red top blood without any additives, listing the tube numbers on the submission form and placing the tubes back in the original container starting from left to right. That recommended procedure saves our laboratory

2010-2014 Rocky Ford BVD Ear Notch Sampling



personnel a considerable amount of time, which translates to accessioning fee savings compared to samples in which ear notches are received in baggies or tube numbers are not listed on the submission form. ▲

CSU VDL in the Field: Case Study

Nitrate Diagnostic as Management Aid

Nitrate has sometimes been considered a silent killer. But it can also be a sneaky killer. We know nitrate can often result in sudden death of a large number of cattle (See "As Livestock Producers Dip into Low-Quality Forages, Beware Nitrate," *LabLines* v19n1, pp 5-6.) But under some conditions, only a few animals may be affected.

Last winter, CSU VDL provided nitrate diagnostic services to help a producer manage this type of nitrate toxicity. The lab received a report of two pregnant cows found dead. Testing showed their aqueous humor was 62 and 264 ppm nitrate. These cows had been in corn stalks for several weeks, and the field was heavily grazed. Sampled stalks from the corn field in which the cows had been and one sample of hay that had been fed was 2.4% (24,000 ppm) and 2.2% (22,000 ppm) nitrate, respectively. Nitrate at those levels would be considered potentially toxic.

The producer had two other fields of corn stalks he wanted to graze, so the referring veterinarian was advised that the stalk of the corn plant is higher in nitrate than the leaves, and the stalk closest to the ground would be the highest in nitrate. With that information the producer submitted stalks and leaves from two different fields. The leaves were below our detection limit (0.1% or 1,000 ppm) while the stalks were

— Dwayne Hamar, PhD, CSU VDL Chemistry and Toxicology Section Head, and Ed Wydallis, DVM, Buckhorn Veterinary Practice, Wellington, Colo.

2.6% (26,000 ppm) and 1.8% (18,000 ppm). Knowing that cattle will eat the corn and leaves before consuming the stalks, the producer decided to graze the corn stalks but not graze them as heavily as he had in the previous field. This targeted diagnostic solution allowed the producer to utilize the inexpensive forage while avoiding any further death loss. ▲



CSU VDL In Press

A Roundup of VDL Faculty Research

SURGERY FOR EXTRASKELETAL OSTEOSARCOMA?

Duffy D, Selmic LE, Kendall AR, Powers BE. Outcome following treatment of soft tissue and visceral extraskeletal osteosarcoma in 33 dogs: 2008-2013. *Vet Comp Oncol*. 2015 Feb 3. doi: 10.1111/vco.12141.

VDL Director Barb Powers participated in this study of the relatively rare extraskeletal osteosarcoma (EOS) in order to help close some of the gaps in knowledge regarding treatment outcomes. Highly malignant, EOS displays distant metastatic spread in up to 64% of cases at necropsy, with reported median survival times from 26 to 90 days.

The study team searched CSU VDL's histopathology submission database for osteosarcoma diagnoses over a nearly six-year period.

The submitting veterinarian on eligible cases was

then contacted to gather additional case information, including whether treatment was undertaken, and whether that treatment qualified as curative-intent surgery—surgery performed with wide or radical excision of the tumor with the intent to completely excise the tumor—or palliative-intent surgery—debulking or marginal excision with aim to remove some of the gross tumor or the entire tumor but leave residual microscopic disease, respectively.

Of the 33 cases that satisfied the study inclusion criteria, surgery was performed in 28, or 84.9%. Palliative-intent surgery was performed in three dogs, or 9%. Dogs that had wide or radical tumor excision had longer survival times compared with dogs that had only marginal tumor excision performed, with a median survival time of 90 days ranging from zero to 458 days, vs. a median survival time of 13 days, with a range of zero to 20 days.

A QUICK HOSPITAL SALMONELLA TEST?

Burgess BA, Weller CB, Pabilonia KL, Bolte DS, Van Metre DC, Morley PS. Detection of different serotypes of *Salmonella enterica* in experimentally inoculated equine fecal samples by commercially available rapid tests. *J Vet Intern Med*. 2014 Nov-Dec;28(6):1853-9. doi: 10.1111/jvim.12440.

Salmonella enterica can have a significant impact on the management of animal facilities; it is reported to be the most common cause of nosocomial outbreaks at equine hospitals. Yet the most common detection

methods— aerobic culture and real-time polymerase chain reaction—are hampered by challenges of cost, limited sensitivity, time needed to obtain results and laborious testing methodologies.

CSU VDL Avian and Molecular Diagnostics Section Head Kristy Pabilonia and Molecular Diagnostics Technician Christina Weller, and Bacteriology Technician Denise Bolte participated in this study to attempt to identify the best rapid field test, based on availability for point-of care, minimal equipment and training demands, results with 24 hours, applicability across settings and useful on specimens relevant to clinical settings. Of the four different commercially available rapid tests—two lateral flow immunoassays, a DNA hybridization test and a real-time PCR—the DNA hybridization detected 94% of isolates, and culture and PCR most reliably detected all. The lateral flow immunoassays detected 84% and 67% of isolates, respectively, but reactivity varied among serotypes, particularly with serotype Cerro, Mbandaka and Montevideo. False-positives were obtained for four negative controls by using DNA hybrid and one negative control by using PCR, while the immunoassays and culture had no false-positive results.

This study suggests culture, PCR, and DNA hybridization still face significant limitations making them unlikely point-of-care tests in veterinary practice. Current pricing suggests immunoassays could give results within 24 hours for about three to five times less expense, making them viable even when faced with higher false-positive rates. Those false-positives could



be ameliorated by concurrent use of culture to phenotypic and genotypic information.

THE NATURE OF EQUINE CUTANEOUS LYMPHOMA

Miller CA, Durham AC, Schaffer PA, Ehrhart EJ, Powers BE, Duncan CG. Classification and clinical features in 88 cases of equine cutaneous lymphoma. *J Vet Diagn Invest.* 2015 Jan;27(1):86-91. doi: 10.1177/1040638714561653.

This study from the CSU Veterinary Diagnostic Lab team retrospectively reviewed cases of previously diagnosed equine cutaneous lymphoma from 1999 to 2010 from the lab's records. This uncommon epitheliotropic neoplasm, similar to the cutaneous epitheliotropic lymphoma variant (mycosis fungoides) observed in human beings and canids, can present with variable clinical signs, immunosuppression and rapid systemic disease progression. The VDL team immunohistochemically labeled stored formalin-fixed, paraffin-embedded tissue samples for T-cell antigen using monoclonal mouse anti-human cluster of differentiation, and for B-cell antigen using a monoclonal mouse anti-human, B-cell-specific activator protein. Each case was then classified into lymphoma subtype according to a previously described veterinary-adapted WHO classification system.

Results from the study confirm T-cell-rich, large B-cell lymphoma (TCRLBCL) as the predominant subtype in equine cutaneous lymphoma. Cutaneous T-cell lymphoma (CTCL) was the second most common phenotype. Less common lymphoma subtypes identified in the study include diffuse large B-cell lymphoma and anaplastic large T-cell lymphoma. Quarter Horses almost exclusively developed TCRLBCL, while the frequency of CTCL was increased significantly in Thoroughbreds compared with other breeds. TCRLBCLs more frequently presented as multiple masses while CTCLs were more often solitary nodules. When multiple regions of the body were affected, 100% of cases were TCRLBCL. Finally, there was an observable trend for longer survival in cases of TCRLBCL without local recurrence, suggesting adequate surgical excision may be an effective treatment modality.

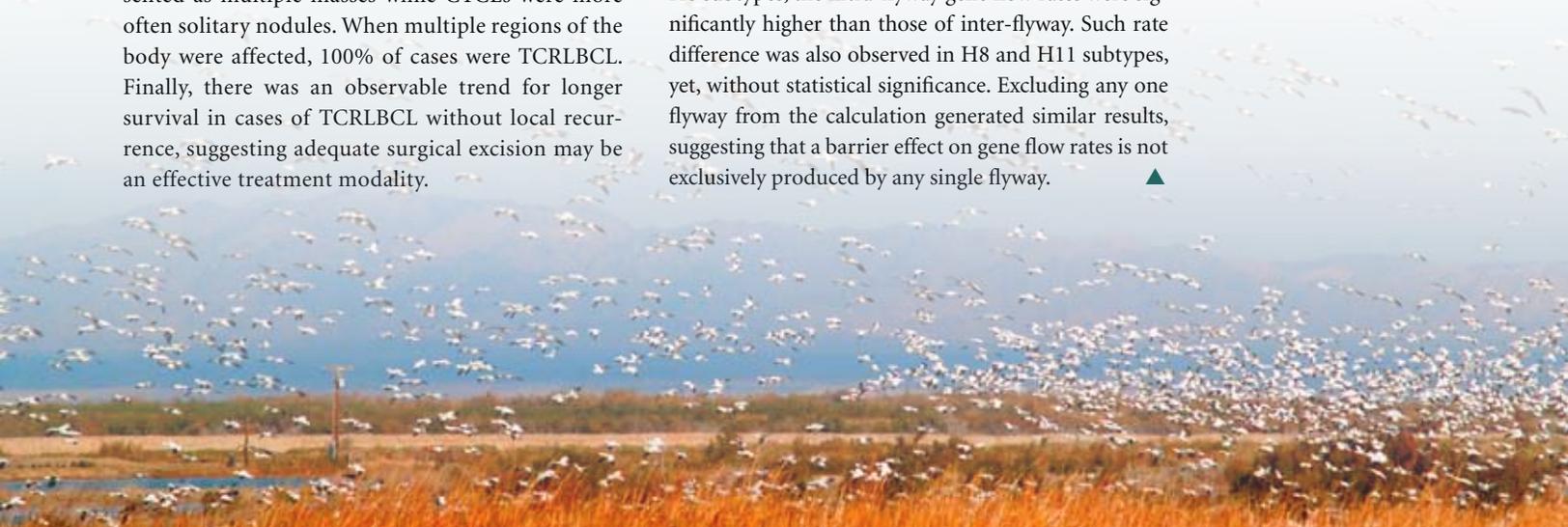


MAPPING THE RISK OF AVIAN INFLUENZA

Scotch M, Lam TT, Pabilonia KL, Anderson T, Baroch J, Kohler D, DeLiberto TJ. Diffusion of influenza viruses among migratory birds with a focus on the Southwest United States. *Infect Genet Evol.* 2014 Aug;26:185-93. doi: 10.1016/j.meegid.2014.05.029. Epub 2014 Jun 6.

CSU VDL Avian and Molecular Diagnostics Section Head Kristy Pabilonia took part in this study using the new field of “phylogeography”— the pairing of genetic sequence data with geo-mapping technology to model geographic diffusion and genetic diversity over time—to assess the genetic diversity of avian influenza within the different migratory flyways of the southwestern United States. The study evaluated 20 influenza viruses— four from Arizona 16 from New Mexico.

Results showed overall evolutionary diffusion of avian influenza viruses is geographically structured, but that diffusion to the Southwest was often from nearby states including California. For H3, H4 and H6 subtypes, the intra-flyway gene flow rates were significantly higher than those of inter-flyway. Such rate difference was also observed in H8 and H11 subtypes, yet, without statistical significance. Excluding any one flyway from the calculation generated similar results, suggesting that a barrier effect on gene flow rates is not exclusively produced by any single flyway. ▲



Feline Histopathology

Detecting Early Feline Kidney Disease

Frequently, chronic kidney disease in elderly cats is made only after renal lesions are irreversible. Our study evaluated the presence and severity of both reversible and irreversible histopathologic changes in the kidneys of cats at each stage of CKD and determined if lesion prevalence and character were different between stages. Within the 46-cat study group, the severity of tubular degeneration, interstitial inflammation, fibrosis and glomerulosclerosis was significantly greater in later stages of CKD compared with early stages of disease. Proteinuria was associated with increased severity of tubular degeneration, inflammation, fibrosis, tubular epithelial single-cell necrosis, and decreased normal parenchyma. The greater prevalence and severity of irreversible lesions in stage III and IV CKD implies that therapeutic interventions should be targeted at earlier stages of disease. ▲

— Shannon McLeland, DVM, PhD, DACVP; and Colleen Duncan, DVM, MSc, PhD, DACVP, DACVPM, CSU VDL Pathologist



McLeland SM, Cianciolo RE, Duncan CG, Quimby JM. A comparison of biochemical and histopathologic staging in cats with chronic kidney disease. *Vet Pathol.* 2015 May;52(3):524-34.

Lab Updates

Choose your Johne's Test Options

The Veterinary Diagnostic Laboratory at Colorado State University offers multiple choices when it comes to diagnostic tests for *Mycobacterium avium* ssp. paratuberculosis. Contact us about the options available to best monitor and benchmark this important herd disease:

- ❑ Liquid culture
\$25 per sample
30-42 day turnaround
- ❑ Solid culture
\$22 per sample
12-16 week turnaround
- ❑ Direct PCR
\$30 per sample
About 1 week turnaround
- ❑ Submit feces or tissues
- ❑ ELISA
Submit 1 mL serum
Volume discount available
Call us for details

■ CSU VDL once again has passed the individual 2014 fecal proficiency panel for *M. avium* ssp. paratuberculosis using ESP liquid media. This allows us to conduct official testing for the National Johne's Program using this method until the end of 2015. This method uses a liquid based system that allows for faster detection than culture using solid media—an average of 36 days vs. an average of 12 to 16 weeks, respectively. Using this method, we can grow the organism faster and use PCR to determine if it is *M. avium* ssp. paratuberculosis rather than another species of *Mycobacterium*.

■ The CSU VDL also has passed the 2014 individual fecal proficiency panel for *M. avium* ssp. paratuberculosis

— Doreene Hyatt, PhD, CSU VDL Bacteriology Section Head

using solid culture. Solid culture allows us to conduct official testing based on a solid culture system. This diagnostic test allows the differential growth of different species of *Mycobacterium* as well as quantitation of the amount of growth seen in the culture. Results for this test typically are available in 12 to 16 weeks.

■ In addition, the CSU VDL has passed the 2014 direct fecal PCR for *M. avium* ssp. paratuberculosis. This allows us to conduct official testing for the organism directly from a fecal sample without the delay of enrichment culture. Results for this test typically are available in less than one week after the samples have arrived at the laboratory.

■ CSU's VDL has passed the 2014 pooled fecal PCR for *M. avium* ssp. paratuberculosis. This allows us to conduct official testing for the organism directly from a fecal sample without the delay of enrichment culture. Results for this test typically are available in less than one week after the samples have arrived at the laboratory.

■ The CSU VDL has passed the 2014 serological (ELISA) proficiency tests for *M. avium* ssp. paratuberculosis, as well. This allows us to conduct official testing for a serological response to the organism from blood as well as milk samples. These results typically are available in less than one week after the samples have arrived at the laboratory. ▲





Years in the making and the dream of VDL Pathologist Tawfik Aboellail, the first annual meeting of the newly minted Middle Eastern division of the C.L. Davis Foundation met in Cairo in late March. In the shadow of the Pyramids of Giza, 50 veterinary pathologists and practitioners participated in lectures on clinical pathology and pathology of small and large animals, lab species, poultry, ruminants and horses. Along with two of his fellow graduates of the University of Cairo and the C.L. Davis Foundation, Aboellail worked tirelessly to create a world-class meeting for local colleagues along with a once-in-a-lifetime experience for non-Egyptian attendees.



Get to Know the Laboratory

New Members Join the Lab Team

Kirsten Reed joins the VDL's Molecular Diagnostics team in Fort Collins. Following a career in the entertainment industry in Los Angeles, Kirsten obtained her bachelor's degree in biology and microbiology at CSU. Upon graduation, she worked as a research associate in the medical device industry, engaged in assay development and product improvement. As a laboratory technician at the VDL, Kirsten is now able to combine her love of animals and science. When not in the lab, she is playing with her three cats, keeping her five chickens out of mischief or experimenting with her sourdough starter in the kitchen.



learning more about animal diseases and diagnostics, especially those that pertain to the health of our planet's wildlife. He looks forward to gaining more lab experience and pursuing potential opportunities for fieldwork experience. His main interests are in climate change effects on wildlife habitat and epidemiology. He enjoys just about every outdoor activity, especially fly fishing and eating and cooking good food.

Evan Cassey recently graduated from CSU with a bachelor's degree in fish, wildlife and conservation biology. He has worked in sample receiving throughout his undergraduate years, and has been inspired by the lab's work to continue



Wendy Cottrell, from Parkersburg, W.V. is an alum of West Virginia University, with degrees in biology and medical laboratory science. She started out in food microbiology and then moved to environmental compliance before getting her histotechnological certification. A resident of 10 states over the course of her life, she moved to Fort Collins from Billings. She has wanted to move to Colorado since she went skiing at Steamboat Springs at 17 years old. She enjoys cycling, skiing and homebrewing. She is a certified beer judge. ▲



Advances in Bacteriology

New ID Methods

CSU's Veterinary Diagnostic Lab has purchased a VITEK® 2 Compact 30 identification system and is validating its use for the bacteriology laboratory. The new system provides the lab a faster and more accurate way to identify bacteria and to run antimicrobial susceptibility by a minimum inhibitory method. This new technology will give you faster and more accurate reports to help you and your patients. Some of the benefits include:

- Testing to genus, species and some sub-species levels.
- Gram-negative identification with results in two to 10 hours, including major enteric and non-enteric micro-organisms.
- Gram-positive identification with results in 2 to 8 hours, including major cocci and rod micro-organisms.
- Bacillus identification and related genera and species
- Yeast identification with results in 18 hours, including major yeast genera and species



— Doreene Hyatt, PhD, CSU VDL Bacteriology Section Head

- Anaerobic identification of major anaerobic genera and species, with endpoint results in 6 hours, including important select *Corynebacteria* species
- Gram-Negative and Gram-Positive Antimicrobial susceptibility tests with average results in 8-12 hours.

Once validation is completed, you may notice the change on your antimicrobial susceptibility results. While we will still report susceptible, intermediate and resistant), we will now be able to report minimum inhibitory concentration. ▲



VDL Lab Coordinator Charlie Davis (left) represented the lab at this year's Colorado Cattlemens Association meeting in mid June in Steamboat Springs. VDL Avian Diagnostics Section Head Pabilonia and Pathologist Gary Mason (above) held a four-day USDA course on ruminant necropsy, diseases, sample selection and collection for Indonesian diagnosticians in Bogor, Indonesia, in May.



Members of the CSU Veterinary Diagnostic Lab toured the Rule Livestock Operation, near Brush, in early March. We are actively looking for other livestock operations to tour throughout the coming year. Tours will help provide information about various livestock business entities and the reasons they utilize the diagnostic services we provide as an integral part of livestock enterprise management. If you're interested in hosting us, contact Charlie Davis, at (970) 297-0370 or (970) 689-1632, or e-mail charlie.davis@colostate.edu

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

VDL Lab Case Coordinator Charlie Davis toured the CSU Research Foundation-owned **Maxwell Ranch**, in Livermore, with five VDL personnel in mid-May. Along with **VDL Director Barb Powers** and **Virology Section Head Christie Mayo**, he also attended the **Protein Producer Summit**, a joint meeting of the Colorado Cattlemen's Association and the Colorado Livestock Association, in June in Steamboat Springs. He represented the CSU VDL at the **Western States Livestock Health Association** meeting in Denver, July 8 through 10, and the **Colorado Woolgrowers Convention**, July 15-16 in Montrose.

VDL Pathologists Chad Frank and EJ Ehrhart will be on hand at the annual meeting of the **American College of Veterinary Pathologists**, Oct. 17-21 in Minneapolis.

VDL Pathologist Terry Spraker Taught two two-day courses to international veterinarians through the **National Wildlife Research Center** in Fort Collins. He also attended the 22nd **Helminthological Days 2015**, in May in the Czech Republic, where he gave an oral presentation on hookworm enteritis with peritonitis in California sea lions. He also presented a

poster on the distribution of the prion associated with chronic wasting disease in the brain stem and peripheral tissues in elk, as well as chaired an afternoon session on prion pathogenesis in animals, at the **Prion 2015 International Meeting** in May in Fort Collins.

Look for **VDL Pathologists EJ Ehrhart** at the annual meeting of the **Comparative Ocular Pathology Society**, Sept. 10 through 11 in Athens, Georgia.

VDL Director Barb Powers, Lab Case Coordinator Charlie Davis, Avian and Molecular Diagnostics Section Head Kristy Pabilonia and **Virology Section Head Christie Mayo** will be at the 2015 annual convention of the **Colorado Veterinary Medical Association**, Sept. 17 through 20 in Loveland.

Powers, Pabilonia and **Mayo** are also scheduled to attend the 58th annual meeting of the **American Association of Veterinary Laboratory Diagnosticians**, Oct. 22 through 28 in Providence, R.I.

REGULAR COLUMNS

- **PUBLIC HEALTH** p. 2
Forensic case work.
- **DISEASE REPORTING** p. 3
Influenza response.
- **DIAGNOSTIC QA** p. 4
EIA form submission
- **LAB UPDATES** p. 14
More new testing options.
- **ON THE ROAD** p. 15
Your chance to meet us.

LAB LINES

INSIDE THIS ISSUE

AVIAN FLU p. 1
 VDL personnel are tracking the latest developments in this pathogenic outbreak.



CURLY CALF p. 6
 VDL diagnosticians help track this potential genetic defect.



VESICULAR STOMATITIS . p. 7
 Warmer weather brings an increase in index cases.

POOLED BVD TESTING . . . p. 8
 Are cattle producers under-using this potential money-saving BVD test strategy?



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

LAB UPDATES p. 14
 Pick your Johne's disease testing options.



Update from the Director

Welcome to this issue of *LabLines*, a little delayed this summer, as we have been extremely busy with multiple disease outbreaks occurring in this state and around the country. The largest disease situation, highlighted on our first page, is highly pathogenic avian influenza that affected Midwest states. As you can see on page 3, we are also monitoring for avian influenza in this state and have been helping out with wild bird surveillance across the country. This effort will ramp up in the fall, as the birds return from the south during seasonal migration and increase the risk of exposing domestic flocks to the virus.



BARBARA POWERS, DVM, PHD, DACVP DIRECTOR

Also in this issue of *LabLines* find articles about vesicular stomatitis, which again has hit our state this year, although this time we have been approved by USDA to do the new PCR testing here at the laboratory. We have also seen a number of cases of tularemia in rodents and cats this year. In this issue of *LabLines* you will find some other interesting articles regarding how we have been seeing increased numbers of forensic cases, especially those related to animal cruelty. You will also find some tips about how to submit equine infectious anemia sam-

ples for rapid reporting.

From the Western Slope, there are articles on curly calf syndrome; from Rocky Ford, on BVD pooled ear notch testing. A number of articles highlight some of the research contributions the Veterinary Diagnostic Laboratories have participated in, as well as articles on nitrate toxicity in feed. We also have some new equipment in the laboratory, which will speed our bacteriology testing, and we also present a reminder about Johne's testing options in the laboratory.

We finished our Annual Report for the laboratory for the year of 2014, which is posted online, and some statistics are within this issue of *LabLines*, as well. Along with the Annual Report in January, we had our annual meeting of our External Advisory Committee, which was one of the best meetings we have had, with very positive feedback from users of the laboratory.

As fall approaches, we hope to see many of you at the Colorado Veterinary Medical Association meeting and will be seeing many of you at the AAVLD meeting in Rhode Island. ▲

Barbara E. Powers