

# LAB LINES

Diagnostic news and trends from the Colorado State University Veterinary Diagnostic Laboratories Volume 23, Number 1 Spring/Summer 2018

Lab Updates

## CSU VDL Earns Full AAVLD Accreditation, Maintains Level 1 Status

The CSU Veterinary Diagnostic Laboratories have earned full accreditation status from the accreditation committee of the American Association of Veterinary Laboratory Diagnosticians.

Accreditation by the AAVLD is based on an on-site audit, which follows a prolonged detailed review of laboratory documents, including the quality-assurance and policy manuals, system standard operating procedures, training records, financial status and information technology policies.

Four members of the accreditation committee and special auditors conducted the audit, reviewing equipment, facilities, safety procedures and compliance with the laboratory's system, as well as testing standard operating procedures in an intense five-day inspection. They also met with members of the laboratory's external advisory committee, Gregg Dean, head of the Microbiology, Immunology and Pathology department, and Mark Stetter, dean of the College of Veterinary Medicine and Biomedical Sciences.

"Accreditation is critical for the CSU Veterinary Diagnostic Lab to remain a level 1 laboratory in the National Animal Health Laboratory Network," says former lab director Barb Powers. "It also assures our clients that results are accurate and of the highest quality by certifying the competence of personnel, the proper function of the facilities and equipment and appropriate documentation of all laboratory tests and processes."

Based on the internationally recognized ISO/IEC 17025 standard, AAVLD accreditation is consistent with the World Organization for Animal Health (OIE) Quality Standard for Veterinary Laboratories. Accreditation is a formal recognition of the competency of laboratories and increases client confidence in diagnostic test results. In order to further demonstrate technical competence between accreditation assessments, personnel from accredited laboratories are also required to participate in relevant proficiency testing programs.

Accreditation contributes to continuous improve-



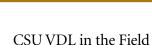
— Barbara Powers, DVM, PhD, DACVP, CSU VDL Former Director

ment and is a management tool that can be used to increase laboratory efficiency, which is critical in times of emergency or limited funding.

Laboratories participating in the USDA's National Animal Health Laboratory Network may be involved in surveillance for early detection of foreign animal disease, surge testing during an outbreak, and testing samples during the outbreak recovery phase. As such, there must be a high degree of confidence in the quality of the laboratories and associated test results. Accreditation is also necessary for assurance and acceptance of test results for live animal export to other countries.

This accreditation extends through 2022.

The AAVLD is a world leader in advancing the discipline of veterinary diagnostic laboratory science to promote global animal health and One Health. Its mission is to promote continuous improvement and public awareness of veterinary diagnostic laboratories by advancing the discipline of veterinary diagnostic laboratory science. In addition to laboratory accreditation, AAVLD provides avenues for education, communication, peerreviewed publication, collaboration, and outreach.



# The New CSU VDL Next Door: Satellite Lab Improvements

Colorado State University's Veterinary Diagnostic Lab at Fort Collins enjoys a world-class reputation. As each issue of *LabLines* attests, our university affiliation, innovative facilities and leading authorities in several research areas all add up to instant recognition of our brand name across the world.

But a different, and perhaps even more important, brand recognition also occurs for thousands of our clients at the grass roots. When those veterinarians, livestock producers, health officials and others think of CSU VDL, they are just as likely to picture our satellite labs located at Rocky Ford and Grand Junction. Those offices, embedded in their local communities and the daily visible face of our extension work, are an indispensable component of CSU VDL's mission to provide relevant, timely, and accurate animal disease diagnostic services to the Eastern and Western Slope of Colorado. Those laboratories play an integral role every day in ensuring the safety of food production, diagnosing zoonotic disease and supporting the management, care and prevention of diseases in animals.

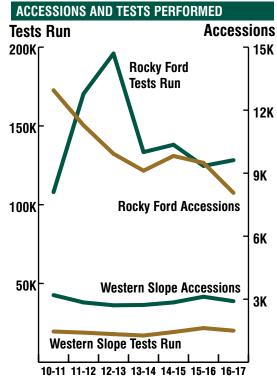
#### **NEW INVESTMENT BRINGS IMPROVEMENTS**

And now they're getting even better. As part of a larger improvement initiative to increase public access to the university, CSU is investing in two satellite campuses that serve research, extension and engagement for local communities. That investment will bring improvements to the services offered at our regional labs.

**CSU Western Campus**. The CSU Western Campus, located at CSU's existing facility in Orchard Mesa, will be home to the brand-new Western Slope Veterinary Diagnostic Laboratory. The planned \$3 million facility, which broke ground this summer, will roughly double the space available at the lab's current, 40-year-old location.



- Barbara Powers, DVM, PhD, DACVP, CSU VDL Former Director



Specific planned improvements, according to Director Raye Walck, include:

- Improved necropsy facilities. Additional space, improved ventilation, facility sizing to better accommodate large-animal work, cooler facilities located within the necropsy lab and relocation of the waste incinerator off-site all will add to the capabilities and efficiency of the new necropsy bay.
- Revamped laboratory space offering improved testing. The additional, improved lab facilities will permit





As the face of CSU VDL in the Colorado community, the regional labs emphasize educational outreach. WSVDL technician Katherine Wadsworth (above), teaches Cedaredge Middle School seventh grade students about food-borne illness and pathogens' cultures, microscopic appearance and specific biochemical testing. Students (right) got hands-on exposure to gross necropsy specimens of livestock cardiovascular systems to study conditions of dilated cardiomyopathy and vegetative valvular endocarditis.

addition of new serology and molecular diagnostic capabilities, specifically on-site Johne's disease PCR and serology testing, as well as other PCR applications. The new lab will also improve biosecurity controls compared to the standards in existence when the original lab was built four decades ago.

■ Shared classroom space to facilitate community involvement. In addition to the lab improvements, the Western Campus will also feature a \$5 million Research and Engagement Building, including office space, two conference rooms and a seminar room, and a commercial kitchen for engagement and Extension education.

"Relocating the lab to centralize us with CSU Ag Extension will really enhance our opportunity to collaborate and improve our already strong outreach efforts to producers and their groups, community and regional colleges, high schools, 4-H and FFA groups and other community members," says Walck.

CSU High Plains Campus. CSU's new High Plains Campus will serve the eastern plains from its home in Rocky Ford.

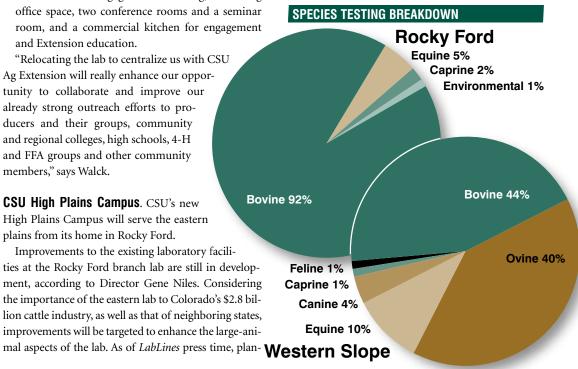
Improvements to the existing laboratory facilities at the Rocky Ford branch lab are still in development, according to Director Gene Niles. Considering the importance of the eastern lab to Colorado's \$2.8 billion cattle industry, as well as that of neighboring states, improvements will be targeted to enhance the large-ani-



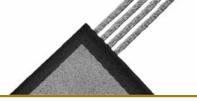
ning was centered on the possibility of adding a large animal storage cooler and a new necropsy building to accommodate livestock work.

The regional lab improvements are part of a combined \$11.65 million infrastructure investment.

"CSU has a strong commitment to agriculture across the state," said CSU President Tony Frank in announcing the initiative last fall, "and this is a testament to that commitment."



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Diagnostic Summary Update

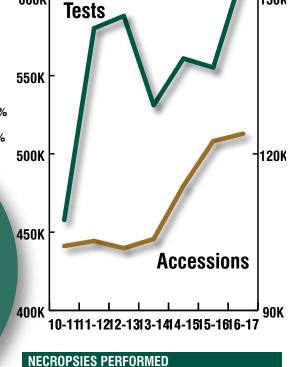
## Annual Report Highlights Activity

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Every year, CSU's Veterinary Diagnostic Laboratories Showcases the progress it has made in meeting its goal of providing timely, accurate, and pertinent animal disease diagnostic services and educational outreach. The following snapshot of testing and disease statistics are by the fiscal year July 1, 2016, to June 30, 2017, and include data from all three of our system labs.

# Caprine 2% Environmental 1% Avian 4% Porcine 1% Wildlife 1% Feline 6% Equine 7% Bovine 34%

Canine 27%

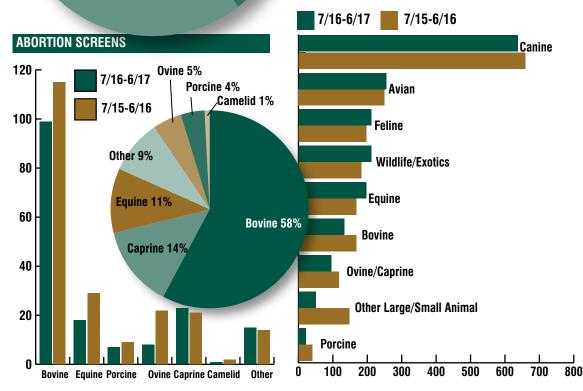


**ACCESSIONS AND TESTS PERFORMED** 



#### **WANT EVEN MORE?**

Review our entire 50-page 2017 Annual Report, under the "Regulations & Resources" tab at www.dlab.colostate.edu



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## Food Animal Production Medicine

## Cyanide Toxicity

Although the number of exposures is relatively small, cyanide poisoning has been the leading cause of cattle deaths reported to the CSU VDL at Rocky Ford.

Over the last five years, the plant causing most of these deaths is poison suckleya. Last summer, we had several reports of deaths due to it, with one cattleman finding nine animals dead within 100 yards of the plants.

Poison suckleya grows along the eastern foothills. Death most often occurs during drought, when dense stands are established around shallow ponds as the water dries up. Prolonged drought leads to increased consumption due to the lack of other, more palatable forages.

Cyanide levels vary greatly within individual plants and from year to year, which is why some authors consider it of minor significance.

Although cyanide accumulation is more common in cultivated flax, blue flax is another plant known to occasionally accumulate toxic levels. Last summer, a cattleman found several cows dead over a period of a week to 10 days. The acute deaths occurred sporadically. None of the animals were observed to be ill before death. On one occasion, he found a dead cow in the road as he was leaving the pasture which had been alive when he entered the pasture just an hour earlier. The cows were grazing a lush stand of blue flax. Gross postmortem examination did not reveal a definitive cause of death, but the veterinarian did note the rumen was packed full of blue flax. Although the rumen content was not kept frozen, it still contained a level of cyanide consistent with cyanide poisoning when analyzed the following day.

Johnsongrass (*Sorghum halepense*) is the only other plant producing cyanide poisoning in beef cattle that has been reported to this laboratory.

Although cyanogenic glucosides, dhurrin (suckleya) and linamarin (flax) accumulate in all parts of the



## from Plants

Gene Niles, DABVT, DVM; Director
 CSU VDL Rocky Ford

plant, seedlings and foliage generally contain the highest cyanide levels. Consumption of fresh green plants, green chop, wilted plants and rapid new growth present the most risk. Second growth flax straw containing green stems is reported to pose a significant risk.

Damage to the plant tissues due to environmental factors or chewing releases the glycosides, initiating a chemical reaction yielding hydrogen cyanide. Undamaged plants do not contain a large quantity of free cyanide.

Cyanide binds with iron in the cytochrome oxidase system, interrupting cellular respiration; therefore, blood does not release oxygen and becomes bright red due to hyper oxygenation. Death results from tissue hypoxia. Postmortem findings are generally nonspecific. While the animal is alive the mucous membranes will be bright pink and the blood cherry red. This color fades rapidly, leaving cyanotic membranes and most often normal blood color. Rumen content may smell like bitter almonds. Care should be taken to prevent inhalation of rumen gas.

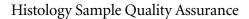
#### **DIAGNOSIS**

Evaluation of clinical signs, necropsy findings and evidence of exposure to plants known to accumulate cyanide are used in diagnosis. Whole blood from live animals can be analyzed for cyanide. Postmortem tissue samples and rumen content should be packaged in air-tight containers, frozen immediately and kept frozen during transit. Plant samples should be placed in an air-tight container and kept moist during transit. Cyanide levels greater than 200 ppm on a wet-weight basis are considered very dangerous, while levels of less than 100 ppm are generally considered safe.

Sodium thiosulfate is used to treat cyanide poisoning. Although it can be used alone, its effectiveness is enhanced by pretreatment with sodium nitrate, which produces methemoglobin that binds the cyanide, restoring cytochrome oxidase system function.

#### **CLINICAL SIGNS**

- Abrupt onset generally within minutes
- Dyspnea
- Ataxia
- Respiratory failure
- Cyanosis
- Bloat rumen contents may smell like almonds
- Convulsions Death



# 'What on Earth is this?' Tips for More Successful Submission Forms

Once that diagnostic sample you just sent off to the CSU VDL makes its way to us, it will have to take many steps before it reaches one of several rotating pathologists who will work diligently to read and report out the correct diagnosis.

Before it heads upstairs to Histology for processing, embedding and staining, prior to making its way to Tissue Trimming, where it is assigned to a pathologist and trimmed into cassettes, first it must go through Sample Receiving. There, the all-important step of checking the sample against the submission form you supplied for tissue type, patient name and other critical information occurs.

We consider ourselves a partner in the care of your patients and their owners; it's a partnership we take very seriously and a trust we strive to protect. A completely and correctly filled out expanded, two-page submission form forms the foundation of that partnership. It helps to:

- Prevent leaving us guessing at what tissue samples are.
- Save money in the amount of cassettes needed to accurately diagnose and treat your patient.
- Indirectly impact your bottom line by saving you time in answering phone calls or inquiring about missed masses.

Check these submission-form quality control steps to make sure you are helping us help you.

Make clinic/client information clear. The clinic information is critical to assigning the correct pathologist to your case. It is very helpful if this section is completely filled out. Please avoid acronyms for your hospital or clinic name. Abbreviations may be a time-saver for the person filling out the form, but considering that the VDL sees hundreds of samples daily, ranging from all over the world, it can be hard to decipher what that acronym stands for. Also please be clear with the city, state, phone number and how the results should be reported. These simple details enable us to assign the correct pathologist, and to reach out to you with any questions that may arise throughout your sample's journey from start to the final report.

**Double-check patient name/species**. After we receive your samples, as well as at multiple times throughout the diagnostic process, we check to match the patient name with the tissue(s) submitted,

— Amy Rich, CSU VDL Laboratory Technician

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## WHERE TO FIND THE SUBMISSION FORM

- Go to www.dlab.colostate.edu
- From the "Client Services" pulldown, select "VDL Forms
- Scroll down to find and click on the form you need

in order to assure the correct jars and tissues are processed in accordance with our quality-control standards. Animal information is vitally important. Patient name discrepancies can cause us to pull your sample from production and move it to quality control, to verify the tissues submitted. That step slows the process. In addition, the species section is important to fill out, as it can aid the pathologist in the diagnosis for species-specific conditions.

**Go digital**. The online submission form can be filled out on your computer as an Adobe Acrobat file and then printed to send with the sample. We prefer this method, to reduce potential for errors due to the inability to read handwritten information.

**Be specific on tissue submissions**. This section is perhaps the most crucial section for the Tissue Trimming department. Non-specific tissue descriptors can cause confusion and slow down the process. Examples include:

■ Vague language, for example, listing out endoscopic biopsies by site—such as stomach and duodenum—yet only enclosing one jar with one cassette. Please make sure the jar labels clearly match the tissue specified on the submission form. In this example of one jar with one cassette and two samples, an easy fix would be to either submit the different sites in different labeled cassettes or write "Stomach and duodenum submitted in same cassette" on the tissue submitted line.

Another vague tissue descriptor that can cause miscommunication is to simply list "masses," with one tissue submitted. Instead, it is valuable to denote several masses are included en bloc, or in one tissue. After fixation, tissue can swell, alter shape and firm up, leaving us unable to see or even palpate certain masses. It can impact both the quality of the results as well as cassette counts.

Feces

**Collection Date** 

- Incorrect tissues. The clearer the tissue is listed and the more accurate its descriptors, the better able we are to make our plan for cutting the tissues. Mark masses if necessary with either ink or suture. (Helpful hint: A spritz of vinegar will help set the ink so it won't bleed over to other margins or areas.)
- Blanks. We know the tissue line on the form is small, so feel free to move down into the history section to list the samples if numerous. Please note on the tissue line to see below for tissue submitted. And you don't need to use a different submission form for each tissue submitted under the same patient; one form is plenty.

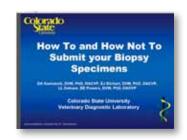
**History**: While we are grateful for complete patient histories, as they help form the overall picture; how-

ever, the most crucial information in this section would be the history involving the tissues submitted. Use the history section to disclose any margin markings, such as ink and suture placement, but avoid using different colored suture, suture diameter or knots as demarcations, as they can be hard to distinguish after fixation. We love your drawings as well! Be clear about whether the sample was excisional vs. incisional, if it was cut down for shipping, whether it was acquired by punch biopsy or Tru-Cut® needle, and so on. History detail can greatly affect the slide counts, which can greatly affect your cost.

**Protect it.** Once you have completed the submission form, please also keep in mind to protect the document from the formalin container by placing it in a separate plastic bag. Formalin has a tendency, despite best efforts, to leak and ruin paperwork. Certain inks will run and bleed, leaving the writing illegible.

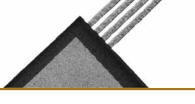
### **ADDITIONAL SUBMISSION REMINDERS**

- If you're new to the CSU VDL, begin by completing a New Client Form available on our forms page. It allows us to speed your process by setting up a personalized account.
- Remember that although page 2 of our expanded submission form lists tests available, a separate submission form is required for Clinical Pathology, Clinical Immunology and Necropsy orders.
- Can't find a test? The two-page form has allowed us to list more of our available tests; however, clients should always consult our User Guide available online for a current, complete list, as well as guidance on proper sample submission.
- More than three animals? Use our Multiple Animal Submission form as a continuation of the General Sample Submission Form if you're submitting samples from more than three animals.



#### **NEED MORE TIPS?**

For more information on how to properly submit a sample, see "How to and How Not to Submit your Biopsy Specimens" on our website, under the "How to Submit a Sample" tab.



## **Innovative PCR Applications**

## Next-Gen Sequencing of MUO

Despite its being believed to account for up to 25 percent of inflammatory central nervous system disease cases in dogs, we still don't know the pathogenic mechanisms that cause Meningoencephalomyelitis of unknown origin, or MUO. An idiopathic inflammatory neurologic disease, MUO includes several inflammatory diseases differentiated histopathologically, including necrotizing meningoencephalitis (NME), necrotizing leukoencephalitis (NLE) and granulomatous meningoencephalomyelitis (GME).

Prior studies have tried a variety of diagnostic tools—polymerase chain reaction (PCR), serology, culture, immunohistochemistry or a combination—to investigate viruses commonly implicated in CNS disease, including herpesviruses, adenoviruses, parvoviruses, canine parainfluenza virus, encephalomyocarditis virus, bunyaviruses, coronaviruses, enteroviruses, flaviviruses, paramyxoviruses and parechoviruses. The overwhelming majority have been negative or inconclusive, limited because they use the traditional approach of targeting their testing to specific agents. Our study approached the problem with a less restricted approach.

ADVANTAGE OF METAGENOMIC SEQUENCING

We used a pathogen-discovery technique that bypasses many of the limitations of specific diagnostics: nextgeneration metagenomic sequencing. Instead of looking only for a specific agent, metagenomic sequencing randomly sequences the total nucleic acids from a  Laura Hoon-Hanks, DVM, CSU VDL Resident; Stephanie McGrath, DVM, CSU Clinical Sciences Assistant Professor; Kenneth Tyler, MD, University of Colorado; Christine Owen, CSU DVM Student; Mark Stenglein, DVM, CSU Assistant Professor

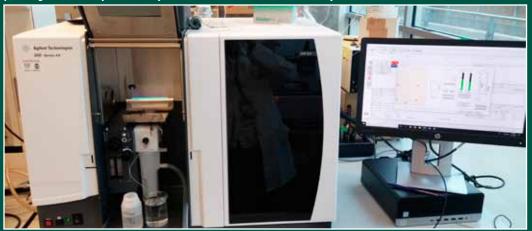
clinical or environmental sample and then categorizes them taxonomically against known sequences in public databases.

In this study, RNA and DNA were extracted from cerebrospinal fluid or brain samples from 11 dogs diagnosed with MUO and 11 dogs without MUO, along with multiple positive controls samples to validate our sequencing and analysis pipelines and to establish detection limits. Nucleic acids were then converted into sequencing libraries, sequenced and filtered, leaving about 2% of the original sequences in each sample. Those were then assembled into longer contiguous sequences and queried against databases of nucleotide and protein sequences to identify possible pathogen-derived sequences.

The result? Sequences from no single organism were found in more than three of 11 MUO samples, and organisms were inconsistent between DNA and RNA from the same tissue as well as brain and CSF collected from the same animal. Although they don't prove it, our results support the hypothesis that MUO is not associated with infectious agents and might instead be an autoimmune disease.

Hoon-Hanks LL, McGrath S, Tyler KL, Owen C, Stenglein MD. Metagenomic Investigation of Idiopathic Meningoencephalomyelitis in Dogs. *J Vet Intern Med*. 2018 Jan;32(1):324-330. doi: 10.1111/jvim.14877.





Spring/Summer 2018

9 LAB LINES



Food Animal Production Medicine

## Field Investigation Unit: An Untapped Livestock Resource

Anyone involved in livestock production as owners, managers or veterinarians finds himself challenged with problems and issues all too frequently. Most would say that facing and solving those challenges is what makes livestock production both an interesting and rewarding endeavor, even when problems occasionally seem almost insurmountable.

If a livestock operation problem presents itself—whether sheep, beef cattle or dairy—all avenues of case workup have seemingly been exhausted and no resolution can be determined, what would be your next step? Certainly, giving up should not be an option at this point.

The Colorado State University College of Veterinary Medicine and Biomedical Sciences, Department of Clinical Sciences and the Veterinary Diagnostic Laboratories, in collaboration with the Colorado State University College of Agriculture, Department of Animal Sciences, has a service known as the Field Investigation Unit (FIU) available to livestock clients and veterinarians in Colorado and the region. I serves as a viable option to help evaluate and solve the more difficult cases that challenge the efforts of a routine client/veterinary interaction in the field. As noted by the make-up of the investigation unit participants above, expertise is top of the line.

Once the client or attending veterinarian agree to engage the Field Investigation Unit, a guideline is delivered them to offer assistance in providing a synopsis of the problem, complete and detailed case/herd history, all records of diagnostic laboratory tests, feed, water and mineral supplement analysis information as well as digital pictures as appropriate. Once the case information submitted is deemed complete, it is dispersed electronically to university personnel who are a part of the FIU as well as animal science and veterinary

- Charlie Davis, DVM, CSU VDL Case Coordinator

students who have interest in livestock production for their evaluation and consideration of the case.

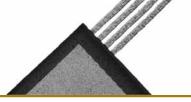
After an appropriate allowance of time to allow FIU personnel to evaluate the case, a teleconference is coordinated and discussion between the owners, attending veterinarians, university personnel, students and any other people who have a relevant part in the case or who the owners approve for participation. The goal is to attain a resolution of the case and give recommendations to the owners moving forward. In the event that the FIU determines more testing or perhaps onsite visitation would be of value to the evaluation of the case, either or both would be completed.

Of importance to note is the fact that there is some time commitment on the part of the owner in putting the case together, but all consultation and recommendations of the university FIU personnel are at **no** charge to the client. The only fees involved would result if further laboratory testing was recommended.

The Field Investigation Unit is a valuable resource for ranchers and practicing livestock veterinarians in Colorado and the regional livestock industry as well, a resource that is underutilized.

How do you engage the Field Investigation Unit?

- Charlie Davis, CSU VDL Case Coordinator. (970) 297-0370 (office), (970) 689-1632 (mobile), Charlie.Davis@Colostate.Edu
- College Of Veterinary Medicine And Biomedical Sciences—Department Of Clinical Sciences Livestock Medicine Office. (970) 297-5000.
- Jason Ahola, Department Of Animal Sciences., 970-491-3312, jason.ahola@colostate.edu

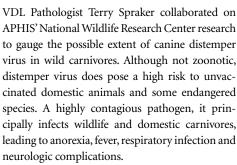


CSU VDL In Press

## A Roundup of VDL Faculty Research

#### **DISTEMPER CLADES IN WILD CARNIVORES**

Wostenberg DJ, Walker N, Fox KA, Spraker TR, Piaggio AJ, Gilbert A. Evidence of Two Cocirculating Canine Distemper Virus Strains in Mesocarnivores from Northern Colorado. J Wildl Dis. 2018 Mar 2. doi: 10.7589/2017-09-238.



The research team opportunistically collected wild and domestic carnivore specimens through a rabies-surveillance program in northern Colorado between 2013 and 2016. They tested a total of 478 animals for the virus, comprised of 10 wild and domestic carnivore species. A total of 24% (71/300)

of raccoons and 4% (1/26) of coyotes tested positive. Spraker also extracted RNA from positive tissues, using reverse-transcription PCR to create complementary DNA. He amplified and sequenced the hemagglutinin gene from 60 CDV-positive tissues, and a median joining network and maximum likelihood phylogenetic tree revealed two major lineages among samples, most similar to the America-2 (n=55) and the America-3 (n=5) distemper lineages circulating in North America.

#### SPOTTING AGGRESSIVE CHONDROSARCOMA

Vinayak A, Worley DR, Withrow SJ, Adams DS, Powers BE.

Dedifferentiated Chondrosarcoma in the Dog and Cat: A Case Series
and Review of the Literature. J Am Anim Hosp Assoc. 2018 Jan/
Feb;54(1):50-59. doi: 10.5326/JAAHA-MS-6566.

VDL former Director Barb Powers participated in

this Veterinary Teaching Hospital case describing seven dogs and one cat from the VDL medical-records database diagnosed with the uncommon, aggressive variant of chondrosarcoma known as dedifferented chondrosarcoma. This, the most aggressive variant in humans which accounts for 10 percent of all human chondrosarcomas, had not yet been described in veterinary patients.

Because a bimorphic pattern consisting of mineralized and nonmineralized areas has been reported in one-third of radiographs, one-half of CT scans, and one-third of MRIs in human patients with dedifferentiated chondrosarcoma, image interpretation is crucial in helping guide multifocal biopsies to obtain an accurate preoperative diagnosis. Definitive diagnosis requires histopathological confirmation of a highgrade noncartilaginous sarcomatous component juxtaposed near a cartilaginous low-grade component. A diagnosis of a conventional chondrosarcoma was made in the four cases in this report with pretreatment biopsies, while re-evaluation by Powers resulted in a diagnosis of dedifferentiated chondrosarcoma. Possible reasons included only the chondroid component being biopsied, missing the dedifferentiated histological component on evaluation, lack of information in the veterinary literature on diagnosing these tumors.

Surgery remains the treatment of choice with this variant in human medicine, with high local recurrence rates with intracapsular and marginal excision. Metastatic disease, primarily to the lungs, remains the cause of death in humans, and the animals in this series appear to have a similar rapid clinical progression to metastasis. This variant exists in veterinary medicine and is likely misdiagnosed as a high-grade conventional chondrosarcoma. Accurate early diagnosis would allow for a tailored treatment plan and a more

SUMMANT OF LIGHT	CASES OF VETERIN	ARY DEDIFFERENTIATED CH	UNDRUGARUC	Progression-	Survival
Signalment	Location	Treatment	Days to presentation	free survival days	time (days)
11 yr SF DSH cat	Right humerus	Amputation	3		225
10 yr CM Australian shepherd	Right femur	Amputation/doxorubicin/ cyclophosphamide	8	47	346
8 yr CM golden retriever	Rostral mandible	Bilateral rostral mandibulectomy	Unknown	217	248
7 yr SF Labrador retriever	Left nasal cavity	Carboplatin/mitoxantrone/ palladia	42	55	241
9 yr SF Labrador retriever	Left and right nasal cavity	Antibiotic therapy	3	N/A	N/A
6 yr SF German shepherd mixed-breed dog	Right nasal cavity	Dorsal rhinotomy	30	Not reached	Not reached
8 yr SF Welsh corgi	Left abdominal wall	Stereotactic radiation therapy	30	143	177
10 yr CM Siberian husky	Right paralumbar region	Marginal excision	3	151	196
CM=castrated male; DSH=domesti	c short hair; SF=spayed female				



accurate prognosis in veterinary patients.

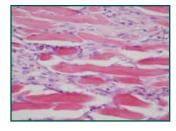
#### KELOIDAL FIBROSARCOMA IN A DOG

Evans SJM, Frank CB, Avery PR, Moore AR. What is your diagnosis? Subcutaneous mass on a dog. Vet Clin Pathol. 2018 Mar;47(1):160-161. doi: 10.1111/vcp.12551.

VDL pathologist Chad Frank helps describe the final histologic diagnosis of low-grade keloidal fibrosarcoma in a 9-year-old, castrated male Boxer, presented for a newly identified 1 cm, soft subcutaneous mass on the dorsum over the right lumbar fat pad. Fine-needle aspiration of the mass was performed and submitted for histopathology at CSU VDL. Histologically, this was a densely cellular, poorly demarcated mass in the subcutaneous tissue composed of small intersecting bundles of moderately pleomorphic mesenchymal cells intermixed with irregularly large and glassy hyalinized collagen fibers. Immunohistologically, the mesenchymal cells were positive for vimentin and negative for smooth muscle actin and CD18. The final histologic diagnosis was a low-grade keloidal fibrosarcoma. As in other dermal fibromas/fibrosarcomas, the spindloid cells in keloidal fibromas/fibrosarcomas were true fibroblasts and not myofibroblasts, as they stained positive for vimentin and negative for SMA. Often admixed with these cells are fusiform or round, reactive CD18+ and CD45+ histiocytes, suggesting the lesion may be initiated by an inflammatory process. In this case, no CD18+ histiocytes were identified.

Keloidal fibromas/fibrosarcomas are rare in dogs. They typically present as poorly demarcated lesions in the dermis, subcutis, or both. The majority are benign keloidal fibromas. A breed or age predilection has not been documented, although both intact and castrated males may be overrepresented. To the authors' knowledge, only one cytologic case of keloidal fibrosarcoma has been previously documented in a subcutaneous mass on a 5-year-old castrated male mixed-breed dog.

Although keloidal fibroma/fibrosarcoma appears to be rare in dogs, the malignant form here was diagnosed based on high cellularity, invasiveness, and cellular atypia. The sensitivity and specificity of cytology for diagnosis of keloidal tumors, and the ability to distin-



Large bundles of mesenchymal cells intermixed with irregularly large and glassy. hyalinized collagen fibers characterize this keloidal fibrosarcoma.



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guish the malignant variant, are unknown. The presence of characteristic hyalinized collagen fibers is a very distinctive cytologic feature which should trigger the primary differential diagnosis when observed in association with mesenchymal neoplasia.

#### IN SEARCH OF LUNG CARCINOMA SPECIFICITY

Beck J. Miller MA. Frank C. DuSold D. Ramos-Vara JA. Surfactant Protein A and Napsin A in the Immunohistochemical Characterization of Canine Pulmonary Carcinomas: Comparison With Thyroid Transcription Factor-1. Vet Pathol. 2017 Sep;54(5):767-774. doi: 10.1177/0300985817712559.

Chad Frank also carried out the histology in this study to compare the reliability of immunohistochemistry for surfactant protein-A (SP-A), napsin A and thyroid transcription factor-1 (TTF-1) as possible specific and sensitive markers for canine pulmonary tumors. Commonly used to diagnose pulmonary tumors in humans, the immunohistochemical markers likewise show immunoractivity in canine lung tissue, although TTF-1 is detected in more than 80% of canine thyroid carcinomas, a tumor that commonly metastasizes to the lung. That nonspecificity has prompted the search for additional markers to improve differentiation of primary from metastatic canine lung carcinomas.

Frank and colleagues applied TTF-1, napsin A and SP-A antibodies to 67 pulmonary tumor samples from the databases of both Purdue and the CSU VDL. The most sensitive marker was SP-A, with detection in 65 of 67 (97%) pulmonary carcinomas. The vast majority of tumors also expressed napsin A (92%, or 62/67) and TTF-1 (91%, or 61/67). Only 1 pulmonary carcinoma was immunohistochemically negative for all 3 markers. Most tumors expressed all 3 markers (88%; 59/67). Although each marker had good sensitivity, only 3% (2 of 67) of lung tumors were negative for SP-A compared with 7% (5/67) and 9% (6/67) for napsin A and TTF-1, respectively. Each antigen was detected in a greater percentage of cells of tumors with acinar or papillary patterns compared with those with squamous

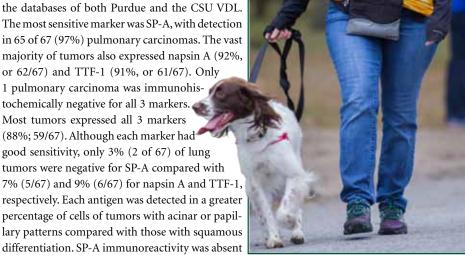


Photo: Flickr/Free to Breathe. Some rights reserved. Used under CC BY-NC 2.0.

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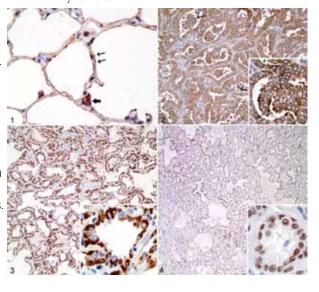
	Surfact	Surfactant Protein A		Napsin A		Thyroid Transcription Factor-1	
	Cell score	Intensity score	Cell score	Intensity score	<b>Cell Score</b>	Intensity score	
Lepidic	2.6	2.3	2.9	2.8	2.9	2.8	
Minimally invasive carcinoma	3.0	1.8	2.0	2.3	1.0	2.8	
Papillary	2.7	2.1	2.5	2.8	2.7	2.8	
Acinar	2.1	2.4	1.7	2.8	2.3	2.3	
Solid	2.0	3.0	2.3	3.0	3.0	3.0	
Adenosquamous	1.3	2.8	0.8	2.1	1.1	2.1	
Squamous cell carcinoma	0.3	0.5	0.3	0.8	2.0	2.0	
All subtypes	2.3	2.1	2.4	2.7	2.5	2.6	

in all 113 nonpulmonary tumors tested. Of 108 normal tissues, SP-A was detected only in lung and in one of six adrenal, one of three endometrial, and one of four hepatic sections.

Based on these findings, SP-A and napsin A are useful markers, and of these, SP-A is the most sensitive and specific (although a possible pitfall is the need to distinguish entrapped normal pulmonary epithelial cells or alveolar macrophages from neoplastic cells). It can be used in combination with TTF-1 or napsin A to improve detection and differentiation of pulmonary carcinomas from metastatic tumors in the canine lung. Using both in combination in canine tissues can also increase specificity by distinguishing a napsin A-positive metastatic tumor of renal origin from a pulmonary carcinoma. Although the reactivity of TTF-1 and napsin A in non-lung tissues is limited, the detection of both in thyroid tumors and of napsin A in renal tumors underscores the importance of using a lungspecific marker to differentiate metastatic from primary tumors.

(Clockwise, from top left)

- Strong labeling for SP-A in the normal dog lung
- Diffuse cytoplasmic labeling for SP-A in tumor cells. Strong granular labeling for SP-A in the cytoplasm of alveolar macrophages (arrow)
- Strong nuclear labeling for TTF-1 in neoplastic cells.
   Inset: Higher magnification showing diffuse nuclear labeling in neoplastic cells.
- Intense granular cytoplasmic labeling for napsin A within neoplastic cells lining acini.



#### WHERE DID THE COXIELLA BURNETII GO?

Oliveira RD, Mousel MR, Pabilonia KL, Highland MA, Taylor JB, Knowles DP, White SN. Domestic sheep show average Coxiella burnetii seropositivity generations after a sheep-associated human Q fever outbreak and lack detectable shedding by placental, vaginal, and fecal routes. PLoS One. 2017 Nov 15;12(11):e0188054. doi: 10.1371/journal. pone.0188054.

Ruminant livestock, particularly small ruminants, are hypothesized to be the primary transmission source of *Coxiella burnetii* to humans. A globally distributed zoonotic bacterial pathogen that causes abortions in ruminants and a potentially fatal influenza-like illness known as Q fever in humans, *C. burnetii* has been reported on four farms in Colorado.

In this study, CSU VDL Interim Director Kristy Pabilonia collaborated with Washington State and APHIS researchers to follow up on the expected level of bacterial shedding and seroprevalence from an Idaho location following a Q fever outbreak in 1984. Currently housing about 1,428 mature ewes, decreased from 5,270 at the time of the outbreak, the facility offered a good opportunity to test shedding patterns, as thousands of ewes have been consistently maintained there, even as the flock has been effectively closed to new introductions for the past decade. Pabilonia tested placental tissue, vaginal swab and fecal samples from 100 sheep by real-time quantitative PCR. Given the large flock size, previous proof that C. burnetii can be shed for multiple parturitions, its extremely high ratio of placental shedding to infectious dose, and demonstrable environmental persistence of the pathogen, the researchers believed still finding shedding should have been a distinct possibility, even 32 years after outbreak.

The surprising result: They found no detectable *C. burnetii* DNA in any placentas, feces or vaginal swabs, and only five or six of the 100 animals showed evidence of seroconversion. The 5% seropositivity they found was not significantly different from the national

LARLINES Spring/Summer 2018

average of 2.7% for the country. While the presence of seropositive individuals demonstrates exposure at some time, PCR results suggest 2016 shedding events were rare or absent.

If the location didn't depopulate after the 1984 outbreak and didn't vaccinate then or since, how did the zero-shedding occur? More work is required to answer, but the research team suggested possible factors might include simple passage of time, the passage of generations, demonstrated by a pedigree analysis showing the average ewe at time of study was 9.21 generations removed from tail-female ancestors that lambed during the epidemic, demolition and replacement of most of the original lambing facilities which might have reduced residual C. burnetii in the lambing area, or changes in husbandry which may have reduced infection from the environment over time. Regardless of the cause, this first documented U.S. progression from outbreak to lack of shedding demonstrates elimination of fetal infection over subsequent generations may be possible.

#### CAN VACCINES STEM BLUETONGUE EPIDEMIC?

Mayo C, Lee J, Kopanke J, MacLachlan NJ. A review of potential bluetongue virus vaccine strategies. Vet Microbiol. 2017 Jul;206:84-90. doi: 10.1016/j.vetmic.2017.03.015.

Noting that little doubt exists now that global distribution and the nature of bluetongue virus infection of livestock have changed recently, CSU VDL Virology Section Head Christie Mayo writes in this review that control efforts aimed at vector insects may grow less and less effective. Climate change's potential impact on the vectorial capacity of populations of Culicoides insects has contributed to at least nine serotypes of

BTV invading and spreading throughout extensive portions of Europe and even Scandinavia, precipitating an economically devastating BT epidemic. Additional novel serotypes of BTV have recently invaded historically endemic countries such as the United States, Israel and Australia, and the first-time detection in Ontario in 2015, represents even further northward expansion of its range.

If vector-midge control is growing less reliable, vaccination may become even more important relatively to control and prevent of BTV transmission. A number of vaccine strategies have been explored and show promise to combat BTV infection of livestock and wildlife; however, only modified-live and killed vaccines are commercially available. Potential inherent problems to effective vaccination exist, including the serotype-specific nature of immunity of animals to BTV, along with the multitude of virus serotypes. Although routinely used in endemic areas, MLV face limitations, from acquisition and transmission by insect vectors leading to their circulation in the environment as field strains, to reassortment of gene segments of attenuated vaccine with field viruses to generate novel strains. Although they have several potential advantages over MLV vaccines, inactivated have their own disadvantages, including a slow onset of immunity and the lack of commercial products for most serotypes. Novel vaccine platforms have been shown to be effective experimentally, but their inherent cost and limited market potential have prevented commercial use.



Photo: Flickr/Virginia State Parks. Some rights reserved. Used under CC BY 2.0.



**CSU Microbiology senior Ciara** Suggs won high honors at April's Celebrate Undergraduate **Research and Creativity awards** for her work on improving reliability of fecal flotation in pinnipeds. Under the mentorship of the CSU VDL Parasitology Section head, Suggs' research modified the technique by adding a detergent step and overnight incubation step to increase visibility in the typically debrisladen samples, improving egg detection.

## **BLUETONGUE VIRUS VACCINE PROS AND CONS**

Heat-, radiation- or chemical-killed virus Inactivated mixed with adjuvant to nonspecifically vaccines stimulate immune system to respond to viral antigens on the killed viruses Virus attenuated by serial passage in Modified alternate cultures replicates only to low level after vaccination; progeny viruses vaccines stimulate virus-specific immunity Non-BTV, non-pathogenic viruses Recombinant genetically modified to produce antigenic proteins to stimulate BTV-specific vaccines antibodies Disabled Essential-gene deletion results in BTV infectious virions that replicate only once after single cycle

live

vector

vaccines

Virus-like

particle

vaccines

vaccination

Genetically modified baculovirus vectors grown in insect cells express BTV proteins, which assemble into empty viral particles which are then advjuvanted

- Relatively safe
- · Can't reassort with field strains

Pro

- Cost effective
- · Single dose immunity possible · Immunity can last for years
- Strong neutralizing immunity possible
- · Potential for single dose immunity
- · Low risk of reversion to virulence
- · High stability · Low risk of side effects
- · No risk of disease
- · Difficult/expensive to design

Con

Multiple doses required

· Local adjuvant reaction

· Reversion to virulence

· Side effects

• Immunity may be transient

· Reassortment with wild strains

Vector transmission possible

- Difficult/expensive to design
- Multiple doses likely necessary
- · Multiple doses required
- · Difficult/expensive to design and manufacture
- · Local adjuvant reaction



Marissa Wilkey, new CSU VDL Bacteriology Section lab technician, grew up in western North Carolina, moved to New Mexico and earned a bachelor's degree in molecular biology with minors in chemistry and art from Fort Lewis College in Durango. She earned her microbiology master's degree from CSU's MIP program.

Five of CSU's anatomic pathology residents passed the board certification examination, recognizing entry-level competency in veterinary clinical pathology and veterinary anatomic pathology. Some have suggested the ACVP certification is the most rigorous and best-designed certification process within all veterinary medical specialties. Congratulations to:

- Mike Betlev
- Kendra Andrie
- Ben Curtis
- Sam Evans
- Cait Martinez

CSU VDL in the Field: Disease Updates

## White Nose Disease in Bats

SU VDL continues to work with the U.S. Geological Survey's National Wildlife Health Center to track the slow but steady westward expansion of white nose syndrome in bats caused by *Pseudogymnoascus destructans*.

The fungus was detected in neighboring South Dakota for the first time this summer during field examination of live bats. Swab samples sent to CSU VDL confirmed the finding. South Dakota joins Mississippi and Texas as states that have detected *P. destructans* presence in bats, but not confirmed white nose syndrome.

According to Interim Director Kristy Pabilonia, CSU VDL has tested more than 100 samples to date using real-time Polymerase Chain Reaction testing. VDL is one of the few labs capable of offering PCR for the fungus, which is capable of specifically detecting the fungus by amplifying DNA from a sample as small

CSU VDL in the Field: Disease Updates



as one cell. PCR can also often successfully detect the pathogen from old or degraded DNA samples.

WNS, named for the powdery, white P. destructans growth that often appears around infected bats' muzzles, has killed millions of bats in North America since it was first seen in New York in 2006. Mortality rates of up to 100 percent have been observed at some colonies. To date, WNS has been confirmed in bats from 32 states and 7 Canadian provinces, although it has not yet been found in Colorado. Bats are important for healthy ecosystems and contribute at least \$3 billion annually to U.S. agriculture through pest control.

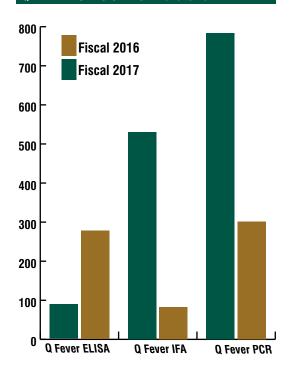
## Q Fever Case Reported in Colorado

SU VDL has seen more than a doubling of its number of tests during fiscal 2017 for *Coxiella burnetii*, the causative pathogen behind the zoonotic disease Q fever. Colorado's Department of Agriculture reported as of mid June that *Coxiella burnetii* has been detected on four farms in 2018 in four Colorado counties. Sites include a raw milk herd-share dairy, a small hobby farm with recent goat abortion and sick neonatal lambs, a meat-goat herd suffering an 80% abortion rate, and a show goat-herd with several abortions. One person associated with one of the farms has been diagnosed with Q Fever.

The state ag department has not determined any epidemiologic links between the four farms and is continuing to trace high-risk movements, such as pregnant animals. Individual farms are implementing best management practices to protect public health and animal health.

Coxiella burnetii is an obligate intracellular gramnegative bacterium distributed worldwide whose primary reservoir is cattle, sheep and goats. It survives for prolonged periods in the environment, and exposure to only a few organisms can result in infection. Most human cases results from contact with cattle, sheep and goats, especially during parturition. Human cases can also potentially result from consumption of unpasteurized milk. Infection is acquired through inhalation or ingestion of the organism. Companion animals are also susceptible and have been documented as a source of human infections. Q fever can result in acute and chronic cases in humans.

#### Q FEVER TESTING CHANGE: 2016 to 2017





Our External Advisory Committee members volunteer their time to meet with us annually and assess our progress, as well as provide input to our future directions. We are grateful for their time and advice, and hope they feel they are an integral part of the laboratory.

Back Row: Ernie Etchart (Sheep Industry), Linda Vap (Section Head), Karen Fox (CPW), Karen Rogers (Feedlot), Jennifer House (Public Health Veterinarian), Tracy Baszler (IT Services), Dean Mark Stetter (Dean of CVMBS), Leesa McCue (Mixed Animal), Ron Kollars (Small Animal), Dwayne Hamar (Section Head), Kellee Smith (CO Livestock Association), Christie Mayo (Section Head). Middle Row: Terry Spraker (Pathologist), Ann Bertschy (Small Ruminant), Larry Mackey (Large Animal), Marv Hamann (Mixed Practice), Joan Bowen (Small Ruminant), Gene Niles (Rocky Ford Director), Norm Brown (Equine), Barb Powers (Former VDL Director), Keith Roehr (State Veterinarian), Ashley Stokes (CSU Extension), Charlie Davis (Case Coordinator), Raye Walck (Western Slope Director), Josh Daniels (Section Head), Michael Lappin (Section Head).

Front Row: Gary Mason (Section Head), Zachary Desmond (Necropsy Technician), Richard Wheeler (Mixed Practice), Kristy Pabilonia (Interim VDL Director), Gregg Dean (Section Head), Janice Inman-Leflet (Business Officer).

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

Plan now to meet VDL Interim Director *Kristy Pabilonia*, Western Slope Lab Director *Raye Walck*, Virology Section Head *Christie Mayo*, Quality Assurance Manager *Dwayne Hamar*, Pathologists *Chad Frank* and *Juan Munoz*, and Quality Assurance Assistant Manager *Kevin Daniels* at this year's annual meeting of the *American Association of Veterinary Laboratory Diagnosticians*, Oct. 18 to 24 in Kansas City.

Interim Director *Pabilonia* will be at the *AAVLD Accreditation* and *Executive Board* meeting in Denver, Aug. 23 to 25. She and VDL Avian Health Coordinator *Heather Reider* were in attendance at the *USDA National Poultry Improvement Plan* meeting, June 26 through 28 in Tennessee. *Pabilonia* and Molecular Diagnostics Technician *Kirsten Reed* were also at the *International Symposium on Avian Influenza* in Brighton, England, April 15 to 18.

Western Slope Lab Director *Walck* will be at the *Colorado Veterinary Medical Association* 2018 convention in Loveland, Sept. 20 to 23. She will also be at the *Stockmanship and Stewardship* educational series in Montrose, Sept. 21 and 22.

She has just returned from the *Colorado Woolgrowers*Association Convention in Montrose, July 11 and 12, the

Colorado Cattlemens Association 2018 Convention in

Loveland, June 18 to 20, and the Western States Livestock

Health Association Meeting, June 10 to 13 in Bozeman.

Walck has also hosted a series of educational outreach meetings for the Western Slope lab, including a lab tour by the Western Colorado Community College animal science class in April, a tour by a Gunnison 4-H goat club in February, and an offsite presentation to the Cedaredge Middle School seventh-grade science class in May, along with Laboratory Technician Katherine Wadsworth.

CSU VDL Parasitology Section Head *Ashley McGrew* plans to be in attendance at September's annual meeting of the *Rocky Mountain Conference of Parasitologists* in Ogallala, Neb. She just returned from the annual meeting of the *American Association For Veterinary Parasitologists* in Denver in July, the 14th Boehringer Ingelheim *Animal Health Symposium on Parasitoses & Arthropod-borne Diseases* in Panama City in June, and the annual meeting of the *International Association for Aquatic Animal Medicine* in Long Beach, Calif., in May.



In May, VDL Interim Director Kristy Pabilonia attended the 86th General Session of the World Assembly of **OIE Delegates in Paris. OIE** is the World Organization for Animal Health. Its World Assembly is composed of one official delegate from each member country and develops international animal health regulations. **Each country brings** additional members with their delegation, and Pabilonia attended with the US Delegation on behalf of the AAVLD. She serves as AAVLD secretary/treasurer, one of the executive officers.



## **REGULAR COLUMNS**

- PCR APPLICATIONS p. 6 Next-gen sequencing.
- FOOD-ANIMAL p. 7

  How to bring our Field

  Investigation Unit in.
- IN THE FIELD p. 14 Q fever returns.
- ON THE ROAD p. 15 Where and when you can meet our faculty.

TO CONTACT

or email

**KRISTY PABILONIA:** 

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DLAB@colostate.edu

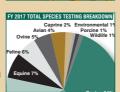
## LABLINES

FULL ACCREDITATION . . . . p. 1 CSU VDL earned full accreditation status from AAVLD. Here's why it's important to you.

THE VDL NEXT DOOR ....p. 2 Big changes are on their way to your neighborhood VDL.

ANNUAL HIGHLIGHTS.... p.4 Every year, the VDL showcases the progress its made. Here's a snapshot of our 2017.





## **INSIDE THIS ISSUE**

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forms leaves us all guessing.
How to make them clear.

LATEST VDL RESEARCH . p. 10 The latest studies from VDL faculty members.

TRACKING WHITE NOSE p. 14 CSU VDL helps track the westward progression of this bat epidemic.





## Update from the Director

It seems like just months ago I was writing my first update from the director, a welcome to the very first issue of *LabLines*. It was summer 1996, and, as I noted then, "The laboratory has gone through many changes."

I had just been named director. We had just hired new section heads for virology and bacteriology. The Veterinary Diagnostic Lab and the Pathology Department had just been combined. We would go on to do about \$700,000 in business that year, and be handling not quite 60,000 annual accessions by the turn of the century.

Now, 22 years later, I am here to report the laboratory is going through changes again. As you can read in the story on page 4, the VDL last year handled more than 120,000 accessions. We grossed more than \$10 million in revenues, which represents more than a *doubling* in our business over the last seven years, contributing approximately 5 percent of the entire College of Veterinary Medicine and Biomedical Sciences' financial support.

It's with mixed emotions that I report that lab is undergoing personnel changes. By the time you read this, I will have left my position as CSU VDL director. I leave the



BARBARA POWERS, DVM, PHD, DACVP FORMER DIRECTOR

VDL in the capable hands of former assistant director Kristy Pabilonia, who is now interim director.

In that first *LabLines* 22 years ago, I set my main goals as maintaining and improving quality and timely service to all our users, working toward rapid development and incorporation of new diagnostic technologies, and improving the quality of education for our studients.

From growing the volume of business we do, to spearheading the opening of the worldclass Diagnostic Medicine Center in 2009, to

the nearly constant addition of innovative technology, to the high level of reputation our brand has across Colorado and the world, I look back on all the VDL's accomplishments over the years with great pride. I know it is our people who have made those goals a reality. Our staff and faculty are passionate about their areas of expertise, about constantly improving, about providing better service, and about furthering the intellectual accomplishment of the entire system. It is with humble pide I thank them—and you—for the opportunity to have served, look forward to seeing you again, and bid you fond farewell.

Babara E. Formas