

Diagnostic news and trends from the Colorado State University Veterinary Diagnostic Laboratories Volume 21, Number 2 Fall/Winter 2016

Get to Know the Laboratory

New Western Slope Director: Renewed Focus on Real-World Diagnostics

As a former practitioner and a dedicated resident of Colorado's Western Slope region, new CSU Western Slope Veterinary Diagnostic Laboratory Director Raye Walck tackles the position with a passion for facilitating the success of the region's veterinary colleagues and agricultural producers. Because of her experience in clinical practice and regulatory medicine, she has an understanding of the real-world challenges that private practitioners and producers face.

Her vision for the Western Slope lab is to merge the cutting-edge diagnostic advances in the veterinary scientific community with the tools and resources of CSU, to create a practical, affordable, real-world diagnostic service that aligns with the needs of a region rooted in agricultural values. Walck plans to support the vision by functioning as a liaison and strengthening ties with CSU's Extension Service.

"Veterinarians faced with diagnostic, therapeutic and financial considerations in the field can find it challenging to juggle progressive and practical approaches," she says. "I see the Western Slope Lab in a position to support its clients and advance their diagnostic capabilities, helping them to become even more successful."

Walck comes to the Grand Junction lab from a diversified career in clinical practice. After earning bachelor's degrees in both biology and Spanish from Western State College in Gunnison, she obtained her DVM from Colorado State in 1998. She has practiced her career almost exclusively on the Western Slope of Colorado, bringing perspective from small-animal practice, livestock production and equine performance and pleasure operations. In addition, she was able to spend the last year working with



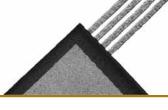
USDA-APHIS Veterinary Services. She received training in regulatory medicine and emergency disease response, after the 2015 Highly Pathogenic Avian Influenza outbreak's devastating effects on the nation's poultry industry.

An intercollegiate volleyball athlete while at Western State, Walck brings a dedicated and enthusiastic teamwork philosophy and approach to disease investigation. Whether at work, on the trail or in the kitchen, learning and applying new skills is the fuel that drives her. Outside of the lab, her personal interests include running, hiking, mountain biking, cooking and attending her children's endless schedule of athletic and school activities.

"It's a very exciting time to join this team, and I'm eager to jump in and see what veterinarians, producers, Extension and CSU can do together," she says.



Please stop by the lab in Grand Junction to meet or reintroduce yourself to Director Walck and the rest of her team, and to find out about upcoming educational outreach activities



Canine Oncology

Splenic Liposarcoma in Dogs

Rarely found in visceral organs like the spleen, canine Rliposarcoma is most often reported in dogs with cutaneous and subcutaneous liposarcomas. In order to better describe the disease in dogs that underwent splenectomy for histologically confirmed splenic liposarcoma, CSU VDL worked with colleagues at Ottawa's Alta Vista Animal Hospital and University of California-Davis, reviewing those institutions' records and ours for dogs with splenic liposarcoma from 2002 to 2012. We found:

Signalment was similar to other splenic tumors.

- Physical examination revealed an abnormality during abdominal palpation in all 13 dogs: 11 dogs had a palpable mass, one had a fluid wave and one had signs of abdominal pain. These findings are not specific to splenic liposarcoma, but they are an indication to recommend further abdominal imaging.
- Splenic mass was found on abdominal radiography or ultrasonography in all dogs; a solitary mass was found ranging in size from 5 to more than 20 cm in diameter. Most splenic liposarcomas were large. The differential diagnoses with a solitary splenic

mass include hematoma, hemangioma, hemangiosarcoma, and nonangiomatous, nonlymphomatous sarcomas (including liposarcoma).

- Anemia was detected in seven of the 13 dogs. The cause was not evaluated, but given the frequency of anemia, splenic liposarcoma should be included in the differential diagnosis list of dogs with anemia and a splenic mass.
- The median survival time was 623 days, ranging from one to 1,283 days. Among the five dogs that died of splenic liposarcoma, survival times ranged from 42 to 369 days. Metastasis at the time of surgery was a negative prognostic indicator: Survival time was 45 days for dogs with metastasis and 767 days for dogs without metastasis. Dogs with grade 1 splenic liposarcoma (0 to 9 mitotic figures per 10 hpfs) had a significantly greater median survival, at 1,009 days, compared to dogs with a grade 2 (10 to 19 mitotic figures per 10 hpfs) or grade 3 splenic liposarcoma (more than 19 mitotic figures per 10 hpfs), at 206 and 74 days, respectively.

LEUKEMIA VIRUS DISTRIBUTION IN A DOMESTIC CAT/LEOPARD HYBRID COLONY

Feline leukemia virus (FeLV) is a retrovirus that causes disease in domestic cats, especially in feral or social populations. Exogenous FeLV (ExFeLV) is a horizontally transmitted virus that causes high morbidity and mortality in a significant percentage of infected animals. Endogenous FeLV (EnFeLV) is a germ-line proviral sequence found only in the Felis species most closely related to the domestic cat (Felis catus) and does not produce an active infection in the host. Other felidae, such as the Leopard Cat (Prionailurus bengalensis) do not have EnFeLV proviral sequences.

The goal of this project was to determine prevalence of FeLV in a colony of privately held domestic cat/leopard cat hybrids with an endemic ExFeLV infection (N=64). The contribution of domestic versus nondomestic

genotypes as well as coinfection with Jordan Powers, Melody Roelke, Elliott Chiu and Sue VandeWoude, Undergraduate Student/ Microbiology, Immunology and Pathology

feline gamma herpesvirus (GHV) and feline foamy virus (FFV) were evaluated as parameters relating to FeLV infection and disease. Thirty-three of 64 cats (51%) of cats were positive for circulating ExFeLV antigen. A subset of 32 animals were tested for FFV and GHV using qPCR. Twenty cats out of the 32 tested positive for FFV (62%) and zero out of 32 cats tested positive for GHV. There was no significant influence of FFV on ExFeLV infection rate. EnFeLV and ExFeLV quantitation is being conducted to relate these parameters to disease state. Information gained from this study will benefit understanding between EnFeLV and ExFeLV interactions in both domestic and nondomestic cats.



Gower KL, Liptak JM, Culp WT, Bravo L, Powers B, Withrow SJ. Splenic liposarcoma in dogs: 13 cases (2002-2012). *J Am Vet Med Assoc*. 2015 Dec 15;247(12):1404-7. **Reportable Disease Updates**

West Nile in Colorado

SU's Veterinary Diagnostic Laboratory diagnosed a horse in Weld County with West Nile virus in August, the first confirmed case of the virus in a horse in Colorado for 2016. Since August, two more cases in Weld County and nine more cases in Adams, La Plata, Larimer, Mesa and Pueblo had been reported to USDA's ArboNET system as of Jan. 3.

CDC also reports as of Jan. 17, 2017, a total of 149 human cases of the zoonotic disease in Colorado for 2016, accounting for eight deaths.

Colorado has conducted statewide surveillance for West Nile since 2001 as part of its Colorado Mosquito-Borne Virus Surveillance Program, which also monitors for western equine encephalitis. West Nile is considered a reportable veterinary disease in Colorado, and any suspected cases, whether laboratoryconfirmed or not, should be reported to the Colorado area USDA office, at (303) 231-5385, or to the Colorado State Veterinarian's Office, at (303) 869-9130. Equine cases of arthropod-borne viral diseases, including West Nile, are reported to ArboNET, CDC's electronic-based surveillance and reporting system used to track and report arboviral activity. ArboNET cap-

Reportable Disease Updates

— Anna Fagre, DVM, MPH, CSU Microbiology, Immunology and Pathology Resident

tures laboratory-confirmed positive cases in humans, horses, other mammals, birds and mosquitoes across the United States.

CSU VDL offers practitioners both IgM ELISA and PCR options in testing for the virus. For antemortem testing, the ELISA may be more reliable than PCR, as IgM antibodies persist for four to six weeks after infection while circulating levels of virus remain low.

WEST NILE TESTING AT CSU IGM ELISA

0.5 mL serum or 0.5 mL cerebrospinal fluid

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- keep refrigerated prior to shipment. Ship with ice pack or on dry ice
- Tests run on Tuesdays and Thursdays only. Results in 24 hours

■ \$17

PCR

- Brain or 1.0 mL whole blood
- Keep cool. Do not freeze or heat blood. Ship overnight in a foam cooler with icepacks
- Results in three business days

\$45

VDL Finds Avian Flu in Wild Duck

SUVDL identified the presence of Eurasian/North American reassortant H5N2 avian influenza in a wild mallard duck from Fergus County, Mont. The sample, taken from a hunter-harvested bird through routine surveillance, was identified by the VDL and confirmed by USDA's National Veterinary Services Laboratories in Ames, Iowa.

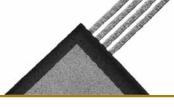
Characterization of the sample is ongoing, but according to USDA, it appears to be one of the strains associated with the outbreak in 2014 and 2015, and is highly pathogenic. During that 2014-2015 outbreak, HPAI H5 detections were reported in 21 U.S. states, including 15 states with outbreaks in domestic poultry or captive birds. No illness or mortalities in U.S. domestic poultry have been reported in connection with this latest finding.

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. The lab ran 11,373 tests

for avian influenza from July 2015 through June 2016, and it routinely tests more than 5,000 domestic and wild birds for the virus annually. The service is available to all bird owners, — Kristy Pabilonia, DVM, DACVM, CSU VDL Avian and Molecular Diagnostics Section Head

whether commercial producers or backyard enthusiasts. CSU VDL also houses the Wildlife Services' National Wildlife Disease Program Wild Bird Tissue Reference Archive, a partnership with USDA's Animal and Plant Health Inspection Services' Wildlife Services. The archive consists of swab samples collected for avian influenza surveillance, and has grown to be a valuable repository now housing over 250,000 samples. This collaboration has been a critical piece of Wildlife Services' effort to establish a network for early detection of highly pathogenic avian influenza. Many of the samples have been critical in characterizing the distribution and movement of low pathogenic avian influenza in North American wild birds, and in assay development. The Wild Bird Tissue Reference Archive is open and accessible to other agen-

> cies, universities, and organizations, and has proven to be an invaluable resource for research studies.



Chemistry and Toxicology

Trends in Canine Liver Copper For The Last 20 Years

In the early 1980s, CSU VDL started determining copper on dog liver biopsies. A method for determining copper on small samples had been developed as part of a research project to determine copper storage in bovine liver biopsies. Initially, the analysis in the dog was a response by the laboratory to a request from a clinician at the veterinary teaching hospital to help evaluate liver disease in Bedlington Terriers. Thus, most of those first samples were from Bedlington Terriers.

We performed the analysis on a dry-weight basis and received samples as fresh or frozen, in formalin and paraffin blocks. After discussions with clinicians and reviewing the literature, it was determined to classify liver copper values in the following manner:

- Deficient < 119 ppm dry weight</p>
- Normal 120 to 400 ppm dry weight
- High 401 to 1,499 ppm dry weight
- Toxic > 1,500 ppm dry weight.

Some researchers have suggested the "normal" amount of copper in the liver is dependant on the breed. Based on histologic and clinical data, toxic effects have been seen in some cases when the hepatic copper content is lower than 1,500 ppm dry-weight

TRENDS OVER THE DECADES

Some people have thought the amount of copper in canine liver has increased in recent years. Thus, we decided to do a retrospective evaluation of the data stored in the diagnostic laboratory data system.

The oldest data in the data base are part of fiscal year 1995 and all of 1996; thus, we combined them as the oldest data we had available. We then evaluated the data for fiscal year 2006 and the last fiscal year, 2016. The bar chart on page 5 gives the percent in the various groups as defined above for the three different time — Dwayne Hamar, PhD, CSU VDL Chemistry and Toxicology Section Head; Kevin Daniels, CSU VDL Laboratory Technician; and A. Russel Moore, DVM, MS, CSU Microbiology, Immunology and Pathology Assistant Professor

periods. The time period for the fiscal years ends June 30 and represents the previous 12 months. The 1995-1996 category represents half of fiscal 1995—the only months for which data were available—and all of fiscal 1996. Numbers above the bar are the number of samples analyzed in each group. The numbers analyzed and percent indicating increased liver copper include:

		Percent either	
	Total analyzed	high or toxic	
1995-1996	91	57%	
2006	638	43%	
2016	2,116	58%	

Does this really represent an increase in liver copper in recent years?

DIAGNOSTIC AND TREATMENT IMPLICATIONS

Although the percent of samples with high and toxic levels of copper analyzed for 2016 increased vs. 2006, the data are probably somewhat skewed in regard to the canine population. The samples analyzed do not represent the population of normal canines; the tested animals were suspected of having sufficient clinical signs and liver dysfunction to warrant biopsy, histology and copper quantitation.

The table below gives the number of samples, average and range of some selected breeds that have been reported as having high liver copper. For 1995-1996, five

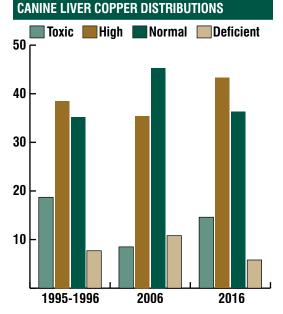
AVERAGE AND RANGE OF LIVER COPPER (PPM DRY WEIGHT) FOR SELECT BREEDS									
	1995-1996		2006		2016				
8	n	Average (Range)	n	Average (Range)	n	Average (Range)			
Bedlington terrier	9	6,890 (179-23,400)	3	268 (124-436)	3	511 (347-658)			
Dalmatian	1	84	10	1,820 (196-6,010)	22	3,900 (194-16,500)			
Doberman Pinscher	15	912 (216-2,430)	50	766 (142-3,330)	71	1380 (71-5,430)			
Retrievers*	8	971 (309-2,210)	76	785 (77-2,550)	426	972 (52-8,000)			
West Highland White Terrier	7	1,879 (250-5,870)	35	982 (128-3,070)	49	1,430 (184-5,490)			

* Retrievers are combination of Retriever, Labrador Retriever, and Golden Retriever

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of the nine Bedlington Terriers were in the toxic range, while three were in the high range. Also note that in 2006 and 2016, a low number of Bedlington Terriers were analyzed. This decrease reflects the breeders' attempts to remove from the gene pool those that carry the defective gene. One Bedlington Terrier in the 1995-1996 group had a liver copper of 23,400 ppm dry weight. We have



had some liver samples from non-Bedlington Terriers since that have been higher. The breed designation is what the client recorded on the accession form.

When assessing liver dysfunction, it is important to perform a histologic evaluation with copper stain as well as the copper quantitation. Histologic evaluation can provide information on the pattern of copper deposition and the amount of inflammation and architectural changes present in the liver. This information can be helpful in determining the cause of increased hepatic copper and in formulating an approach to treatment.

HEPATIC COPPER CONCENTRATIONS AND HISTOLOGICAL CHANGES IN THE DOG

— Dan Moezzi, CSU CVMBS student; Barbara Powers, DVM, PhD, DACVP, CSU VDL Director; and David Twedt, DVM, DACVIM, CSU Professor Clinical Sciences

Abnormal hepatocyte copper concentrations are associated with oxidative stress resulting in cell death and subsequent necroinflammatory hepatic changes. The accumulation of hepatic Cu with inflammatory liver changes in the dog has been linked to certain breeds, due to suspected excessive dietary copper intake and the result of cholestatic disorders. We hypothesized that Cu concentrations would be higher in dogs having necroinflammatory liver biopsies compared to those with non-inflammatory hepatopathies or other changes that did not fulfill the criteria of inflammatory or non-inflammatory. A second aim was to determine if the level of hepatic Cu concentrations are related to the extent of necroinflammatory changes.

We examined the CSU VDL records of 675 samples obtained in 2014 having both liver histopathology and hepatic Cu quantitation. Normal hepatic Cu concentrations are reported to range from 200 to 400 μ g/g dry weight. Inflammatory groups were characterized by being lymphoplasmocytic or suppurative and grouped as being mild, moderate or severe. Non-inflammatory samples were characterized by hepatocellular cell swelling, hyperplasia, or lipidosis. A third "other changes" group was characterized either as nonspecific reactive hepatopathies, fibrosis or hepatic neoplasia. The mean hepatic Cu concentrations (in parts per million) were higher in the inflammatory groups; mild (553), moderate (1013) and severe (1143) compared to the non-inflammatory (353) and other group (471). These preliminary findings suggest the highest hepatic copper concentrations are associated with inflammatory liver disease and the severity of inflammatory changes tends to increase with hepatic Cu concentrations.

Canine Virology

Canine Distemper a Growing Threat to Animals in the Front Range

Canine distemper virus (CDV), the etiologic agent of canine distemper, may result in the rapid manifestation of multisystemic clinical signs ranging from respiratory to neurologic disease and has been responsible for multiple outbreaks across the United States.¹ Canine distemper affects all carnivores, and the disease is well-known for its ability to quickly attack the respiratory, gastrointestinal and neurologic systems. The virus is easily shed in respiratory secretions, and urine and remains endemic in some locations due to the presence of susceptible wildlife species contributing to transmission, including ferrets, foxes, most large cats, coyotes, badgers and otters.

In 2015, the CSU Veterinary Diagnostic Laboratory detected 166 canine distemper cases, of 833 submitted, via real-time reverse transcription PCR (rRT-PCR). Since 2016, 272 of 995 submitted cases have been detected. Reports of CDV nationwide have increased, and clinicians should be aware of the diverse array of clinical signs animals may present with, as early identification and supportive treatment are essential for patient recovery.

— Anna Fagre, DVM, MPH, CSU Microbiology, Immunology and Pathology Vet Resident

CLINICAL SIGNS AND DISEASE PROGRESSION

Due to the rapid progression of clinical disease and fair to poor prognosis, CDV should remain a differential for any febrile dog with systemic clinical signs. Unvaccinated dogs are generally affected between 3 to 6 months of age, when maternal antibodies begin to wane. The disease is most often diagnosed in areas with low vaccination rates or settings with high densities of naïve dogs, such as shelters.

The virus initially replicates in the respiratory epithelium before moving to the tonsils and lymph nodes. Following respiratory infection, viremia causes leukopenia and subsequent immunosuppression. Subsequently, the virus spreads systemically to the gastrointestinal, urinary and central nervous system, should the dog not mount an immune response sufficient to curtail the course of the disease. Neurologic signs generally occur weeks to months after the disease and may occur in the absence of previous clinical disease. "Old dog encephalitis" may

DETECTION TARGET	RESULT INTERPRETATION	SAMPLE TO SUBMIT	PRICE	TURN- Around Time
Antigen; detects presence of nucleic acid in tissue or sample	Reported as positive, suspect or negative	whole blood, 0.5 mL urine, conjunctival, preputial swab or nasal swab in 0.5 mL sterile saline, cerebrospinal fluid, or lung tissue (postmortem diagnostic).	\$45	3 business days
Antigen; detects presence of antigen in tissue of sample	Reported as positive or negative	conjunctival smear; fresh lung, kidney, or cerebellum tissue; or a swab of conjunctival cells	\$14	24 hours
Detects presence of antibodies in serum or CSF	Reported as titer; indicates recent infection	0.5 mL serum or CSF	\$18	24 hours
	Reported as titer; difficult to interpret without both acute and convalescent samples	0.5 mL serum or CSF	\$18	24 hours
	Reported as titer; preferable to run acute and convalescent samples	0.5 mL serum or 0.3 mL CSF	\$17	1 week
	TARGETAntigen; detectspresence of nucleicacid in tissue orsampleAntigen; detectspresence of antigenin tissue of sampleDetects presence ofantibodies in serum	TARGETINTERPRETATIONAntigen; detects presence of nucleic acid in tissue or sampleReported as positive, suspect or negativeAntigen; detects presence of antigen in tissue of sampleReported as positive or negativeDetects presence of antibodies in serum or CSFReported as titer; indicates recent infectionReported as titer; difficult to interpret without both acute and convalescent samplesReported as titer; difficult to run acute and convalescent	TARGETINTERPRETATIONSAMPLE TO SUBMITAntigen; detects presence of nucleic acid in tissue or sampleReported as positive, suspect or negativewhole blood, 0.5 mL urine, conjunctival, preputial swab or nasal swab in 0.5 mL sterile saline, cerebrospinal fluid, or lung tissue (postmortem diagnostic).Antigen; detects presence of antigen in tissue of sampleReported as positive or negativeconjunctival smear; fresh lung, kidney, or cerebellum tissue; or a swab of conjunctival cellsDetects presence of antibodies in serum or CSFReported as titer; indicates recent infection0.5 mL serum or CSFReported as titer; difficult to interpret without both acute and convalescent samples0.5 mL serum or 0.3 mL CSFReported as titer; preferable to run acute and convalescent0.5 mL serum or 0.3 mL CSF	TARGETINTERPRETATIONSAMPLE TO SUBMITPRICEAntigen; detects presence of nucleic acid in tissue or sampleReported as positive, suspect or negativewhole blood, 0.5 mL urine, conjunctival, preputial swab or nasal swab in 0.5 mL sterile saline, cerebrospinal fluid, or lung tissue (postmortem diagnostic).\$45Antigen; detects presence of antigen in tissue of sampleReported as positive or negativeconjunctival smear; fresh lung, kidney, or cerebellum tissue; or a swab of conjunctival cells\$14Detects presence of antibodies in serum or CSFReported as titer; indicates recent infection0.5 mL serum or CSF\$18Reported as titer; difficult to interpret without both acute and convalescent samples0.5 mL serum or 0.3 mL CSF\$17Reported as titer; preferable to run acute and convalescent0.5 mL serum or 0.3 mL CSF\$17

result years later due to the persistence of the virus within central nervous tissues.

DIAGNOSIS OF CANINE DISTEMPER VIRUS

The CSU VDL offers several assays to aid in the diagnosis of distemper in dogs, including both antigen/ nucleic acid tests and antibody tests. They include:

- Real-time reverse transcription PCR (rRT-PCR). Our rRT-PCR is a highly sensitive and specific diagnostic assay used to detect virus in tissues, respiratory secretions, urine or whole blood. Certain sample types may be pooled together in one rRT-PCR assay, not including whole blood, which must be run individually. Submission of urine with a conjunctival or preputial swab is indicated to increase sensitivity; these two samples may be pooled and run at the cost of one assay plus a pooling charge. It is important to note that canine distemper virus may be shed in the urine and other secretions for several months after resolution of clinical signs.
- Fluorescent antibody (FA). The second option for canine distemper virus antigen detection is FA testing, which utilizes a fluorescent-labeled antibody to detect antigen within the cells of particular tissues or swabs from infected animals. This test is not as sensitive as rRT-PCR, as it relies upon the antigen's presence in the specific area of submitted tissue. As a result, a false negative is more likely. Additionally, it should be noted that the only acceptable antemortem sample type is conjunctival smear or swab. Lung, kidney, or cerebellum may be submitted for post-mortem diagnosis.
- Indirect immunofluorescence antibody (IFA) serology. Utilizing the IFA assay, serum or cerebrospinal fluid (CSF) may be tested for the presence of IgG or IgM antibodies to the virus. This assay requires a single serum/CSF sample, with results reported as an IgG/IgM antibody titer. IgM antibodies persist for about five to 12 weeks in natural disease, while IgG antibodies may be present for years. IgM titers in serum or CSF above 1:2 may indicate recent or active infection, aiding in the diagnosis of CDV. In a previously vaccinated animal, the IgG serum titers should be interpreted cautiously due to long duration of antibody persistence. The presence of IgG antibodies within a CSF sample is highly suggestive of exposure, as antibodies mounted during a post-vaccine immune response should not traverse

the blood/brain barrier. However, when looking for IgG antibodies in a CSF sample from a previously vaccinated animal, note that any trace amount of blood contamination occurring during collection may result in a false positive.

Serum neutralization (SN) serology. In using the SN assay, CDV is incubated with serial dilutions of the patient's serum to quantify the titer of neutralizing antibody for the virus. This is the preferred assay for interpretation of acute and convalescent titers.

TITER INTERPRETATION

When using titer levels to detect disease, we recommend submitting paired titers, also referred to as acute and convalescent titers. SN is the preferred assay for testing acute and convalescent titers, as it directly measures the concentration of circulating IgG antibodies. A four-fold rise over the course of two weeks is highly suggestive of active or recent infection.

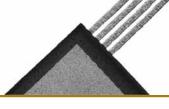
Due to our limited understanding of IgG titer levels and corresponding protection against CDV, it is difficult to determine an appropriate cutoff titer that may protect against disease. Further, titer levels fluctuate and lapses in protection may occur in ill, stressed or immunocompromised animals. Some clients may request CDV titers in lieu of re-vaccination, and in these cases it is crucial to discuss risks associated with unknown protection and the importance of annually checking titers.

CASE CONTROL AND OUTBREAK PREVENTION

In the event a case of canine distemper is diagnosed, reducing contact with other dogs is critical to curb further spread of the disease. This disease is particularly devastating in shelter situations, as an infected dog entering from the community may rapidly transmit the disease to other naïve shelter dogs. Early diagnosis and treatment via supportive care are critical to prevent disease progression. Subsequent isolation of clinically affected animals, coupled with close monitoring of susceptible dogs, helps reduce the chances of a shelter-wide outbreak. The variable incubation period also highlights the importance of quarantining all incoming animals, and maintaining barrier and isolation precautions for affected animals due to potentially substantial shedding periods. In light of the complex epidemiology of distemper and the multiple species susceptible to the virus, vaccination is crucial to maintaining herd immunity and preventing future outbreaks.

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CSU VDL in the Field: Case Studies

Locoweed Toxicosis Control, Diagnosis and Management

Although there are over 375 species of the perennial herbaceous plant *Astragulus* spp, less than 40 are listed as toxic or potentially toxic in the 2013 edition of *Toxic Plants of North America*. Of these, *A. miser* (timber milk-vetch), *A lentiginosis* (specked loco), A *mollimssimus* (woolly loco) and *A. allochorus* (halfmooned milkvetch) along with *Oxytropis lambertii* (Lamberts crazyweed, purple loco) cause most of the locoweed toxicosis in the United States.

Astragulus mollissimus and O. lambertii are the major concern in the Rocky Mountain region and Great Plains. These biannual or perennial plants are found anywhere from below sea level up to more than 10,000 feet in altitude. They are acclimated to many soil types and rainfall patterns. Their seeds remain viable for years and can survive prolonged droughts which contributes to the cyclic nature of locosim, leading to increased occurrence during the years following droughts.¹

A LONG HISTORY OF CONTROL EFFORTS

Astragulus mollissimus and O. lambertii have plagued livestock producers in the great plains and eastern Front Range of the Rocky Mountains since 1870. It was such a serious problem that in the late 1800,s the state of Colorado offered a bounty for each pound of locoweed that was dug below the root line. This program cost the state up to \$50,000 per year until it was repealed in 1885. In 1904, the USDA established a research laboratory in Hugo, Colo., with the specific purpose of investigating A. mollissimus and O. lambertii effects on livestock. Locoweed toxicosis was so important to the livestock industry that an additional Locoweed Research Laboratory was established in Alpine, Texas, in 1930 to study ways to control livestock losses due to locoism. Locoweed research continues today at the USDA Poisonous Plants Research Laboratory in Logan, Utah.1

The palatability of the various locoweeds is variable with some non toxic varieties providing valuable protein sources for wildlife and livestock. Although toxic locoweeds are generally unpalatable, some animals start eating locoweed early in the spring as it greens up early, become habituated to the plants and continue to eat them after palatable forages become available. Consumption of locoweed is also a learned behavior as cattle that have not been exposed to locoweed learn — Gene Niles, DVM, Director, CSU VDL Rocky Ford Branch; and Charlie Davis, DVM, CSU VDL Lab Coordinator

to eat it when placed with cattle already eating locoweed.¹ A cattleman whose cows had clinical locoism last summer said "the affected cows sought out locoweeds when moved to new pastures and they couldn't be driven off."

METABOLISM OF TOXINS

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

Swainsonine is the toxic principle responsible for the neurologic signs seen in animals. Locoism is documented in horses, cattle, sheep, goats, pigs, deer, elk, antelope and poultry. Horses are the most susceptible to swainsonine. Swainsonine can cause reproductive failures in all species. It is produced by fungal endophytes present in all parts of the locoweed plant.

Swainsonine inhibits alpha-mannosidase, resulting in proliferation of lysosomes within the cell that contain incompletely processed waste materials. These vacuoles interfere with cell function, resulting in failure of the cell to eliminate the waste materials. Microscopically they are seen as foamy vacuoles that cause vacuolar degeneration of the cells within the nervous, endocrine, reproductive, immune and gastrointestinal systems.²

The toxins in locoweeds are dose and time dependent. Cases that involve adult animals generally require continuous ingestion of large quantities of plant material over an extended period of time. Locoism is most often seen in young animals. One reason for this effect may be that swainsonine is water soluble and eliminated in milk, urine and feces. The amount of swainso-

MORE ON LOCOWEED TOXICOSIS IN THE PREVIOUS ISSUE OF LABLINES

CSU VDL's Rocky Ford laboratory has been involved with several cases involving locoweed toxicosis. All these cases demonstrate some of the varied clinical presentations of locoweed toxicity. For more, see csu-cvmbs.colostate.edu/ vdl/lablines nine eliminated in milk is sufficient to cause toxicosis in nursing animals before clinical signs occur in their dams. If nursing animals are also grazing locoweeds, clinical disease is observed much sooner. Swainsonine remains toxic when dry and may pose problems in 2-year old hay.⁵

DIAGNOSIS

Five clinical syndromes are associated with locoweed toxicosis. Four occur in North America which include locoism, selenosis, photosensitization and nitrotoxicosis. The fifth disease process is thiamine-related neuro-toxicosis affecting sheep in Morocco.¹

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

Diagnosis of locoism in live animals is generally based on the clinical syndrome coupled with evidence That locoweed has been eaten. Analyzing blood samples for swainsonine and alpha-mannosidase may be beneficial when the animals are still consuming the plant, but the levels of these compounds change dramatically as soon as the animals stop eating locoweed.

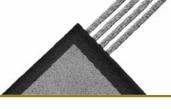
The half-life (T1/2) for serum swainsonine is 20 hours and 60 hours in the liver. Vacuolar degeneration in neurons and other organs can also resolve quickly with low dose, short exposures but neurologic changes are often permanent with chronic exposure. With locoweed-induced abortions, vacuolar degeneration may be observed in the fetal tissues with special attention to the brain, adrenal gland, pancreas and thyroid.²

If animals are removed from access to locoweeds at the first sign of illness, the signs of disease and developing lesions will generally return to normal within a few weeks. The withdrawal time for swainsonine to clear the body is 28 days.

Locoweed toxicosis increases following periods of prolonged drought. Swainsonine affects multiple tissue and produces multiple clinical syndromes. The concentration of swainsonine in locoweeds is highly variable with most plants containing <0.5-0.1%.¹ Disease can occur even though the total population of locoweeds within a pasture hasn't changed, if the concentration of swainsonine significantly increases. In this area of Colorado, locoweed toxicosis should be included in the differential diagnosis when other causes of disease are eliminated.

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Get to Know the Laboratory

New Members Join the Lab Team

Ben Curtis, new anatomic pathology resident, originally comes from Grand Rapids, Mich.—"Beer City, USA." He received his bachelor of science degree in biology at Grand Valley State University on the Lake Michigan shore in 2009 while also working at a small-



animal clinic for about 6 years. He attended Michigan State University to earn a DVM in 2016. Curtis' interest in anatomic pathology developed early in his DVM training, and he spent numerous clinical rotations exposing himself to pathology as well as collaborating on small research projects and manuscripts.

His research projects have included development of new processing techniques for evaluation of scleractinian (hard) corals histologically, and use of MALDItof as a potential prognostic tool in dogs with diffuse large B-cell lymphoma. He his goal is to find a PhD project that may include development of new diagnostic tools that can be used to help clinicians provide meaningful information to their clients and their animals. After being accepted to the combined anatomic pathology residency and PhD program at CSU, Curtis made the journey west with wife, Abby, and new son, Grayson. He is excited to take advantage of the hiking and camping Colorado has to offer. Additionally in his spare time he enjoys cycling, running, playing games, following Michigan sports teams and trying new beers.

Mike Betley was predisposed to becoming a veterinarian after being raised by an equine veterinarian father and animalloving mother. He spent the majority of his childhood in the St. Louis area, playing sports, fishing and catching reptiles. Always intrigued by biology, he



made the decision to choose a veterinary career during his undergraduate education at St. Louis University. During vet school at University of Missouri-Columbia, he participated in the typical student experience of choosing a different veterinary career path every six to eight weeks, until a summer research project and experience in the comparative pathology club ultimately drove him to pursue a life in biomedical research and pathology. Following vet school, Betley moved to California to work on a doctorate in neurosciences at Stanford. His thesis focused on the physiologic mechanisms through which aerobic exercise beneficially increases neurogenesis in the adult hippocampus, a brain region vital to spatial learning and memory. After completing his dissertation work in spring 2016, he joined CSU VDL for a three-year term as an anatomic pathology resident. He hopes to combine his neuroscience research experience and pathology training to pursue a career that will improve the understanding of neurological disease in both animals and people. In his free time, he spends time outdoors, riding and racing bikes, skiing and traveling.

Kendra Andrie, anatomic pathology resident, grew up in Michigan, where she spent much of her childhood playing ice hockey. Her interest in pathology began between an undergraduate education at Salve Regina University, in Newport, RI, and the begin-



ning of veterinary school while working for Neurotech Pharmaceuticals. Her position as a research and development intern with the company introduced Andrie to histology during investigations into intraocular implants to treat retinal degenerative diseases. This early exposure to histology and pathology continued in her veterinary curriculum, which further strengthened her general interests, ultimately leading her to pursue a career in anatomic pathology. In 2016 she earned her DVM, along with a masters degree in food safety from Michigan State University.

Through her experiences , she has developed interests in infectious disease pathology and respiratory pathology, but she is also eager to learn more about the various research opportunities within oncological pathology, as well.

She has a passion for learning and traveling, and is always looking to pursue unique life experiences. Since moving to Colorado most of her free weekends have been spent hiking in the mountains, but she also enjoys playing ice hockey, biking, skiing, traveling and spending time with her dog, Ruby, as well as family and friends. **Cristy Dice**, CSU VDL's new research program coordinator, has been involved in agriculture her whole life, with the main focus of her 15-year professional career being in the commercial swine industry. Most recently, she worked at the Colorado Department of Agriculture in



the State Veterinarian's Office, where her main responsibilities were project management, livestock disease emergency response and planning, livestock movement and disease program/response data management, and serving as liaison with external stakeholders.

Dice is passionate about agriculture and working to ensure farmers and producers are able to successfully raise animals while still being able to provide for their families. In her free time she enjoys backpacking, hiking, running, cooking and baking, reading and spending time with her husband, sister, three dogs and two cats. **Alysia Cozza**, new sample receiving technician, grew up in the sunny state of Florida and discovered a love for animals at age 7. She started to work on cattle and horse farms in Florida, by which she found a love of riding horses. She competed at a young age



in barrel racing and hunter/jumper competitions. During her final years in Florida she started to train horses for jumpers and also went to Edison State College, where she received her associate of science degree in pre-vet. Cozza has worked in veterinarian clinics since the age of 16. In 2011 she transferred to CSU, where she received her bachelor's degree in equine science, with a minor in business administration. Alysia plans to pursue a masters degree in equine sciences.

NEW VDL PARASITOLOGY SECTION HEAD WILL EMPHASIZE DIVERSE, APPLIED PARASITOLOGY

During her undergraduate years studying biological sciences at University of Northern Colorado in Greeley, new CSU VDL Parasitology Section Head Ashley McGrew developed a strong interest in parasites. At the same time, the Fort Collins native gained experience in the field of aquatic-animal medicine, participating in cetacean rehabilitation efforts through the Sarasota, Fla., MOTE Marine Laboratory & Aquarium's Dolphin & Whale Hospital, along with research on the health of bottlenose dolphin populations through Connecticut's Mystic Aquarium.

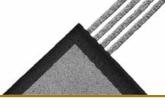
When she came to CSU in 2004 to pursue the DVM-PhD dual-degree program, her graduate work involved exploring the uptake of non-essential elements by parasites infecting marine mammals and gray wolves. After graduating from veterinary school, a post-doctoral fellowship in the CSU VDL Parasitology Section, where she helped developed the lab's revolutionary new PCR test for detecting and speciating ruminant strongyles, which is helping prevent parasite resistance to anthelmentics by better targeting parasiticides.

Now a Diplomate of the American College of Veterinary Microbiologists, specializing in parasitology, McGrew looks forward to continued opportunities on faculty in the Department of Microbiology, Immunology and Pathology, and hopes to grow her research and teaching interests in marine parasitology, as well as to broaden the services offered in the Parasitology Section in this area.



MCGREW'S RESEARCH HIGHLIGHTS

- Exploring the Bone Proteome to Explain Altered Bone Remodeling in Hibernating Marmots.
- Ecotoxicoparasitology: Mercury concentrations in gut contents, intestinal helminths and host tissues of Alaskan gray wolves.
- Anthelmintic fishmeal polymer baits to control baylisascaris procyonis in free-ranging raccoons.
- Mercury in gray wolves in Alaska: increased exposure through consumption of marine prey.



CSU VDL In Press

A Roundup of VDL Faculty Research

HUMAN HISTO MARKERS IN CANINE TUMORS Ramos-Vara JA, Frank CB, DuSold D, Miller MA. Immunohistochemical Detection of Pax8 and Napsin A in Canine Thyroid Tumors: Comparison with Thyroglobulin, Calcitonin and Thyroid Transcription Factor 1. J *Comp Pathol.* 2016 Aug 24. pii: S0021-9975(16)30099-8. doi: 10.1016/j. icena.2016.07.009.

VDL pathologist Chad Frank participated in this study to evaluate the usefulness in canine thyroid neoplasm immunohistochemistry of the human oncology marker Pax8 and napsin A. Pax8 is used in human oncology mainly to diagnose renal and ovarian carcinomas and those of mullerian duct origin. Napsin A is an aspartic proteinase detected in normal type II pneumocytes, alveolar macrophages, renal proximal tubular epithelium and exocrine pancreatic cells, as well as many pulmonary and renal neoplasms.

Using 114 cases of canine thyroid lesions selected from the VDL database archive between 2004 and 2014, the study evaluated the immunohistochemical expression of the two markers compared to the immunoreactivities of thyroglobulin, calcitonin and TTF-1. Of the 114 cases studied, all 81 follicular tumors expressed thyroglobulin and were negative for calcitonin; 79 of 81 (98%) of these tumors expressed TTF-1 and Pax8, and 60 of 81 (74%) expressed napsin A. All 25 C-cell lesions expressed calcitonin and were negative for expression of thyroglobulin, 22 (88%) were positive for TTF-1, 13 (57%) were positive for Pax8 and all 24 of 24 for napsin

A. Six mixed follicularemedullary carcinom a s

expressed all five markers. Both carcinosarcomas expressed TTF-1 and napsin A, and one each of these tumors expressed thyroglobulin, calcitonin or Pax8.

The study found Pax8 was as sensitive as TTF-1 and slightly less sensitive than thyroglobulin for identification of follicular tumors, but had low sensitivity for C-cell tumors. Napsin A was as sensitive as calcitonin for C-cell neoplasms, but was less sensitive than thyroglobulin for follicular neoplasms. Thus, these markers are sensitive and, except for renal cell carcinoma (for both Pax8 and napsin A) and pulmonary adenocarcinoma (for napsin A), are specific thyroid tumor markers.

USEFUL NEW MARKERS FOR B-CELL LYMPHOMA? Curran KM, Schaffer PA, Frank CB, Lana SE, Hamil LE, Burton JH, Labadie J, Ehrhart EJ, Avery PR. BCL2 and MYC are expressed at high levels in canine diffuse large B-cell lymphoma but are not predictive for outcome in dogs treated with CHOP chemotherapy. *Vet Comp Oncol*. 2016 Aug 11. doi: 10.1111/vco.12263.

VDL Pathologists Paula Schaffer, Chad Frank and EJ Ehrhart participated in this study of the VDL system's diagnostic records for dogs treated for diffuse large B-cell lymphoma by conventional cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) chemotherapy protocol, to test the value of immunuhistochemical expression of the human protooncogenes MYC and BCL2 as possible canine indicators. BCL2 is an anti-apoptotic factor that plays a crucial role in normal B-cell development and differentiation, which when over-expressed can result from either chromosomal translocations or amplifications. MYC is involved with cellular proliferation and cell cycle progression, and both chromosomal translocations and increased expression independent of translocations have been reported in human cases of diffuse large B-cell lymphoma.

A total of 43 dogs' cases from 2007 to 2014 were selected from the VDL archive. Results found a generalized pattern of high expression of MYC and BCL2. Based on reported expression ranges in human DLBCL, the vast majority of our canine cases would have been considered positive for BCL2 and MYC, and no cases would have been considered negative for both markers. They indicate the condition is a more homogenous tumor type in dogs than in people. Compared with human patients, the canine cases had high IHC expression of both MYC and BCL2, and relative expression levels of one or both markers were not associated with clinical outcome.

ERYSIPELAS BEHIND NEW ARCTIC FOX OUTBREAK? Spraker TR, White PA. Shaggy Lame Fox Syndrome in Pribilof Island Arctic Foxes (Alopex lagopus pribilofensis), Alaska. Vet Pathol. 2016 Aug 19. pii: 0300985816660745.

While conducting mortality studies of northern fur seals in the Pribilof Islands of Alaska between 1986 to 2014, VDL Pathologist Terry Spraker encountered arctic foxes with shaggy, unshed winter coats late into Fall/Winter 2016

13 LAB LINES



the spring and summer months. These foxes also were severely lame and emaciated. Although the condition has only been documented so far on St. Paul Island, it is suspected to be present on other islands in the Pribilof Archipelago, based on Spraker's observations. Since 1995, the Pribilof arctic fox population of St. Paul Island appears to be decreasing.

In order to learn more about the underlying cause of the condition, which is now termed shaggy lame fox syndrome, and its possible importance as a populationlimiting factor, this study carried out necropsies on 24 foxes found dead or euthanized in summers between 1986 and 2014. Serum samples were also collected for study during a separate behavioral study.

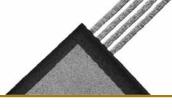
Although the cause of SLFS remains unknown, the gross and histological lesions were similar to those described in domestic dogs and pigs infected with Erysipelothrix rhusiopathiae. Both culture and PCR of the foxes were positive for E. rhusiopathiae type 2 using synovial membrane from a swollen stifle joint and kidney. Vascular lesions identified in the foxes resembled those described in Swedish ranch-raised arctic foxes infected with E. cuniculi, a condition similar to polyarteritis nodosa in dogs. The gross and histological lesions combined with the microbiological results suggest SLFS may be a primary bacterial infection with a secondary immunemediated vasculitis. Although the mode of transmission of SLFS is unknown, if the cause is bacterial, a possible source of infection could be ingestion of bacteria-laden food, including the foxes' natural diet of northern fur seal placentas, scavenged dead pups and adults.

TRACKING EQUINE ENCEPHALITIS VIRUS ROUTES Phillips AT, Rico AB, Stauft CB, Hammond SL, Aboellail TA, Tjalkens RB, Olson KE. Entry Sites of Venezuelan and Western Equine Encephalitis Viruses in the Mouse Central Nervous System following Peripheral Infection. J Virol. 2016 May 27;90(12):5785-96. doi: 10.1128/JVI.03219-15.

CSUVDL Pathologist Tawfik Aboellail collaborated with faculty from CSU's Arthropod-Borne and Infectious Disease Laboratory and the Department of Environm e n tal and Radiological Health Sciences to determine precisely where alphaviruses like the mosquito-borne Venezuelan and western equine encephalitis viruses enter the central nervous system of infected animals. The team bioengineered a recombinant western equine encephalitis virus and a recombinant Venezuelan equine encephalitis virus to express the lab reagent firefly luciferase, or fLUC. They then injected the engineered viruses into the footpads of outbred CD-1 mice and tracked the virus' entry into the nervous system through in-vivo and ex-vivo bioluminescent imaging, immunohistochemical exam, and enhanced fluorescence imaging of whole brains.

They found that VEEV, WEEV, and perhaps EEEV gain entry into the nervous system at specific areas where the blood-brain barrier is naturally absent. Peripherally injected alphaviruses enter by hematogenous seeding of the brain's circumventricular organs (CVOs), followed by centripetal spread along the neuronal axis The CVOs have not been described previously as sites of neurotropic-alphavirus entry into the CNS, increasing the significance of the findings and suggesting other neuroinvasive viruses might gain entry into the CNS through similar entry sites.





Diagnostic Sample Quality Assurance

Biopsy Sample Submission Tips

Shipping samples for biopsy often presents unique challenges for clinics. Here are a few quick tips on how to—and now *not to*—submit those samples:

Large samples. Samples like whole spleens or leg amputations require special consideration for fixation and shipping. These samples can be held to fix for at least 24 hours at the clinic and the formalin poured off prior to packaging for shipping. This in-clinic fixing not only minimizes the risk of leaking formalin in transit, but also saves on shipping charges.

We do not require the volume of formalin to be ten times the sample volume when shipping. Avoid shipping five-gallon buckets filled with formalin; buckets often leak and eventually end up at the landfill. An alternative method is to soak paper towels in formalin, place the sample in the towels and package for shipping. Triple bagging is recommended for all tissue samples to minimize the risk of leaking formalin. Shipping tissue in saline is not recommended.

Small samples. Samples like endoscopic tissues are best shipped in high-screen cassettes placed in formalin. The VDL will supply these upon request. Using pencil, label each cassette with the tissue site. If tissue cassettes are not available, formalin-filled red-top tubes can also be used. If neither cassettes nor red-top tubes are avail-

— Linda DeBuse, CSU VDL Histology Technician

able, placing samples in a small histology sample container is more than acceptable. Endoscopic samples do well free floating in the sample containers. It is best to submit endoscopic samples with no foreign materials. Gauze, tissue paper or tongue depressors are not needed.







Thanks to our VDL Advisory Group which met again in January to help guide the lab's direction. Members include (front row, from left) Gene Niles, Marv Hamman, Kenny Rogers, Dwayne Hamar, Leesa McCue, Gary Mason, Joan Bowen, Kristy Pabilonia, Keith Roehr, Jennifer House, Larry Mackey, Barb Powers, Jessica Timian, Tim Hackett, Don Beckett, Charlie Davis, Richard Wheeler, (back row, from left) Raye Walck, Mark Stetter, Linda Vap, Ron Kollars, Chad Frank, Karen Fox, Karen Rogers, Norm Brown, Ashley McGrew, Connie Heighes, Christie Mayo, Kellee Mitchell, Gregg Dean and Tracy Baszler.

Always feel free to contact us with questions or refer to our website: csu-cvmbs.Colostate. Edu/documents/vdlbiopsy-submission.Pdf

<u>₁₅</u> LAB **LINES**

Guardians of Public Health

Helping Ensure Pet Food Safety

Pet food contamination and recalls have become a highly visible news item. Consider, for example, the February U.S. recall by Illinois-based Evangers over concerns some of its products might have been contaminated with pentobarbital. As a member laboratory of the U.S. Food and Drug Administration's Veterinary Laboratory Investigation and Response Network (Vet-LIRN), CSU VDL plays an integral role in ensuring the safety of the estimated \$24 billion worth of pet foods sold in this country annually. The 38 member labs of Vet-LIRN support the concept of one health and promote human and animal food safety by conducting investigations into reports of problems with animal foods or animal drugs.

Veterinarians or consumers can submit reports identifying suspected food or drug related illness using the FDA's electronic Safety Reporting Portal or by calling their state's FDA Consumer Complaint Coordinators.

Once a complaint is lodged,

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

Vet-LIRN partners with the pet owner's veterinarian to evaluate the animal's feeding history and medical records. It may then request additional blood, urine, necropsy or suspect product testing, and arrange shipment of samples to the VDL for testing. VDL then reports results back to Vet-LIRN, which works with the veterinarian to resolve the case with the client. ▲

> Report a pet-food complaint using FDA's reporting portal, at www.safetyreporting. hhs.gov, or call Colorado's FDA consumer complaint coordinator, at (303) 236-3044

Safety alling umer dged,

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

Parasitology Section Head **Ashley McGrew** attended the **47th Annual Meeting of the Rocky Mountain Conference of Parasitologists**, Sept. 8 through 10 in Lincoln, Neb. She and VDL Pathologist **Paula Schaffer** sit on a master's committee of a biological sciences student who won best student poster at the meeting. McGrew is scheduled to attend the 17 International Association for Aquatic Animal Medicine Conference, May 20 to 24 Cancun, Mexico, where she and veterinary student Jake Rodgers will present their research on optimizing diagnostics in marine parasitology. She also plans to attend The Ohio State University's Acarology Summer Program this summer.

Rocky Ford Branch Director *Gene Niles* has been traversing the state during the last quarter of 2016, giving presentations on VDL laboratory updates, poisonous weeds, polioencephalomalacia and more. He also was on hand at the *Academy of Veterinary Consultants Winter Meeting*, in December in Denver. You can catch up with him at the *Academy's Spring* 2017 meeting, March 30 to April 1 in Irving, Texas, or the *Summer 2017 meeting*, Aug. 3 to 5 in Denver.

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

VDL Lab Coordinator *Charlie Davis* conducted recent staff field trips at *Long's Peak Dairy*, in Pierce, and at *Harper Feeders* sheep feedlot, in Eaton. He also led several CSU *Field Investigation Unit* cases around the state. He attended the *Larimer County Stockgrowers Association* meeting in January. Davis will be available to meet with you at the *Colorado Cattlemen's Association* annual convention, June 12 to 14 in Grand Junction, at the *Colorado Wool Growers Convention* this summer, and at the *Colorado Veterinary Medical Association* convention, Sept. 21 to 24 in Loveland.

VDL Pathologists *Chad Frank* and *Paula Schaffer* attended the annual meeting of the *American College of Veterinary Pathologists*, Dec. 3 through 7 in New Orleans.

VDL Avian Diagnostics and BSL3 Operations Section Head *Kristy Pabilonia* and Poultry Programs Coordinator *Cristy Dice* attended USDA's Live Bird Marketing System Working Group 2017 meeting, Feb. 22 and 23 in San Antonio

Pabalonia, along with VDL Director Barb Powers, Virology Section Head Christie Mayo, Chemistry and Toxicology Section Head Dwayne Hamar were at this year's 59th annual meeting of the American Association of Veterinary Laboratory Diagnosticians, Oct. 13 to 19 in Greensboro, N.C.



Veterinary Diagnostic Laboratories College of Veterinary Medicine and Biomedical Sciences Fort Collins, CO 80523-1644 Nonprofit Organization US Postage PAID Fort Collins, Colorado 80523 Permit Number 19

INSIDE THIS ISSUE

- CANINE ONCOLOGY p. 2 Splenic liposarcoma.
- REPORTABLE DISEASE p. 3 West Nile update.
- IN THE FIELD p. 9 More on locoweed.
- LAB UPDATES p. 10 New team additions.
- SAMPLE QA p. 14 Biopsy sample submission.

WESTERN SLOPE HEAD ...p. 1 New Western Slope Director Raye Walck will put focus on practical diagnostics.

RETURN OF AVIAN FLU?..p.3 VDL identified a case of highly pathogenic avian flu in January. Is it back?

CANINE COPPER TRENDS p.4 With two decades of data, what we know about liver copper.



DISTEMPER ON THE RISE p. 6 Canine distemper has been on the increase. Here are all your testing options.

VDL'S LATEST RESEARCHp. 12 From mosquito-borne disease to arctic foxes, the latest studies from VDL faculty members.

PROTECTING PET FOOD . p. 15 As a Vet-LIRN member lab, VDL helps track pet-food problems.





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Update from the Director

Welcome to this issue of *LabLines*, a little bit belated this winter, as we have been very busy. We finished up the year with a nearly 10% increase in accessions, which has kept us all quite busy. At the same time we have had a fairly significant amount of faculty and staff turnover. In this issue, meet some of our new faculty, including Dr. Raye Walck at the Western Slope Diagnostic Laboratory as the new director after Dr. Don Kitchen retired. Our new Parasitology Section Head, Dr. Ashley McGrew, is taking



BARBARA POWERS, DVM, PHD, DACVP DIRECTOR

over for Dr. Ballweber who has moved over into a teaching assignment. In the next issue of *LabLines*, we will be introducing our new Bacteriology Section Head, Dr. Josh Daniels, who will start July 1, after the previous section head, Dr. Doreen Hyatt, also moved over into a teaching assignment. We also have an ongoing search for a new pathologist after Dr. E.J. Ehrhart left for private consulting services. Every year we have a new group of anatomic pathology residents, and we have some other new staff in various different sections.

I hope you enjoy the articles in this issue of *LabLines*, as there are different articles affecting a wide variety of spebers who help evaluate our laboratory and suggest new avenues for improving service to everyone. We greatly appreciate their advice and assistance in keeping our laboratories moving forward and meeting their needs.

cies, including companion animals, livestock,

equine, and wildlife, including our research

activities. Topics range from oncology to

In January, we had our annual meeting

with our External Advisory Committee mem-

infectious disease to toxicology.

Finally, I hope you'll take a moment to review our latest Annual Report. It is now available on

the VDL website, at csu-cvmbs.colostate.edu/vdl, under the "Regulations & Resources" tab. In it, you'll find a comprehensive review of all the activity the VDL has pursued over recent history to satisfy our mission of providing timely, accurate and pertinent diagnostic services, supporting important research and contributing to the education of tomorrow's veterinary professionals. If you get a chance, I would always welcome your feedback—positive or negative—on how we are doing at accomplishing that important mission.

Sincerely,

Barbara E. Powers