

LAB LINES

Diagnostic news and trends from the Colorado State University Veterinary Diagnostic Laboratories
Volume 17, Number 1
Spring/Summer 2012

Lab Updates

Coming Soon: New Website Services Will Speed and Simplify Access

Coming soon this fall, CSU's Veterinary Diagnostic Lab will roll out our brand new website, which we have been refining for the last six months to one year. This new website will allow you to much more quickly find information you need. Look for these highlights and more: **24/7 online results.** For instance, you can create an account for on-line results so that you can view your results at any time. This is password-protected so only you can view your clinic's results, depending on how you wish to set it up. Please call (970) 297-1281 or email us at www.dlab.colostate.edu for instructions on how to obtain a password-protected access to your results.

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

Searchable test catalog. This new website will more easily list all of our tests and have a search function so you can find the appropriate test for the disease you are interested in. It will highlight all our sections and briefly describe the laboratory personnel as well as the operations of the different laboratory sections.

Scheduling and pricing. Of course, our price list will also be readily available on the website as will be the test schedule, so you can see when different tests are run.

Submission guidelines. Information on how to submit



samples and what types of samples are needed for each specific test will be easily available.

News you can use. Finally, all interesting news items such as access to our newsletter *LabLines* and our Annual Report, as well as other interesting news tidbits will be available on this new website. ▲

Visit the new website at dlab.colostate.edu

ANY WAY YOU WANT IT

Web-based improvements notwithstanding, you can still get your lab results by any means you want:

- Hard copy by snail mail
- Fax
- Email
- On-line via password-protected portal on our website

Let us know how you wish to receive your results. Call (970) 297-1281 or Email us at www.dlab.colostate.edu to tell us your personal preference.

Don't miss the improvements to CSU's Molecular Pathology Website, too, at CSUAnimalCancerCenter.org.

In addition to a wide array of information for the general public about the center's facility, consulting, clinical oncology service, education, outreach and research programs, it also offers direct access to molecular pathology diagnostic information for professionals, such as the cutaneous canine mast cell tumor panel and the list of available immunocytochemical tests.



Laboratory Updates

Farewell to Our Colleague, and Friend, Jim Kennedy

One of my saddest duties as VDL director was to announce the unexpected death Dec. 31 of our colleague and friend Jim Kennedy, Director of the Rocky Ford Branch. In his short ten years in the position, Jim was dedicated to serving all clients of the laboratory and focused on improving the practical diagnostics to benefit cattle producers. A pioneer in pooled BVD and Trich testing, he led Colorado's voluntary BVD control program that is the model for the nation. Named Colorado Veterinarian of the Year in 2007, widely published in peer-reviewed veterinary journals, and a prolific public speaker within the state, Jim will be fondly remembered and will be missed by all of us in the laboratory and by many more across the state. ▲

— Barbara Powers, DVM/PhD/DACVP, CSU VDL Director;
Photos courtesy Heather Kennedy, Stan Oswald.



Clockwise, from above. Before earning his veterinary degree in 1979 from University of Missouri, Jim served as Captain in the US Air Force between 1970 and 1974, from which he earned a lifelong love of flying. In 1966, Jim married his first wife, Diana Vernon, who preceded him in death. Jim's many professional recognitions, too numerous to list, included this Diamond Service Award from 1979. Jim owned many beloved dogs over the years, including Duke, pictured here. Vacations with his family included this shot, from Buckskin Joe's in Canon City, Colo. Even during those family vacations, Jim took time out to check animals, as shown here while on a family trip in South Dakota.



Exotics Medicine

Exotics Pathology Service Now Available at CSU VDL

The pathology service at the Veterinary Diagnostics Laboratory has been a long-time leader in the pathology of wildlife species. Now, we are excited and proud to introduce a pathology service especially for exotics and zoo animals.

The new exotics service includes surgical biopsy interpretation, necropsy and disease diagnosis as well as



ancillary diagnostic testing for pet,

zoo or wild non-domestic and non-traditional mammals, reptiles, amphibians, fish and birds.

This new service is covered by three board-certified VDL pathologists who share a special interest and dedication to non-traditional species: Sushan Han, Colleen Duncan and Terry Spraker. They will work in collaboration with CSU Veterinary Teaching Hospital Zoological Medicine Clinical Sciences Assistant Professor Matthew Johnston.

FULL RANGE OF SERVICES

Our new service offers one- to five-day turnaround of preliminary reports on surgical biopsies, with finished full written reports. We offer competitive prices for multiple tissues submitted, high quality histochemical stains and immunohistochemical stains, and no charge for tissue decalcification. Additionally, we offer discounted FedEx mailers for rapid overnight shipping of specimens to the laboratory and same-day processing of tissues upon receipt at the laboratory. At the request of the referring DVM, pathological cases can include a clinical consult by our CSU zoological medicine faculty, also board-certified in avian medicine. We offer full necropsy services at our facility with possible on-site visits upon request.

It is our goal to offer, as always, excellent client communication, which is paramount in critical and complicated exotics and zoo cases. As a leader in veterinary pathology and veterinary education, we have a strong interest in promoting disease investigation, publishing unique cases and findings, and educating veterinary students and residents. Cases submitted to the laboratory will be used in monthly interactive exotics rounds, which help train students, residents, clinicians and pathologists. We would eventually like to open these rounds to interested referring DVMs via teleconference, as well.

We hope to grow this new, exciting service and to

— Sushan Han, DVM, PhD, DACVP, CSU VDL Pathologist and CSU Microbiology, Immunology and Pathology Assistant Professor



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meet the needs of state and regional exotics and zoo referring veterinarians. Please help us better serve you by participating in our service and letting us know what specific services you may need or special interests you may have.



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HOW TO SUBMIT

Exotic specimens and whole cadavers can be submitted using our current accession form, available at dlab.colostate.edu/webdocs/services/forms/General_062011.pdf. Mark the required information as you would for any submission, and specifically include species information and a detailed history. Please also mark “exotics pathologist” under the section titled “Pathology” and “Pathologist requested” at the bottom of page 2.

We look forward to serving your exotics and zoo species needs! ▲

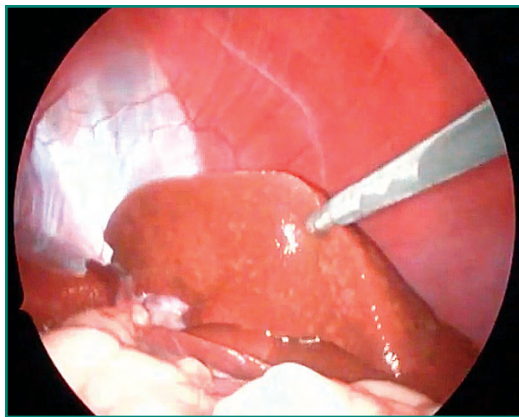
For any additional information or questions, please contact
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Diagnostic Interpretation

How to Interpret and Report Liver Biopsy Standard Examination

Liver biopsies constitute a significant portion of our Laboratories' submissions. Similar to standardization of evaluating and interpreting gastrointestinal biopsies, pathologists at CSU standardized how to examine and interpret a liver biopsy based on World Small Animal Veterinary Association standardization.

— *Tawfik Aboellail, BVSc, MVSc, PhD, DACVP, CSU VDL Pathologist, and Sushan Han, DVM, PhD, DACVP, CSU VDL Pathologist and CSU Microbiology, Immunology and Pathology Assistant Professor*



© KuschLife Veterinary Centre

piece. These sample sizes will provide us with greater than 10 mg of tissue on a dry-weight basis. Although our method is validated for samples larger than 10 mg, we will analyze samples that are smaller. Keep in mind that weighing samples smaller than 10 mg will introduce error into the final Cu, Fe or Zn concentration.

Liver biopsies may be submitted fresh, formalin fixed or in paraffin blocks. Fresh tissue is preferred and may be placed directly into a red-topped tube without any preservative. The sample does not need to be kept cold.

WHAT WE OFFER

- Timely results
- Morphological diagnosis
- Special stains to better characterize pathologic lesions (e.g. copper, Masson's)
- Pathologist and clinician consult when applicable
- Differentials will be provided; however, you should not anticipate an exact cause in all cases
- Reversible or irreversible changes will be indicated, but, in some cases, we can not provide an exact prognosis

INDICATIONS:

A liver biopsy is indicated when the patient presents one or more of the following criteria:

- Abnormal liver enzymes or function tests for longer than a month
- Hepatic enlargement due to undetermined etiology
- Involvement of the liver as a part of multisystemic disease, such as jaundice
- Staging of hepatic neoplasia
- A follow-up biopsy to assess progression of chronic hepatic disease or response to treatment

SPECIAL STAINS

A panel of special stains can give more insight as to the severity of the disease and distribution of the lesion, and it sometimes offers a clue as to the inciting cause.

- Stains for fibrosis (Sirius red, Masson's trichrome). Sirius red is a better stain for detecting fine fibrous strands in an incipient fibrosis and reticulin framework stain is employed when lobular collapse is suspected

SOURCES

1. WASAVA liver standardization group. *Standards for clinical and histological diagnosis of canine and feline liver disease*. 1st. Ed. (Spain: Elsevier, 2006).
2. Cullen JM, Van Winke, TJ. *Pathology of the liver: What's new and what's still true*. (Savannah, Ga.: The C. L. Davis Foundation and the American College of Veterinary Pathologists, November 2007).

ADDITIONAL QUESTIONS?

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WHAT WE NEED

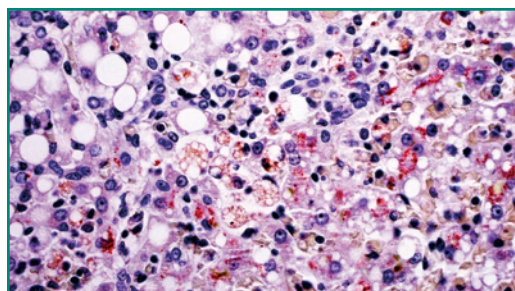
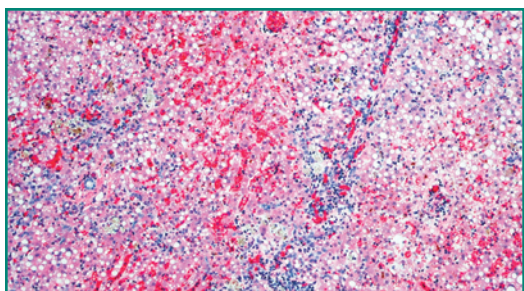
Most liver diseases can be diagnosed based on histological examination, especially parenchymal liver diseases (inflammatory, toxic or metabolic), biliary diseases and hepatic and biliary tumors. Diagnosing circulatory diseases, however, is more challenging and largely depends on clinicopathologic teamwork. Pathologists need to know:

- Signalment (species, breed, age and gender)
- History (clinical symptoms duration and progression)
- Biochemical alterations (exact values)
- Diagnostics (ultrasound examination, exploratory laparoscopy and gross appearance of the liver)
- Previous histological reports if available

QUANTITATIVE CU, FE AND ZN TESTING

Liver biopsies may be utilized for quantitative copper, iron and zinc testing, because the normal concentration ranges of these elements in liver tissue are in the hundreds of parts per million. Much larger sample sizes are required for elements, such as selenium, that naturally occur at lower concentrations.

Liver biopsies may be obtained laparoscopically or via ultra-sound guided needle. Samples obtained laparoscopically need not be larger than a split pea. Samples obtained via ultra-sound guided needle should consist of tissue 3 cm in length from a 14-gauge needle, although the tissue does not need to be one contiguous



Copper staining correlates well with copper quantification and gives a better idea as to the distribution of pathologic lesions. The good quality liver biopsy from a 10-year-old West Highland white terrier presenting with chronic ALP/ALT elevation and extrahepatic shunts (left) reveals arteriolar hyperplasia with portal bridging fibrosis and mild portal lymphoplasmacytic hepatitis. Copper stain (rhodanine) (right) shows high levels of copper pigment granules diffusely throughout the section, but more concentrated in the centro-lobular region. The copper level was toxic at 2820 ppm.

- Stains for reticulin network (Gordon and Sweet) indicated for prognostication
- Mineral pigment stains (Rhodanine for copper and Perl's Prussian blue for iron)
- Other pigment stains (Schmorl's and/or modified Ziehl-Neelson for lipofuscin)
- Glycogen and other carbohydrate stains (PAS+/- diastase)
- Stains for amyloid (Congo red)
- Stains for bilirubin (Hall's bilirubin stain)
- Stains for infectious agents (Fite's stain for acid fast

stain and silver stains for spirochetes [*leptospira*] and clostridia [*C. piliforme*])

IMMUNOHISTOCHEMISTRY

Immunohistochemistry is most often requested when differentiating lymphoma from lymphocytic cholangitis is a challenge in older cats or confirming histogenesis of poorly differentiated tumors (epithelial vs. mesenchymal). Ruling in lymphoma is possible via PCR to detect monoclonality of the infiltrative lymphocytes. ▲

Advances in Oncology Diagnostics

Alkaline Phosphatase CytoChem

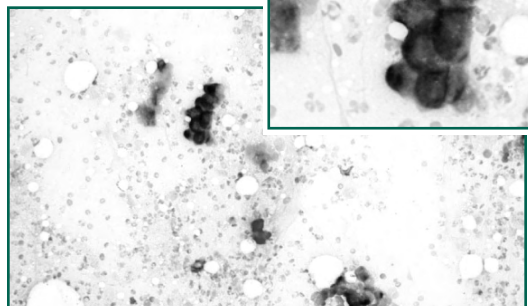
Cytologic diagnosis of primary lung neoplasia in dogs and cats can be confounded by the morphologic overlap between the neoplastic cells in primary lung tumors and those in other pulmonary/pleural neoplastic processes, including metastatic neoplasia and mesothelioma. This study investigated the ability of alkaline phosphatase (ALP) cytochemistry (CC) to distinguish primary lung neoplasia from other lung and thoracic lesions in dogs and cats.

A database search identified 29 lung aspirate and pleural fluid cases from 23 dogs and five cats in which cytologic slides were available and a corresponding histopathologic diagnosis was made. For ALP CC, Wright-Geimsa stained slides were treated with 5-bromo, 4-chloro, 3-indolyl phosphate/nitroblue tetrazolium phosphatase substrate and assessed for ALP activity.

Our results suggest ALP CC is a useful tool to differentiate primary lung neoplasia from other pulmonary disease. The sensitivity of ALP CC to identify primary lung neoplasia in cases of canine and feline lung neoplasia was 91 percent and 100 percent, respectively. ALP reactivity was seen in 10 of 11 canine and three of three feline primary lung tumors. Meanwhile, specificity considering all lung neoplasia was 100 percent. Most

— Maureen Emanuelli, DVM, CSU VDL Clinical Pathology Resident; Andrea Bohn, DVM/PhD/DACVP, CSU VDL Clinical Pathologist, and Davis Seelig, DVM, CSU Microbiology, Immunology and Pathology Resident

non-primary lung neoplasia cases, including inflammatory disease (n = 7), histiocytic sarcoma (n=4), mesothelioma (n=2), hemangiosarcoma (n=1) and anaplastic sarcoma (n=1) were ALP negative (except for a few normal respiratory cells).



ALP staining of neoplastic epithelial cells, showing (inset) the brownish cytoplasmic color indicating ALP activity.

PRICES

Prices vary depending on complexity of the hepatic disease and the number of special stains necessary to better characterize pathologic changes.

- Standard histological examination \$45
- Standard histological examination, copper stain and copper quantitation \$67
- Standard histological examination, copper stain with copper and iron quantitation \$81
- Histology, mineral quantitation and a battery of special stains . . . \$110
- Immunohistochemical stain \$45 each
- Lymphosarcoma immunohistochemistry \$65

View the online poster presented during the Annual Meeting of the American Society for Veterinary Clinical Pathology, Dec. 3-7, 2011, in Nashville, at www.asvcp.org/meeting/2011/onlinePosters.cfm

CSU VDL in the Field: Disease Updates

Infection with Porcine Reproductive and Respiratory Syndrome Virus

A submitting clinician euthanized a sow initially presenting for dystocia and a caesarian-section of nine stillborn fetuses following a three-week history of a dry nonproductive cough. He reported respiratory disease in all ages of pigs on the farm and several sows that had aborted underdeveloped fetuses. Tissues from the field-necropsied sow were submitted for bacteriology and virology testing and microscopic examination.

Histologically, the sow had lymphohistiocytic interstitial pneumonia with vasculitis and suppurative bronchopneumonia with fibrinous pleuritis. Polymerase chain reaction testing of the lungs and tracheobronchial lymph node was positive for porcine reproductive and respiratory syndrome (PRRS) virus and negative for swine influenza virus. *Pasteurella multocida* was cultured from the lungs.

The PRRS virus is a small, single-stranded, enveloped RNA virus within the genus *Arterivirus*, family *Arteriviridae*, which also includes lactate dehydrogenase-elevating virus, equine arteritis virus, and simian hemorrhagic fever virus. Now distributed worldwide, PRRS is considered one of the most economically significant diseases of the swine industry. It causes clinical disease ranging from female reproductive failure to respiratory disease in neonates, weaned pigs and occasionally grower-finisher pigs. The virus can be transmitted by direct nasal, oral, or coital contact with saliva, oropharyngeal mucus, urine, semen, feces and mammary secretions. Following exposure, the virus infects macrophages in the tonsils, nasal cavity and lungs, and then through viremia infects macrophages throughout the body.

The most consistent gross and microscopic lesions include interstitial pneumonia, generalized lymphadenopathy and lymphocytic inflammation in various visceral organs. Other less frequently identified microscopic lesions

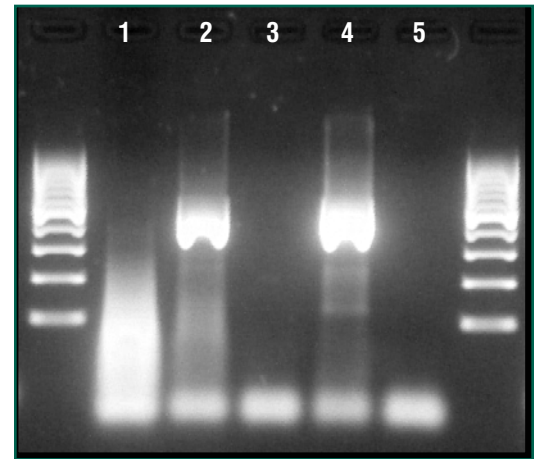
— Chad Frank, DVM/MS/DACVP, CSU VDL Pathologist, and Jeanette V. Bishop, CSU VDL Molecular Diagnostics Research Associate

include systemic vasculitis, myocarditis, and encephalitis. Secondary bacterial infections are common due to viral-induced alterations in alveolar macrophage function. The histologic lesions are highly suggestive of PRRS viral infection, but definitive postmortem diagnosis requires virus isolation, fluorescent antibody testing, PCR or immunohistochemistry. The most sensitive of these tests is PCR, which we offer at the laboratory. Clinicians experiencing respiratory disease outbreaks or reproductive failure in swine herds can submit pigs for necropsy examination or lungs and lymph node for microscopic evaluation and PRRS virus PCR testing. ▲



SOURCES

1. Rossow KD. Porcine Reproductive and Respiratory Syndrome. *Vet Pathol.* 1998 Jan;35(1):1-20.
2. Caswell JL, Williams KJ. Respiratory system. In: Jubb, Kennedy, and Palmer's *Pathology of Domestic Animals*. 5th ed., vol. 2. Edited by Maxie MG. Saunders-Elsevier, Philadelphia, PA, 2007:579-581



1.) Undiluted DNA
 2.) 1:10 dilution of DNA
 3.) Negative extraction control
 4.) Positive amplification control
 5.) Negative amplification control

YOU WANT THAT WHEN?

Many times, you need to receive results rapidly. We process samples as fast as possible, but some tests are run on certain schedules. You may request a STAT processing, and we will run your sample out of our normal schedule for a minimum \$25 fee. If we have to call personnel into the laboratory on weekends to process the sample, an additional \$25 charge will be added for a total STAT fee of \$50. Some samples cannot be run STAT, but you can request them ASAP. For instance, histopathology samples require

a 24-hour processing time, but if you are anxious for your results and need them ASAP, let us know. The pathologist on duty can read them at the beginning of duty rather than at the end of the stack of slides. Similarly, designation of ASAP means we will run your sample on our regular test schedule but will make sure it is properly lined up in the next run to be reported as soon as results are available. There is no fee for an ASAP sample. Questions? Call us at (970) 297-1281. ▲





EXTERNAL ADVISORY COMMITTEE

Front row, from left

- Dr. Marv Hamann, Mixed Practice, Pueblo West
- Mr. Ed Hansen, Beef Cattle, Livermore
- Dr. Don Kitchen, VDL Western Slope
- Dr. Keith Roehr, State Vet, Dept of Agric, Denver
- Dr. Larry Mackey, Large Animal, Greeley
- Dr. Pete Hellyer, Clinical Sciences Associate Dean
- Dr. Steve Wheeler, Small Animal, Englewood
- Mr. Norm Brown, Equine, Wellington
- Dr. Joan Bowen, Small Ruminant, Wellington
- Dr. Mike Gotchey, Equine, Steamboat Springs
- Dr. Chris Orton, CSU Clinical Sciences Department Head
- Dr. Dean Hendrickson, Director CSU Veterinary Teaching Hospital

Back row, from left

- Dr. Ron Kollers, Small Animal, Fort Collins
- Dr. Del Miles, Beef Cattle, Greeley
- Dr. Lance Perryman, CSU College of Veterinary Medicine Dean (retired)
- Dr. Tolani Francisco, USDA/AVIC/APHIS, Lakewood
- Dr. Elisabeth Lawaczek, Colo. Dept of Public Health and Environment, Denver
- Dr. Jan Carroll, CSU Extension, Fort Collins
- Dr. Leesa McCue, Mixed Animal, Limon
- Dr. Gregg Dean, Microbiology, Immunology and Pathology Department Head

Get to Know the Laboratory

New Members Join the Lab Team

Katherine Dirsmith, an Honors Undergraduate Research Scholars program freshman in biomedical sciences and conservation biology, hopes to eventually assist in international wildlife conservation efforts. Along with VDL faculty, Katherine has been working on a research project concerning *Coxiella burnetii* in northern fur seals, which she presented at the CVMBS Research Day in January and the Celebrate Undergraduate Research and Creativity Showcase in April.



Tracy Baszler, originally from Milwaukee, graduated from Colorado State with a BS in



computer science. She has worked as a software engineer for many years with companies such as Lockheed Martin and Qwest and now works on the VDL's server software.

Patrick McDougale grew up in Las Cruces, N.M., and completed a BS in computer science at New Mexico State University. While at NMSU he interned at Walt Disney World's research biology department at Disney's Animal Kingdom. Since graduation, he has worked as a government contractor on navigational software for military aircraft, military simulations and bioinformatics. He also teaches game programming for Baker College Online. ▲



CSU VDL In Press

A Roundup of VDL Faculty Research

Webb BT, Norrdin RW, Smirnova NP, Van Campen H, Weiner CM, Antoniazzi AQ, Bielefeldt-Ohmann H, Hansen TR. Bovine Viral Diarrhea Virus Cyclically Impairs Long Bone Trabecular Modeling in Experimental Persistently Infected Fetuses. *Vet Pathol.* 2012 In Press.

A research team from CSU's Animal Reproduction and Biotechnology Laboratory, including Veterinary Diagnostic Laboratory Virology Section Head Hana Van Campen and pathology graduate student Brett Webb, inoculated 46 BVDV-naive pregnant 18-month old Hereford heifers with noncytopathic BVDV type 2 containing media or media alone on day 75 of gestation. The inoculation produced BVD-persistently infected (PI) and control fetuses, respectively, which were then collected via Cesarean section on days 82, 89, 97, 192, and 245 of gestation. The study first detected radiographic and histomorphometric abnormalities on day 192, at which age PI fetal long bone metaphyses contained focal densities (four of seven fetuses) and multiple alternating transverse radiodense bands (four of seven fetuses).

Fetuses at day 245 were similarly affected. Histomorphometric analysis of proximal tibial metaphyses from day 192 fetuses revealed transverse zones with increased calcified cartilage core and trabecular bone volumes in regions corresponding to radiodense bands. Numbers of tartrate resistant acid phosphatase positive osteoclasts and bone perimeter occupied were both decreased. Mineralizing surface, a measure of tissue level bone formation activity, was reduced in PI fetuses. The research concluded that PI BVDV induces cyclic abnormal trabecular modeling, which is secondary to reduced numbers of osteoclasts. The factors responsible for these temporal changes are unknown but may be related to the time required for osteoclast differentiation from precursor cells.

Chavez DJ, Levan IK, Miller MW, Ballweber LR. *Baylisascaris procyonis* in raccoons (*Procyon lotor*) from eastern Colorado, an area of undefined prevalence. *Vet Parasitol.* 2012 Apr 30;185(2-4):330-4.

VDL Hughes Undergraduate Research Scholar Deanna Chavez and Parasitology Section Head Lora Ballweber collaborated on this study to help fill knowledge gaps about distribution of the zoonotic parasite *Baylisascaris procyonis* in eastern Colorado, a region where no published incidence information exists. *B. procyonis* has been documented in raccoons throughout much of the

United States; however, no published information on its occurrence is available for the transition zone from the Great Plains to the Rocky Mountains. The parasite presents a public health concern because it can cause neural larva migrans and diffuse unilateral subacute neuroretinitis in humans and other hosts.

The study collected 53 raccoons throughout eastern Colorado during 2007 to 2010; 46 were examined by necropsy, seven by fecal flotation. When available, feces were further processed to detect *Giardia* and *Cryptosporidium* using a direct fluorescent antibody detection method.



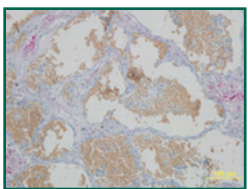
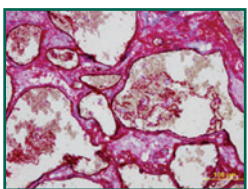
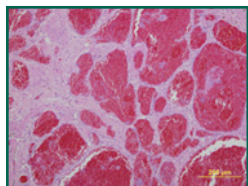
Chavez and Ballweber's study found both *B. procyonis* and *Cryptosporidium* spp. appear to be prevalent. *B. procyonis* was found in 31 of 53 raccoons. Mean intensity was 11.7, with a range of one to 49 worms per infected individual. *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts were detected in 25 percent and 6.9 percent of the animals, respectively.

Miller AG, Halsey CH, Miller MD, Bohn AA. What is your diagnosis? Intracranial mass in a dog. *Vet Clin Pathol.* 2011 Dec;40(4):563-4.

Former VDL Clinical Pathology Resident Amy Miller, Clinical Pathologist Andrea Bohn and pathology graduate student Chuck Halsey detail the first cytologic characterization of a cerebral cavernous hemangioma in a dog. Magnetic resonance imaging (MRI) of a 6-year-old male neutered Rhodesian Ridgeback dog presented for a 6-week history of uncharacteristic aggressive behavior, left-sided circling, pacing, seizure activity and other symptoms revealed a mass measuring 5.0 cm by 2.8 cm by 2.7 cm. The mass extended from the right olfactory lobe with involvement of the right and left frontal lobes, hypothalamus and portions of the basal nuclei.



Fine-needle aspirates and impression smears of an excised portion of the mass were collected at eventual necropsy for cytopathologic exam. Histopathologic examination revealed a compressive mass containing multiple variably-sized blood-filled vascular spaces lined by a single layer of well-differentiated endothelial cells and separated by connective tissue that contained hemosiderin-laden macrophages. Proliferations of small capillaries were also present. The diagnosis was cavernous hemangioma or vascular hamartoma. Immunohistochemical staining for factor VIII-related antigen and glial fibrillar acidic protein was performed. The cells lining vascular spaces as well as most mesenchymal cells separating vascular spaces demonstrated intense immunoreactivity for factor VIII-RA, supporting endothelial cell origin. Rare intervening stromal cells expressed GFAP and represented entrapped glial cells in the mass.



Histologic section of cerebral mass showing H and E (top), immunohistochemical staining for factor VIII-RA (middle) and immunohistochemical staining glial fibrillar acidic protein (bottom).

Shoeneman JK, Ehrhart EJ 3rd, Eickhoff JC, Charles JB, Powers BE, Thamm DH. Expression and function of survivin in canine osteosarcoma. *Cancer Res.* 2012 Jan 1;72(1):249-59.

Lab Pathologist EJ Ehrhart and Director Barb Powers cooperated in a study demonstrating that elevated survivin expression in primary canine osteosarcoma tissue is correlated with increased histologic grade and mitotic index and a decreased disease-free interval. Survivin attenuation in canine osteosarcoma cells inhibited cell-cycle progression, increased apoptosis, mitotic arrest, and chemosensitivity, and cooperated with chemotherapy to significantly improve in vivo tumor control. The study's findings illustrate the utility of a canine system to more accurately model human osteosarcoma and strongly suggest survivin-directed therapies might be highly effective in its treatment.

Survivin is an inhibitor of apoptosis family member protein that blocks apoptosis and drives proliferation in human cancer cells where it is commonly elevated. The study showed a canine osteosarcoma model is superior as a translational tool for evaluating survivin-directed therapies, owing to the striking similarities in gross and microscopic appearance, biologic behavior, gene expression and signaling pathway alterations.

Rhyan JC, Miller MW, Spraker TR, McCollum M, Nol P, Wolfe LL, Davis TR, Creekmore L, O'Rourke KI. Failure of fallow deer (*Dama dama*) to develop chronic wasting disease when exposed to a contaminated environment and infected mule deer (*Odocoileus hemionus*). *J Wildl Dis.* 2011 Jul;47(3):739-44.

VDL Pathologist Terry Spraker collaborated on a National Wildlife Research Center study that exposed a herd of fallow deer to the chronic wasting disease (CWD) agent from mule deer by housing them in a paddock considered contaminated with infectivity from its history of housing CWD infected deer and by comingling them with infected mule deer. In contrast to at least eight of 12 sentinel mule deer they confirmed infected with CWD, none of the 41 exposed fallow deer showed clinical signs suggestive of CWD, IHC staining of disease-associated prion in lymphoid or brain tissues, or evidence of spongiform degeneration in sections of brain stem at the level of the obex when sampled 18 months to seven years after entering the contaminated mule deer paddock. ▲



Feline Virology

Strain distribution of feline AIDS

Similar to human immunodeficiency virus (HIV), the pathogenesis of feline immunodeficiency virus (FIV) involves infection of lymphocytes and macrophages, and results in chronic progressive immune system collapse and death. Neuropathologic correlates of FIV infection have not been elucidated, and may be relevant to understanding HIV-associated disease

To examine viral genetic determinants that might contribute to neuropathogenicity, cats were exposed to two well-characterized FIV strains with divergent clinical phenotypes and a chimeric strain. A sham inoculum control group was also included. At termination of the study (350 days post-inoculation), brain sections were obtained from four anatomic locations known to be involved in human and primate lentiviral neuroAIDS. Histological and immunohistochemical evaluation with seven markers of inflammation revealed that Pcnv infection resulted in mild inflammation of the CNS, microglial activation, neuronal degeneration and apoptosis, while C36 and PPR strains induced minimal neuropathologic

— Craig Miller, DVM, CSU VDL Pathology Resident, Helle Bielefeldt-Ohmann, University of Queensland, Martha MacMillan, CSU Microbiology, Immunology and Pathology Research Associate, Salvador Huitron-Resendiz, Scripps Research Institute, Steven Henriksen, Western University of Health Sciences, John Elder, Scripps Research Institute, and Susan VandeWoud, CSU Microbiology, Immunology and Pathology Associate Dean

changes. Conduction velocity aberrations were noted peripherally in all three groups at 63 weeks post-infection. Pcnv viral load in this study was intermediate to the parental strains. Results suggest that 3_C36 genomic elements contribute to viral replication characteristics, and 5_PPR genomic elements contribute to CNS manifestations. This study illustrates the potential for FIV to provide valuable information about neuroAIDS pathogenesis related to genotype and viral kinetics. ▲



FELINE

Exotics Epidemiology

Canine Hepatitis in a Sun Bear?

Adiagnosis of canine adenovirus-1 (CAV-1) infection, the causative agent of Infectious Canine Hepatitis (ICH) in dogs, was made in a zoo's sun bear (*Ursus malayanus*). CSU-VDL's Molecular Diagnostic section detected it by using a CAV-1 polymerase chain reaction (PCR) test. CAV-1 infects a wide variety of species including members of the Ursidae (bears), Procyonidae (raccoons), Mustelidae (skunks) as well as Canidae (foxes, coyotes, wolves). Serologic surveys of free-ranging grizzly, brown and black bears and virus isolation in polar and black bears indicate these bears are susceptible to infection with CAV-1. To our knowledge, this is the first report of CAV-1 infection in a sun bear.

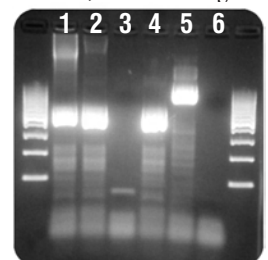
In dogs, CAV-1 is usually ingested followed by replication of the virus in lymphoid tissue in tonsils. The virus infects monocytes, macrophages and endothelial cells. Infected monocytes enter the blood, spreading CAV-1 to multiple organs including the brain, liver, spleen, kidneys and adrenal glands. The virus replicates in endothelial cells, leading to vascular damage and hemorrhage, which in turn causes tissue injury and the resulting clinical signs. The organs affected and the clinical signs of CAV-1 infection vary with the species of animal infected. Hepatitis commonly occurs in dogs; whereas, encephalitis is common in foxes.

— Hana Van Campen, DVM/PhD/DACVM, CSU VDL Virology Section Head and Jeanette V. Bishop, CSU VDL Molecular Diagnostics Research Associate

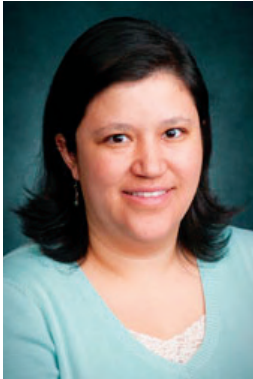
Although this disease has become uncommon where vaccination is used in a high proportion of domestic dogs, CAV-1 is likely to be kept in circulation by feral dogs and wild animals. Controlling the spread of CAV-1 is difficult as the virus is shed in saliva, respiratory secretions, feces and urine; viral shedding can occur for prolonged periods of time, up to 200 days, particularly in urine. Adenoviruses are moderately stable in the environment and can also be spread via contaminated fomites. Prevention of disease due to CAV-1 in susceptible zoo animals involves physical separation of zoo animals from feral dogs, wildlife and high human traffic areas. ▲



EXOTICS



1.) Undiluted DNA from blood
2.) 1:10 dilution of DNA
3.) Negative extraction control
4.) CAV-1 + amplification control
5.) CAV-2 + amplification control
6.) Negative amplification control



PABILONIA WINS INTERNATIONAL YOUNG SCIENTIST AWARD

VDL Avian Diagnostics and BSL3 Operations Section Head Kristy Pabilonia received a Young Scientist Award for her presentation, "Avian Influenza Virus in Domestic Ducks in West Java, Indonesia" at the 8th International Avian Influenza Symposium. The symposium, which took place at Royal Holloway, University of London, was hosted by the Animal Health and Veterinary Laboratories Agency, whose mission is to help safeguard animal health and welfare and public health, protect the economy and enhance food security through research, surveillance and inspection. ▲

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

VDL Parasitology Section Head **Lora Ballweber** will present two talks at *the 2012 American Veterinary Medical Association Annual Convention*, Aug. 3 through 7, in San Diego; will give one long talk at the *Colorado Veterinary Medical Association* annual meeting, Oct. 4 through 7, in Loveland; will attend the American Association of Veterinary Parasitologists 57th Annual Meeting, Aug. 4 through 7, in San Diego; and will attend the Association of American Veterinary Medical Colleges' Veterinary Educator Collaborative Symposium, July 27 and 28, in Fort Collins.

VDL Director **Barb Powers**, Bacteriology Section Head **Doreene Hyatt**, Microbiologist **Christina Weller**, Chemistry and Toxicology Section Head **Dwayne Hamar**, Virology Section Head **Hana Van Campen**, and Pathologist **Chad Frank** will all be in attendance at the 55th Annual American Association of Veterinary Laboratory Diagnosticians Annual Meeting, Oct. 18 through 24, in Greensboro, N.C.

VDL Pathologist **Sushan Han** will review small ruminant abortions at the *Colorado Veterinary Medical Association* annual meeting, Oct. 4 through 7, in Loveland.

VDL Pathologist **Tawfik Aboella** lectured on gross pathology of avian diseases and gross pathology of camelid diseases during the *C.L. Davis Foundation's Gross Pathology Review Course*, July 11 and 12, in Fort Collins.

VDL Microbiologist **Christina Weller** participated in the *National Laboratory Training Network's Train the Trainer: Designing and Conducting Training for the Sentinel Laboratory workshop*, June 21 and 22, in Houston.

VDL Director **Barb Powers**, Virology Section Head **Hana Van Campen**, Pathologist **Colleen Duncan** and Avian Diagnostics and BSL3 Operations Section Head **Kristy Pabilonia** will attend the *Colorado Veterinary Medical Association* annual meeting, Oct. 4 through 7, in Loveland.

Lab News

How To Get Samples to Us

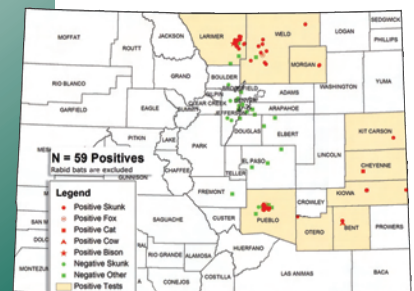
Samples may be sent to the laboratory in a number of different ways.

- If you are local, you can drop them off at the sample receiving area any time between 7:30 a.m. and 5 p.m.
- Locally, we also have a courier service which runs through the Fort Collins area. If you are interested in establishing a courier pick-up, call 297-5415.
- If you are more distant from the lab, we encourage use of either UPS or Fed Ex, both of which deliver samples to our doorstep in the mornings. We have an account with Fed Ex which allows a discount price to be applied to your invoice. Both UPS and Fed Ex are very useful because they provide tracking numbers to monitor the status of your sample in the mailing process.
- We do have mailers for histopathology samples that go through regular mail. These sometimes, however, get delayed as they are not processed with any priority through the Postal Service and may take between three days to more than two weeks to reach our laboratory. They also cannot be tracked. As always, it is very important to package your samples correctly.

Please see our website for instructions on how to best pack and ship, or feel free to call us at (970) 297-1281

CONTINUE TO BE WATCHFUL OF RABIES

For the last three years, we have seen increasing numbers of positive rabies cases in species other than bats — primarily skunks, but also horses, cattle, deer and, recently, a bison. This year, more than 16 cases of skunk rabies have been diagnosed in Larimer County. We have tested 208 animals, with 33 positive — more than three times the number of positives as last year. For more information, visit cdphe.state.co.us. Keep animals vaccinated!



REGULAR COLUMNS

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ALP use for differentials.
- LAB UPDATE p. 7
Meet new staff members.
- FELINE VIROLOGY p. 10
Feline AIDS strains.
- LAB NEWS p. 15
How to get samples to us.
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Rabies update.

LAB LINES

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VDL IN THE FIELD p. 6
Swine respiratory disease or reproductive failure may call for PRRS testing.

VDL IN PRESS p. 8
A roundup of recent work by VDL faculty members.

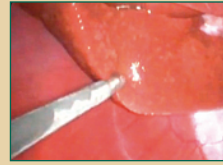
EXOTICS EPIDEMIOLOGY p. 10
VDL virologists discuss the first report of CAV-1 infection in a sun bear.



LAB NEWS p. 2
Farewell to VDL colleague and friend Jim Kennedy.

EXOTICS MEDICINE p. 3
We are excited and proud to introduce a new pathology service especially for exotics.

INTERPRETATION p. 4
CSU pathologists standardized how to examine and interpret a liver biopsy.



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Update from the Director



WEEKEND BACTERIOLOGY IS BACK

VDL is bringing back Saturday morning bacteriology service, to allow better completion of cases from the week and facilitate inoculation of samples arriving on Saturdays. Service will begin as soon as training ends, with a goal of full service by the end of August.

Welcome to this delayed issue of *LabLines*. Please accept my apologies for this delay, but the Colorado wildfires, especially the High Park Fire in Fort Collins, has caused disruption to some of our normal routines, resulting in delay of this issue of *LabLines*. I would like to take the opportunity to acknowledge and thank all of the firefighters for their help in assisting Colorado through these difficult times. Despite the High Park fire in Colorado that burned over 87,000 acres, forced many people from their homes in the foothills west of Fort Collins (including yours truly), and destroyed 259 homes, the laboratory work goes on, and we continue to provide quality service to our clients.

I hope you'll take a few minutes to look inside this issue for interesting articles regarding recently diagnosed cases of PRRS in Colorado, canine hepatitis, and research advancements that our laboratory personnel have contributed to. In February, we had a very successful meeting with our External Advisory Committee, which you can find pictured on page 7. We had



BARBARA POWERS, DVM, PHD, DACVP DIRECTOR

a new format for the meeting, this time using breakout groups to help provide us with many helpful suggestions, most notably suggesting improvements in our website, which is in progress. You'll find details of those improvements on page 1.

In this issue, we also reflect upon the untimely, unexpected, sudden death of our Rocky Ford branch laboratory director, Dr. Jim Kennedy. We continue to miss

Jim's presence in the laboratory system, and hope you will enjoy the tribute in his honor. We are currently in progress of searching for a director to fill this position.

We look forward to seeing many of you at the Annual Colorado Veterinary Medical Association meeting in September and the Annual American Association of Veterinary Laboratory Diagnosticians meeting in October.

Respectfully,

Barbara E. Powers

LAB NEWS

