

LAB LINES

Diagnostic news and trends from the Colorado State University Veterinary Diagnostic Laboratories
Volume 22, Number 1
Spring/Summer 2017

Meet the Lab

New Bacteriology Section Head: Pushing Frontiers in Diagnostic Tech

In his decision to move to Colorado State University's Veterinary Diagnostic Laboratories, the opportunity to join a world-class group of diagnosticians at a premier veterinary laboratory was one Joshua Daniels could not pass up. The large and diverse caseload of the CSU VDL, combined with the proximity of the Veterinary Teaching Hospital, will provide an advantageous position for making impactful contributions to diagnostic medicine in his new role as Bacteriology Section Head.

In addition, it will offer the chance to interface with a larger group of practitioners who want to get the most out of their diagnostic submissions.

"Frequently, interpreting bacteriology results requires the integration of additional information: details about patient history; clinical status (and that of the herd, if applicable); how specimens are collected; and very importantly—concurrent cytopathological or histopathological data," he says. Daniels is excited about assisting clients of the laboratory with their bacteriology workups, including providing input on antimicrobial selection.

It's a process he's familiar with, after serving for the past nine years as director of the Clinical Microbiology Laboratory at The Ohio State University College of Veterinary Medicine. There, his primary focus was providing direct consultation to clinicians in a tertiary referral facility, as well as educating house officers and DVM students. He believes it provided him with diverse experience and interests in clinical diagnostics and research.

Daniels received his DVM from University of Wisconsin's School of Veterinary Medicine in 1999. He then spent three years as a small animal practitioner, mostly in Albuquerque. Following private practice, he trained at Washington State University in a combined clinical microbiology residency and PhD program, where his research focus was in antimicrobial resistance.

He looks forward to continuing his research in that area and collaborating with CSU faculty on a variety of projects.

"Like many other microbiologists," he says, "I'm sort of a jack-of-all-trades in this era of rapidly evolving

diagnostic technology. With a focus on bacteriology, it is essential to have a firm grasp on classical cultivation methods, while also working with molecular tools. It is this sort of straddling of the Pasteurian and molecular approaches to diagnostics that keeps me on my toes and makes what I do very challenging."

Daniels also brings with him a passion for microbiology education and clinical training. As chair of the



examinations committee of the American College of Veterinary Microbiologists, he has an investment in certifying the veterinary microbiologists of tomorrow. "CSU is one of a handful of U.S. Veterinary Colleges that has a post-DVM microbiology training program. I couldn't imagine a better fit for me."

When not in the lab, Dr. Daniels enjoys playing the guitar, fiddling with ham radio and spending time with his family and cats. He looks forward to exploring the front range on two feet, two wheels and perhaps two skis. ▲

Advances in Molecular Diagnostics

Protein Characterization Using Electrophoresis and Immunofixation

ELECTROPHORESIS AND IMMUNOFIXATION TESTING AT CSU

■ Samples

Serum:
250 μ L serum
(preferred) or 0.5 mL
non-additive whole
blood

Urine:
10 mL urine

■ Protect shipment from high temperatures

■ Turnaround 24 hours if submitted by 11 a.m. Tuesdays and Fridays

■ Cost Protein electrophoresis: \$45 Immunofixation: \$73 Plus \$9 per case accession fee*

Most biochemical profiles measure total protein and albumin and then calculate the amount of globulin as the difference between the two. Although this practice is adequate for many purposes, protein electrophoresis and immunofixation can further characterize protein changes when abnormal protein levels are encountered or suspected. You can now order these clinical pathology tests through CSU's Veterinary Diagnostic Lab .

Protein electrophoresis passes electrical current through an agarose gel, causing proteins to migrate at differing rates dictated by their relative size and charge. After staining, the density of stain uptake in the gel quantifies the amount of the proteins present in the sample. Because the stain binds to any protein present in the gel, this technique can be performed on samples from many species, including small animals, large animals and exotic species. Protein electrophoresis is most commonly performed on serum or urine. Serum proteins are typically divided into albumin, alpha 1, alpha 2, beta 1, beta 2 and gamma globulin fractions. Specific proteins are expected to be found in each fraction. Significant glomerular disease can allow serum proteins to pass into the urine, and these can be detected with urine protein electrophoresis. Immunoglobulin light chains are small enough to pass through the renal glomeruli and into the urine; these light chains may be detectable by electrophoresis of concentrated urine.

—Russell Moore, DVM, MS CSU Microbiology, Immunology and Pathology Assistant Professor; and Paul Avery VMD, PhD, CSU Microbiology, Immunology and Pathology Associate Professor

Serum protein electrophoresis available in CSU VDL's clinical pathology lab includes a total protein measurement, preparation of the electrophoretic gel and densitometric tracing and interpretation by a board certified clinical pathologist who attempts to categorize the sample into one of several patterns:

■ **Normal.** Typical serum contains a predominance of albumin with lower amounts of various globulin subsets.

■ **Acute phase protein response.** Inflammation stimulates production of positive acute phase proteins, such as haptoglobin, ceruloplasmin, serum amyloid A and C-reactive protein. These proteins are typically found in the alpha and beta globulin fractions. Inflammation also is associated with decreased amounts of negative acute phase proteins, most notably albumin. While this pattern helps explain the pathologic process, it is not specific for the cause of inflammation.

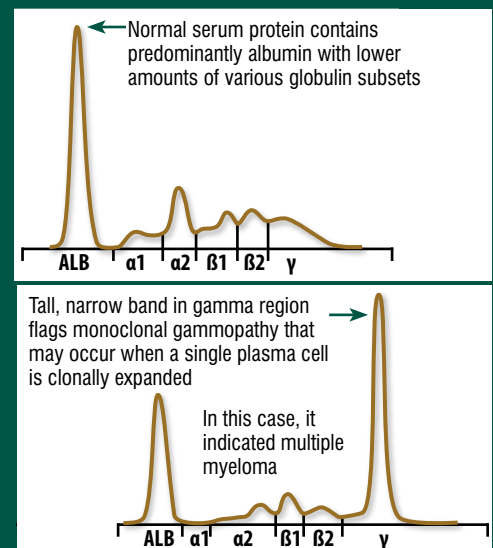
■ **Polyclonal gammopathy.** When inflammation, infection or immune stimulation persist for an extended period of time, multiple types of immunoglobulins are produced. This is seen as a broad increase in the beta 2 and gamma globulin region.

CASE 1

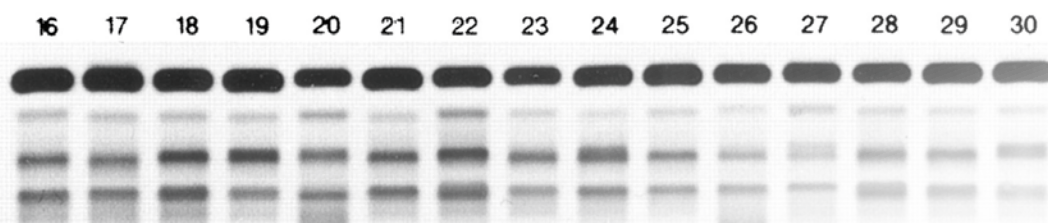
A 9 year old female spayed mixed breed dog had several cutaneous plasma cell neoplasms. Mild hyperglobulinemia occurred at 4.0 g/dL, reference interval (RI) = 1.5 - 3.2 g/dL. Serum protein electrophoresis was within normal limits. The lack of a monoclonal gammopathy suggested the masses were likely to be a cutaneous plasma cell tumor. These tumors typically are benign and can often be handled with surgical excision. Clinical progression confirmed this. Rare cases of cutaneous plasmacytomas which behaved as malignant neoplasms have been reported.

CASE 2

A 13 year old domestic short haired cat was presented for acute lameness on the left hind leg. Survey radiographs detected multiple lytic bone lesions, splenomegaly and hepatomegaly. Hyperproteinemia (11.4 g/dL, RI = 6.3-8.0 g/dL) and marked hyperglobulinemia (8.4 g/dL, RI = 2.7-4.2 g/dL) was found. Serum



* Subject to change; please verify on our website



Most polyclonal gammopathies are associated with infectious agents, but occasionally they will be seen with chronic liver or dermatologic disease.

■ **Monoclonal gammopathy.** Individual plasma cells produce a single type and subtype of immunoglobulin. When a single plasma cell is clonally expanded, a monoclonal gammopathy can occur, which is seen as a narrow, distinct band. A monoclonal gammopathy can produce a significantly high protein concentration which overshadows all other protein bands, including albumin, and alters plasma viscosity or interferes with clotting factor function. The most common causes of a monoclonal gammopathy are myeloma related disorders, including multiple myeloma or cutaneous or non-cutaneous extramedullary plasma cell tumors. Less common causes of a monoclonal gammopathy include immunoglobulin secreting lymphoma/leukemia or a clonal, but non-neoplastic, response associated with infectious agents such as Ehrlichiosis and other rickettsial diseases, leptospirosis and, rarely, FIP. Human medicine advocates monitoring monoclonal gammopathy as an indicator of response to treatment of myeloma-related diseases; this practice is beginning to be investigated in veterinary medicine.

POWERFUL TOOL FOR CHALLENGING CASES

Immunofixation allows specific detection of immunoglobulin heavy and light chains. It operates on

similar principles to routine electrophoresis, but the gel is labeled with species-specific antibody to IgG, IgA, IgM and immunoglobulin light chains. Our lab offers immunofixation on serum for canine and feline patients only. Immunofixation can be used to classify the type of immunoglobulin in a monoclonal gammopathy or confirm that a high concentration, restricted protein band is immunoglobulin.

Immunofixation can also be used to detect a monoclonal gammopathy when no readily apparent band on electrophoresis exists due to a low-concentration monoclonal gammopathy which is overshadowed by other normal proteins, or buried within a polyclonal expansion. In human medicine, about 5% of multiple myeloma cases will have a monoclonal gammopathy that is not detectable by electrophoresis but which can be found by other methods, including immunofixation. The exact incidence of this event in veterinary medicine is uncertain. Several cases evaluated through CSU demonstrating the usefulness of immunofixation have been published.

In most cases, serum protein electrophoresis is sufficient and will provide diagnostically and prognostically useful information. The combination of protein electrophoresis and immunofixation is a powerful tool that can be very helpful in challenging cases. Clinical pathologists are available to discuss cases and the application of these tools. ▲

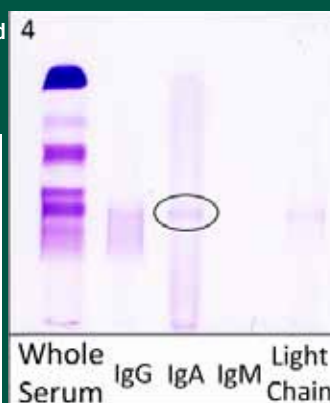
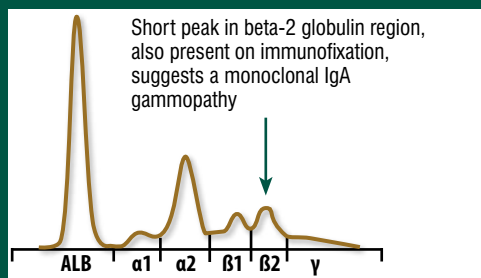
FOR MORE

- Igase M, Shimokawa Miyama T, Kambayashi S, et al. Bimodal immunoglobulin A gammopathy in a cat with feline myeloma-related disorders. *J Vet Med Sci.* 2016 May 3;78(4):691-5.
- Rout ED, Shank AM, Waite AH, et al. Progression of cutaneous plasmacytoma to plasma cell leukemia in a dog. *Vet Clin Pathol.* 2017 Mar;46(1):77-84.
- Seelig DM, Perry JA, Avery AC, Avery PR. Monoclonal gammopathy without hyperglobulinemia in 2 dogs with IgA secretory neoplasms. *Vet Clin Pathol.* 2010 Dec;39(4):447-53.

protein electrophoresis found a tall narrow band in the gamma-globulin region. The diagnosis was multiple myeloma. Feline myeloma related disorder is often extraosseous, infiltrates the spleen and is treated differently from other lymphoid neoplasms.

CASE 3:

A 12 year old male neutered Chihuahua had a history of anemia and lameness. Bone marrow evaluation suggested a plasma cell tumor or lymphoma. PARR analysis confirmed a B-cell lineage tumor but could not distinguish between a plasma cell neoplasm or lymphoma. Although globulins were within normal limits (4.0 g/dL, RI=1.5 - 3.2 g/dL), serum protein electrophoresis showed a short peak in the beta-2 globulin region, thought to be part of an acute phase protein response. Immunofixation labeled the peak as a monoclonal IgA gammopathy consistent with multiple myeloma.



CSU VDL in the Field

Is Intensive Fattening Causing Fed-Cattle Cardiac Remodeling?

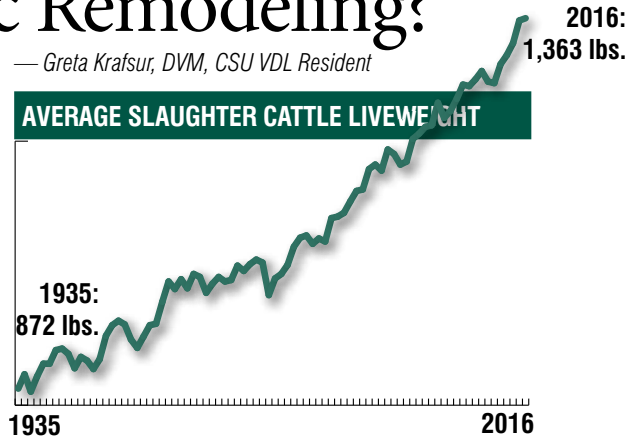
— Greta Krafsur, DVM, CSU VDL Resident

The expanding American waistline is recapitulated in modern beef production; the average live market weight has increased 44%, from 882 pounds in 1944 to over 1,300 pounds in 2016. In humans, obesity and its comorbidities are considered risk factors for pulmonary hypertension (PH) secondary to left heart disease (PH-LHD), the most common form of PH.

As in obese humans, PH and heart failure are increasingly recognized in U.S. fed cattle, associated with their intense feeding and fattening regimens intended to augment growth performance as beef cattle transition from the weanling to fattened phase. Features of metabolic syndrome are strongly associated with cardiac fibrosis, LV diastolic dysfunction and pulmonary venous hypertension (PVH). The pro-inflammatory, pro-fibrotic milieu associated with adiposity may initiate structural and functional alterations in the cardiopulmonary axis of fattened beef cattle.

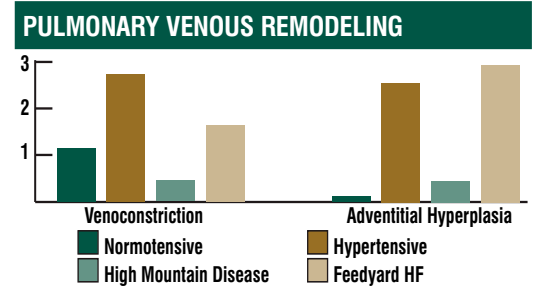
To investigate the possibility that fattening is leading to bovine heart disease, we conducted an autopsy on post-mortem cases from feedlots at either high altitude or in the Great Plains that met veterinary criteria for heart failure or were confirmed by necropsy. We then evaluated histology and cardiac and pulmonary remodeling according to human clinical-pathologic criteria.

In a collateral field study, we selected steers from two ranches at high altitude for testing through the feeding phase. Steers with the highest and lowest mPAP scores at weaning were selected for sequential right heart



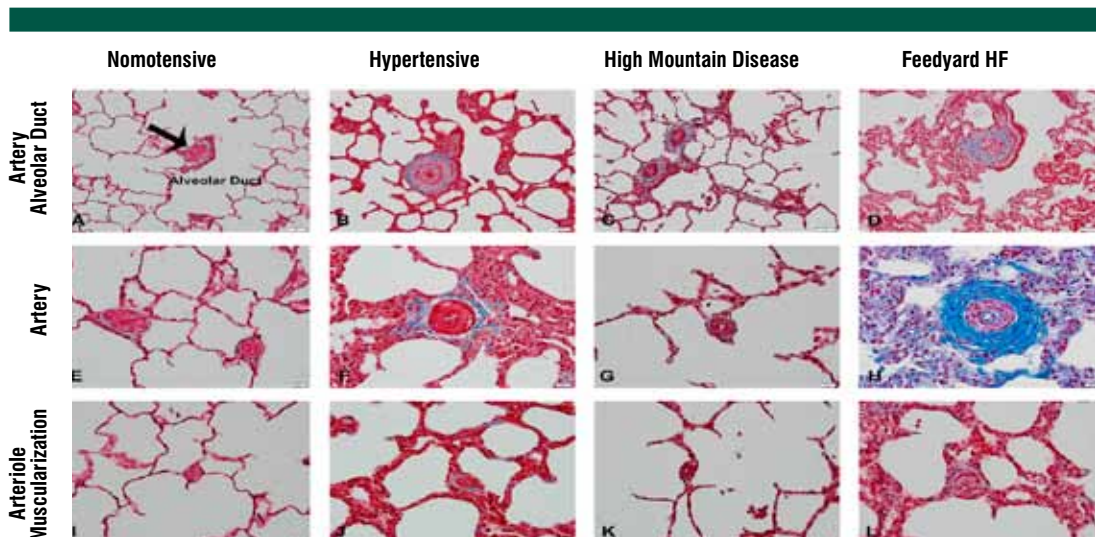
catheterization and blood sampling throughout the feeding period. We found:

- The mPAP increased strongly in response to metabolic and anabolic demands imposed by the fattening phase, particularly in steers most sensitive to environmental hypoxia.



Krafsur M, Brown RD, Holt TN, et al. Intensive Feeding and Fattening Regimens Augment Pulmonary Hypertension, Pulmonary Venous and Cardiac Remodeling in Beef Cattle: A Natural Large Animal Model of Pulmonary Hypertension with Left Ventricular Dysfunction. American Thoracic Society annual conference. Washington: 2017 May 19-24.

In contrast to predictably inconspicuous smooth muscle and adventitial layers in the pulmonary arterial circulation of normotensive steers (column 1, at right), obvious pulmonary arterial vasoconstriction, luminal narrowing and adventitial fibrosis were present in (columns 2 through 4, respectively) hypertensive, high mountain disease and feedlot HF cattle.



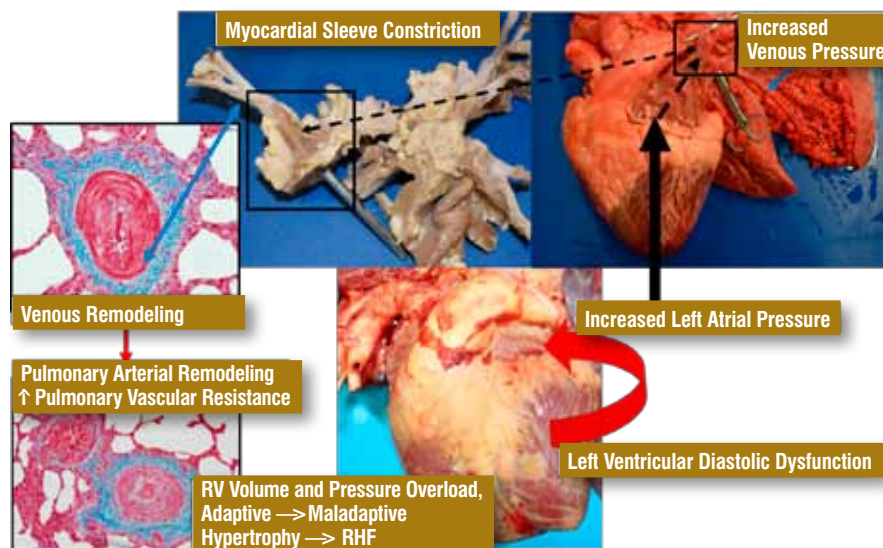
PATHOGENESIS OF PVH IN FATTENED CATTLE

■ Pulmonary arterial wedge pressure (PAWP) exceeded 15 mm Hg in all steers, but was greatest in the hypertensive steers. Hypoxemic stress elicited a robust hyperventilatory response resulting in hypocapnia and compensatory metabolic alkalosis. Elevated O_2 extraction fraction and arterial-venous difference in select steers implied either increased tissue demand for O_2 or decreased O_2 delivery due to reduced cardiac output.

■ Beef cattle exposed to chronic hypoxic environments exhibit PH selectively associated with pre-capillary PH, pulmonary arterial, and right heart remodeling. In contrast, fattened beef cattle, even in the absence of environmental hypoxia, exhibit features of PH with LHD.

■ LV fibrosis was measurably increased in the feedyard cattle succumbing to right heart failure compared to cattle dying from high mountain disease, and significantly greater than the hypertensive study animals.

LV fibrosis and pulmonary venous remodeling we observed implies LV stiffening and diastolic dysfunction cardiac fibrosis. The degree of LV fibrosis was similar to beef cattle experiencing right heart failure



associated with chronic environmental hypoxia. Normotensive animals had minimal fibrous connective tissue in the interstitium.

Similar to the case in obese humans, adverse interactions between metabolic dysfunction and inflammation may play a role in the pathogenesis and disease progression of pulmonary vascular remodeling, pulmonary hypertension and heart failure. These adverse interactions may predispose susceptible individuals to comorbidities including bovine respiratory disease, and worsened disease outcomes. ▲



CSU Western Slope Veterinary Diagnostic Laboratory Director Raye Walck and Laboratory Technician Martina Svetlik conducted a series of outreach efforts to local public-school students in April in May. Presentations included an information booth at Science Night at nearby



Cedaredge Middle School offering hands-on anatomical specimens and parasites, along with information on backyard poultry biosecurity. At right, Svetlik presents heart and lung anatomy and bacteriology to members of the Cedaredge science classes.


Lab Updates


Answers to Seven Common Questions about our New Invoicing

— Janice Inman Leflet, CSU VDL Business Office

In order to provide better customer service and to more efficiently manage the CSU VDL's accounts-receivable process, the VDL is bringing our process for invoicing and managing customers' accounts receivables in-house. We sincerely hope the changes will make it easier and more efficient for you, as well as our staff.


Here are some answers to the common questions we've received about the new invoicing system:


 **Who?** Your statements will now come from the Diagnostic Lab, instead of from the central Colorado State University office, as they used to. Please direct questions directly to the VDL at dlab@colostate.edu, or reach out to Janice Inman Leflet at (970) 297-5061 or janice.inman@colostate.edu if you have any questions or concerns.


 **Where?** All payments should now be sent to the VDL rather than to the university's central office. Your new remittance address is:


Veterinary Diagnostic Laboratory
Attn: Accounts Receivable
300 West Drake Bldg C
1644 Campus Delivery
Fort Collins, CO 80523-1644


Sending payments straight to the lab will allow us to track and post payments against invoices and provide you with a more accurate representation of invoices paid vs. invoices outstanding.

 **What?** To best serve you and to make this implementation successful, we need you to include with your payment a list of invoices the payment covers. If you pay by check, a list of invoice numbers is essential. If you pay via ACH, please send us an e-mail with the date, amount of payment and invoices included in the payment. For credit card payments via phone or in person, we will ask for the invoice information when payment is made.

 **How much?** Customers are expected to pay accounts in full by the date listed on the invoice/statement. Any balance outstanding after the payment due date will be assessed a 1.5% late payment charge on the unpaid balance. The 1.5% late payment charge will be assessed on the principal balance each month that the balance goes unpaid.

 **How?** Currently we are able to accept payment in person or via a phone call. Credit cards will, in the future, be accepted for payment on line. When payment on line becomes available; information on submitting payment by credit card will be included with the monthly invoice.

 **When?** These changes took effect July 1, but there will be a time of transition in which you will receive two statements—one from CSU central accounting for the outstanding charges on the current account that were processed under the old system and one from the VDL for invoices that were processed for the new charges on the new system. Please send payment directly to the VDL and we will determine if the payment is for old or new balances and get your payment to the proper office. Invoices will be considered due upon receipt.

 **Why?** This change to in-house billing will allow the VDL to communicate directly with customers rather than having an intermediary in the form of the CSU central office, who may not have enough information to properly answer questions about invoice amounts. As always, our goal is to provide the best service possible to VDL customers. We hope these changes will help us best serve you. ▲

Please reach out to Janice Inman Leflet at (970) 297-5061 or VDL_AR@colostate.edu with any questions or concerns.

GET YOUR RESULTS ANY WAY YOU WANT THEM

Our lab's web-based improvements have made it easier than ever to get your test results where, when, and how you want. Let us know how you wish to receive your results. Call (970) 297-1281 or e-mail us at dlab@colostate.edu to tell us your preference:

- Online, via password-protected portal on our website. Create your own personal account and view results any time, 24/7.
- Hard copy, mailed to you.
- Faxed hard copy.
- Electronic file delivered by e-mail.

Annual Diagnostic Summaries

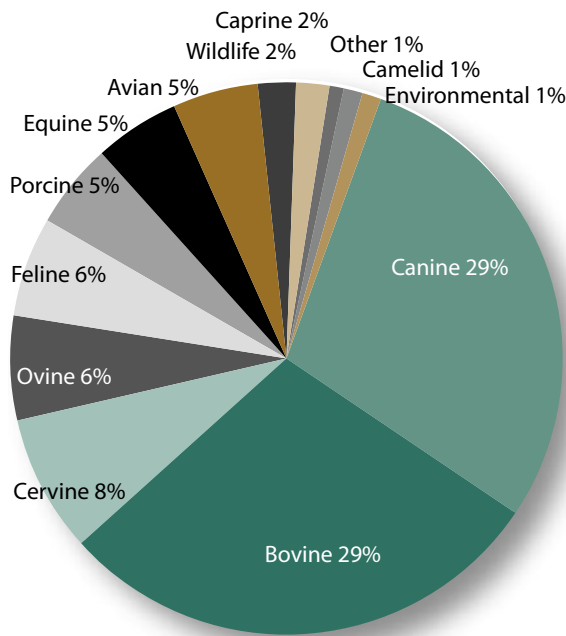
Annual Diagnostic Lab Report Statistical Snapshot

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

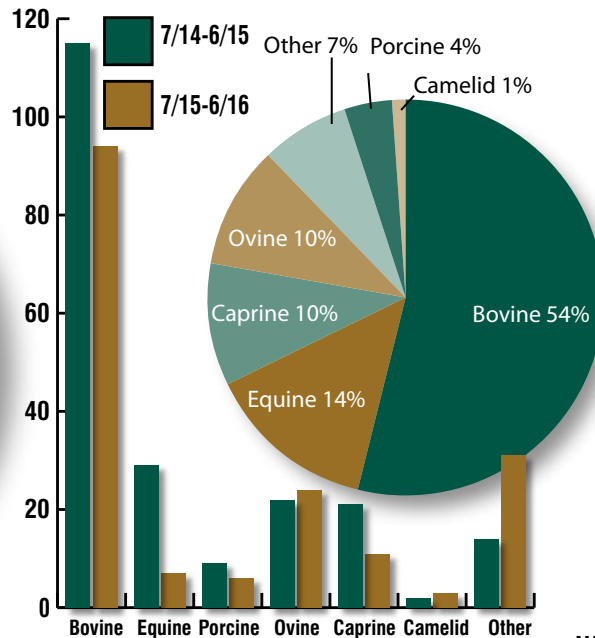
— Barb Powers, DVM, PhD, DACVP, CSUVDL Director

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

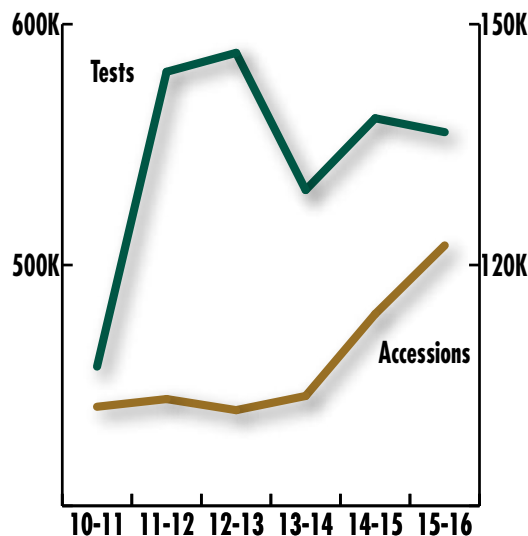
FY 2016 TOTAL SPECIES TESTING BREAKDOWN



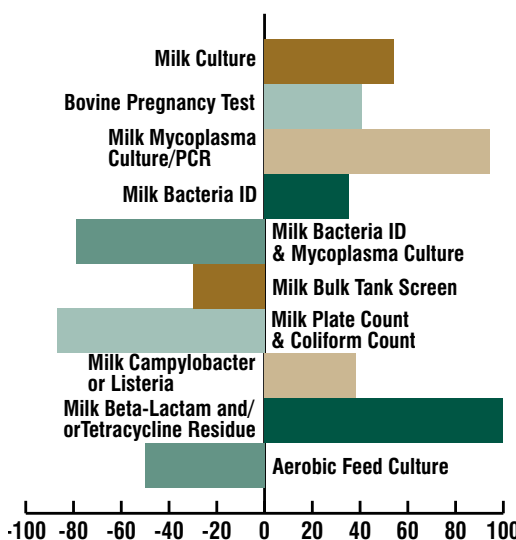
ABORTION SCREENS



ACCESSIONS AND TESTS PERFORMED



MASTITIS TESTING: PERCENT CHANGE



WANT EVEN MORE?

Review our entire 49-page 2016 Annual Report, under the "Regulations & Resources" tab at www.dlab.colostate.edu

COLORADO STATE UNIVERSITY
VETERINARY DIAGNOSTIC LABORATORIES
ANNUAL REPORT 2016



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CSU VDL in the Field: Case Studies

Bovine Abortion Due to Heart Failure Resulting from Selenosis

In spring 2016, a rancher in central eastern Colorado submitted a near-term, stillborn fetus for necropsy. It was the third stillborn fetus within a week in a group of second-calf heifers. The heifers had been commingled with a group of mature cows two months previously. None of the mature cows aborted.

The carcass was in good condition and exhibited significant lesions. Extensive ecchymosis was present on the heart and thymus. The heart was round and had a rough, irregular texture. Circumscribed areas of pallor were evident on the cut surface. Extensive edema was present in the lungs and mesentery. The liver was large with round edges and had a nutmeg appearance.

Histopathology revealed severe myocardial degeneration, necrosis and mineralization with multifocal fibrosis. The liver was chronically congested, evidenced by centrilobular hepatocellular necrosis with fibrosis. Thymic and intramyelinic hemorrhage within the brain was observed. Renal tubular degeneration and interlobular edema were additional findings.

Heart failure was the cause of the fetal death. Vitamin E/selenium deficiency, ionophore toxicosis and exposure of the dam to cardiotoxic plants were included in the differential diagnosis.

Fluorescent antibody analyses of the lung, liver and spleen were negative for BVD and IBR. The ocular fluid was negative for nitrate. Mineral analysis of the fetal liver for vitamins A and E, copper and selenium revealed 10.02 ppm vitamin E (reference range 7.00-20.00 ppm) and 526.44 ppm copper (reference range 150-650 ppm). The liver contained 6.65 ppm vitamin A (reference range - 8.00 -40 ppm) and 42.34 ppm selenium (reference range 1.10-5.90 ppm).

This presence of greater than seven times the upper end of normal selenium in the liver, coupled with the pathologic lesions, support selenium toxicosis as the cause of heart failure. According to the owner, these cattle had not received any supplemental selenium other than what is normally present in range mineral supplements. The heifers grazed a pasture that contained significant stands of locoweed before they were commingled with the mature cows. Abortions/stillbirths occurred only in the heifers.

SEASONALITY OF SELENIUM EXPOSURE

This past spring, the rancher presented two fetuses for postmortem examinations. They were from first-calf heifers that had been managed similarly to the

— Gene Niles, DVM, Director, CSU VDL Rocky Ford Branch; and Chad Frank, DVM, VDL Pathologist

heifers from the previous year. The only significant findings were elevated liver selenium levels (7.6 and 16.35 ppm dw).

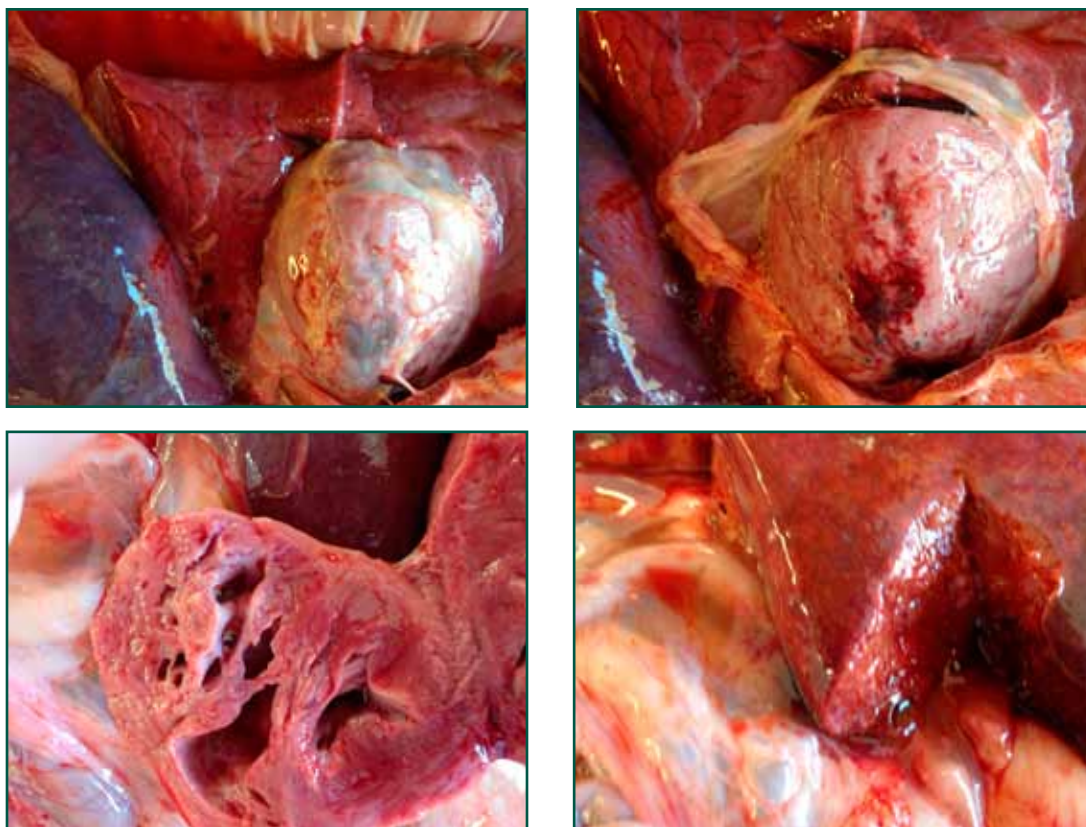
Toxic Plants of North America authored by Burrows and Tyrl lists nineteen species of *Astragalus* as selenium accumulators. *Astragalus* sp and other accumulator plants are generally unpalatable but can act as converter or translocator plants. These plants translocate selenium from deeper soil levels into the root of zone of neighboring forages and cereal grains leading to levels of 5-10 ppm selenium the palatable plants adjacent to the converter plants. Plant levels of 5 ppm selenium are considered significant. Consumption of palatable forages containing 5 -20 ppm selenium is considered the major source of selenium when selenosis does occur. Selenium levels peak just before flowering and decline as the plant matures, but selenium can significantly increase during rapid new growth periods.

SELENIUM ACCUMULATORS

Although the specific species of locoweed was not identified in this case, a neighboring rancher with a similar history of abortions and clinical selenosis in a group of first calf heifers identified two-grooved milkvetch as the predominate species of locoweed in his pastures. More palatable than other *Astragalus* sp., two-grooved milkvetch translocates selenium and can accumulate swainsonine with resulting locoism. In 1935, a USDA researcher considered *A. bisulcatus* the most important toxic plant in Wyoming.

Selenium accumulates in fetal tissues and can reach levels up to 2-4 times greater than in the dam. Analysis of liver tissue is the best indicator of selenium status. In cattle, whole blood selenium values are up to 3.33 times greater than serum values and are better indicators of long term selenium status, whereas serum samples give a better indication of current status. Hair and hoof wall also are used to confirm selenium toxicosis and differentiate between acute and chronic selenosis.

Selenosis can occur in all species of livestock and wildlife. Raisbeck lists the susceptibility to natural sources of selenium as: swine >horse> cattle> sheep. Sudden death is common in all species with misapplication or misformulation of injectable and oral preparations of selenium supplements. ▲



Necropsy of a near-term, stillborn fetus from a group of heifers suspected of selenosis demonstrated (clockwise, from top left):

- Pulmonary edema and passive hepatic congestion
- Myocardia Hemorrhage

- Myocardial Necrosis
- Passive congestion of the liver

Advances in Wildlife Diagnostics

Are Fecal Floats Reliable for Pinnipeds?

It is unclear whether recent efforts to optimize veterinary fecal flotation to detect helminth eggs in domestic species are applicable to marine parasites. To test the hypothesis whether helminth eggs of pinnipeds would have different specific gravities than those of terrestrial domestic mammals, we collected fecal samples from 27 live or necropsied pinnipeds that stranded and were brought to The Marine Mammal Center, in Sausalito, Calif., in spring and summer 2015.

We carried out modified double centrifugal flotations on 1-gram fecal samples and then examined the sediments. Sugar-gradient modified centrifugal flotations were performed on ten samples to determine the specific gravity of helminth eggs.

Results showed trematode, ascarid, and cestode eggs were detected in 14 of 27 (52%), 10 of 27 (37%) and four of 27 (15%) individuals, respectively. Trematode eggs per gram ranged from one to too numerous to count; whereas, ascarid counts ranged from three to 482 eggs per gram. Because trematode egg

— *Jacob Rodgers, CSU CVMBBS DVM Student; Paula Schaffer, DVM, PhD, CSU VDL Pathologist; Cara Field, DVM, PhD, The Marine Mammal Center; Lora Ballweber, DVM, MS, CSU Clinical Parasitologist; and Ashley McGrew, DVM, PhD, CSU VDL Parasitology Section Head*

counts were too numerous and cestode eggs are not typically quantified, only the ascarid egg counts were analyzed statistically.

We found that while ascarid eggs typically had a specific gravity of 1.00 to 1.15, the ascarid eggs from two of seven (29%) individuals had a broader range, extending from 1.05 to 1.27. Trematode eggs consistently had a high specific gravity of 1.15 to 1.27, and four of nine (44%) individuals had a specific gravity ranging from 1.05 to 1.27. We concluded the specific gravity of trematodes and ascarids is similar to that seen in terrestrial hosts, and a flotation media with a specific gravity greater than 1.25 may be most appropriate for use in marine species. ▲

**Second Place—Undergrads/
Vet Students/Masters Students
International Association for
Aquatic Animal Medicine,
2017 Conference Presentation:
Jacob Rodgers, Veterinary
Student, Colorado State
University, Optimization of
Diagnostic Approaches in
Marine Parasitology and
Specific Gravity Determination
of Helminth Eggs in Pinnipeds**

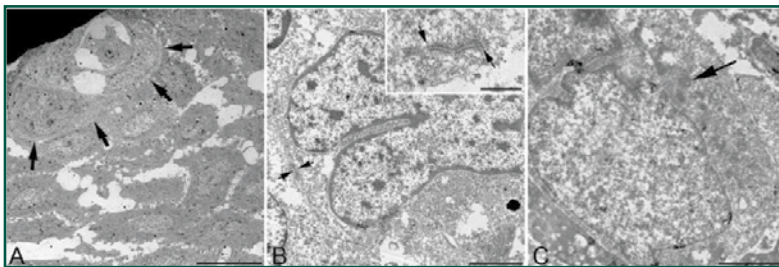
CSU VDL In Press

A Roundup of VDL Faculty Research

ATYPICAL ACANTHOMATOUS AMELOBLASTOMA

Malmberg JL, Howerth EW, Powers BE, Schaffer PA. Acanthomatous ameloblastoma with atypical foci in five dogs. *J Vet Diagn Invest.* 2017 Mar;29(2):154-159. doi: 10.1177/1040638716682565.

VDL Pathologist Paula Schaffer, Pathology Resident Jennifer Malmberg and Director Barb Powers report on five cases of variant acanthomatous ameloblastoma with atypical foci, identified from the case database of the CSU Veterinary Diagnostic Laboratory. Unlike



Unlike typical neoplastic epithelial cells, atypical cells (A, bounded by arrows) are round with less discrete borders. Atypical cells (B) have round, irregular or cleaved nuclei with clumped chromatin, and they contain occasional electron-dense cytoplasmic structures (C) consistent with secretory granules or lysosomes (arrow).

typical acanthomatous ameloblastoma—a common, locally invasive, nonmetastasizing tumor of the canine oral cavity which offers good long-term prognosis if complete excision can be achieved—these cases raised concern for poor clinical outcome due to the pleomorphic features of cells within atypical foci. Those atypical foci, or nests of large round-to-polygonal cells with modest amounts of cytoplasm and large nuclei with prominent nucleoli, presented unclear histogenesis. Based on histologic morphology, initial considerations included possible collision tumor between a typical acanthomatous ameloblastoma and melanosarcoma, other sarcoma or round cell tumors. However, the multifocal distribution was not typical for a collision event.

The atypical population was immunonegative for vimentin, melan A and PNL2, making melanocytic origin highly unlikely. The atypical cells were additionally immunonegative for CD18, CD3, and Pax5, making histiocytic or lymphocytic histogenesis unlikely. Although neuroendocrine origin was considered based on the ultrastructural presence of spherical electron-dense cytoplasmic structures, the atypical cells were immunonegative for chromogranin A and synaptophysin. The negative immunoreactivity for vimentin also made manifestation of epithelial-to-mesenchymal transition unsupported.

The five cases represented 0.3% of all histologic diagnoses of canine acanthomatous ameloblastomas diagnosed during the 10-year search period. Although the atypical nature raised concerns, with the exception of one patient that died of unrelated causes 20 months

following complete excision, all patients lived with no evidence of local recurrence or metastasis at least six to 30 months after diagnosis. These cases suggest complete excision alone by mandibulectomy or maxillectomy may be curative. In fact, two incompletely excised tumors failed to recur after six and 12 months, respectively, suggesting less aggressive oncologic behavior.

IS LEPTO SHEDDING A THREAT IN FERAL SWINE?

Pedersen K, Anderson TD, Bevins SN, Pabilonia KL, Whitley PN, Virchow DR, Gidlewski T. Evidence of leptospirosis in the kidneys and serum of feral swine (*Sus scrofa*) in the United States. *Epidemiol Infect.* 2017 Jan;145(1):87-94.

VDL Avian Section Head Kristy Pabilonia and Archivist Ted Anderson collaborated with APHIS wildlife specialists to continue gauging the incidence of *Leptospira* prevalence in wild swine, which now has been reported in 39 of 50 states. Lepto is just one of numerous viral and bacterial pathogens raising concern that zoonotic infection could occur as outdoor workers and recreationists grow more likely to contact feral swine and as reports of feral swine in golf courses, city parks, backyards and cemeteries expand.

To conduct this first-ever quantitative national study of the probability that feral swine could shed infectious leptospires, the study collected kidneys and paired serum when possible from 677 swine in 124 counties of 29 states. These counties had already been identified as antibody positive for six serovars of *Leptospira inter-* *rogans*.



Although exposure to the same six serovars continued to be high in the counties sampled, at 53%, results showed only 23, or 3% to 4%, of kidneys were positive. Corresponding sera from the feral swine with positive kidneys was antibody-positive for all animals from which there was serum available to test, and all of these tested positive for at least one serovar except for two. Prevalence based on kidneys was highest in Mississippi, at 26.4%. Of the antibody-positive samples, 47.1% were positive for multiple serovars. The most common were Bratislava, at 30.7%, Icterohaemorrhagiae, at 25.7%, Pomona, at 13.6%, Canicola, at 9.7%, Grippotyphosa, at 9.4%, and Hardjo, at 5%. Based on these results, it appears the zoonotic risk may be relatively low.

MORE ON BLUETONGUE OVERWINTERING

Mayo C, Mullens B, Gibbs EP, MacLachlan NJ. Overwintering of Bluetongue virus in temperate zones. *Vet Ital.* 2016 Sep 30;52(3-4):243-246. doi: 10.12834/VetIt.521.2473.3.

Virology Section Head Christie Mayo reviews her work on the mystery of how bluetongue virus overwinters and becomes infective to livestock in the warm months. (See *LabLines* 19[2]:2-3.) Her recent studies compared the population dynamics of *Culicoides sonorensis* midges, the major vector of Bluetongue in California, and seasonal reinfection patterns of sentinel dairy cattle. Bluetongue virus infection of parous female midges captured in traps set during daylight hours was detected during the interseasonal period of virus activity; whereas, no concurrent active infection of sentinel cattle during the overwintering period was found. The finding of BTV-infected vector midges



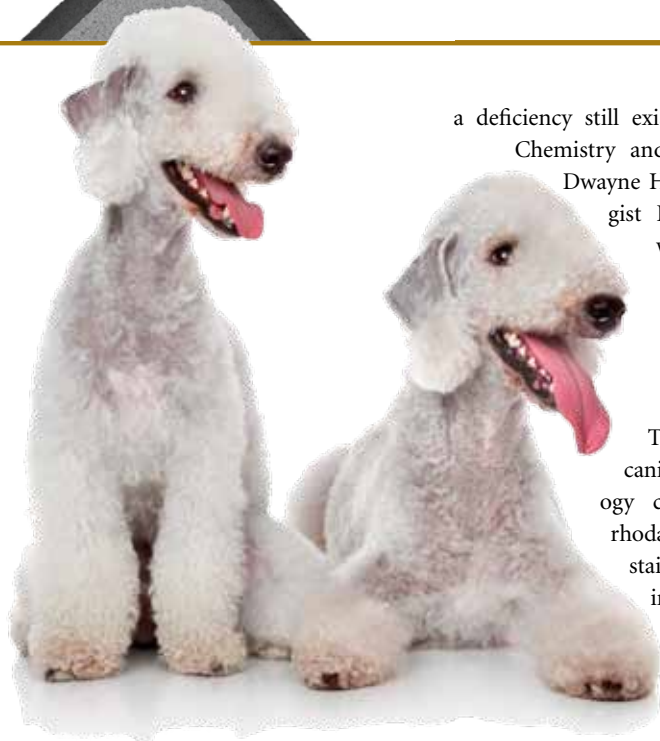
during mid-winter suggests bluetongue can overwinter in Northern California by infection of long-lived female midges that were infected during the prior seasonal period of virus transmission and which, then, entered a quiescence in the fall but re-emerge sporadically during the overwintering period. In addition to defining the mechanism of BTV overwintering, the studies Mayo reviewed in this article also provide precise documentation of temporal changes in the annual abundance, dispersal and dynamics of the virus. The findings are critical to accurately predicting and abating bluetongue livestock infection.

RHODANINE-STAINING FOR HEPATIC COPPER?

Moore AR, Coffey E, Hamar D. Diagnostic accuracy of Wright-Giemsa and rhodanine stain protocols for detection and semi-quantitative grading of copper in canine liver aspirates. *Vet Clin Pathol.* 2016 Dec;45(4):689-697. doi: 10.1111/vcp.12401

Although a cytologic copper grading system using rubeanic acid-stained, fine-needle hepatic aspirates has been shown to draw acceptable correlation between histologic and cytologic findings to diagnose primary copper hepatopathy, this study's authors believed

PROPOSED MECHANISM	SCIENTIFIC EVIDENCE TO DATE
Transovarial transmission of BTV in vector insects	Unproven definitively for BTV with any <i>Culicoides</i> species in either field or laboratory studies. We found no evidence of vertical transmission of BTV to the progeny of <i>C. sonorensis</i> infected with BTV in the laboratory or in larvae, male or nulliparous female <i>C. sonorensis</i> collected in the field.
Complex cycle with unidentified intermediate host	Unlikely in California because <i>C. sonorensis</i> feed principally on ruminants. Blood meal analysis of <i>C. sonorensis</i> midges collected during these studies confirmed that these vectors fed only on cattle and black-tailed deer (unpublished data).
Prolonged infection of ruminant livestock	Highly unlikely as multiple recent studies confirm viremia in BTV-infected livestock is transient, and the duration is considerably less than the approximately 6-month overwintering period. Viremia duration in livestock infectious to vector midges is typically <60 days.
Prolonged survival of adult <i>Culicoides</i> insects	Plausible in temperate regions, as female midges are infected for life but become infectious only after a temperature-dependent extrinsic incubation. Much remains to be learned regarding impact of temperatures at potential refugia where adult midges may naturally rest during interseasonal period.
Ongoing low-level infection cycle among cattle and midges	Potentially plausible explanation for the overwintering of BTV; however, sentinel cattle at our study sites never became infected during the interseasonal overwintering period.



a deficiency still existed in the literature. VDL Chemistry and Toxicology Section Head Dwayne Hamar and Clinical Pathologist Russell Moore collaborated with CSU colleagues to retrospectively study the diagnostic accuracy of routine WG-stained, hepatic fine needle aspirates for increased hepatic copper. They rhodanine stained 40 canine hepatic WG-stained cytology cases using Wright–Giemsa rhodanine staining. A rhodanine-stained cytologic copper grading system was developed. Prospectively, 67 canine liver samples with quantitative copper measurement, a WG-then rhodanine-stained slide, and a non-WG rhodanine-stained slide were used to assess the performance of the grading system and the effect of previous WG staining. Copper was not described in 40 retrospective cases on initial cytologic evaluation; eight of 40 cases had increased copper content after rhodanine staining or quantitative copper assessment.

Prior WG staining and destaining significantly affected the cytologic copper grade but not the diagnostic performance as measured by receiver-operating characteristic curve analysis. Quantitative copper concentration and previous WG-stained copper grade were moderately correlated. For detection of ≥ 600 ppm of dry-weight copper, sensitivity was 0.75 and specificity was 0.97. For detection of ≥ 1500 ppm, sensitivity was 1.0 and specificity was 0.97.

Their study concludes Wright–Giemsa staining alone does not reliably detect hepatic copper, but grading of rhodanine-stained canine hepatic cytologic samples demonstrates acceptable diagnostic performance for detection of copper content.

DIAGNOSTIC PICTURE OF ANAL SAC MELANOMA

Vinayak A, Frank CB, Gardiner DW, Thieman-Mankin KM, Worley DR. Malignant anal sac melanoma in dogs: eleven cases (2000 to 2015). *J Small Anim Pract.* 2017 Apr;58(4):231-237. doi: 10.1111/jsap.12637.

VDL Pathologist Chad Frank represented the CSU lab in this five-laboratory study of the signalment, clinical signs, staging, cytology, histopathologic analysis, and surgical and nonsurgical treatment of 11 dogs with malignant anal sac melanoma from 2000 to 2015. This first-known report to describe the complete clinical progression of MASM with specifics on origin, clinical signs, tumor size, diagnostics, histopathological findings, time to local recurrence, response and survival

times with different treatment modalities found MASM associated with poor survival times.

Bloody discharge from the anal sac is the most common presenting complaint. The disease is characterized by a high mitotic index, high rate of local recurrence with surgery, regional lymphatic metastasis, and short progression-free survival and overall survival. Dogs that had complete histologic margins following surgery had longer periods of clinical remission and progression-free survival. Mortality of dogs in this study was due to euthanasia from disease progression regardless of treatment. Only one dog survived more than a year after diagnosis.



Penile melanoma metastasis in a dog that presented for stranguria. Diffuse blue-black discoloration not apparent during initial consultation was noted during attempted urinary catheterisation.

NEOPLASTIC DISEASE SURVEY IN DONKEYS

Davis CR, Valentine BA, Gordon E, McDonough SP, Schaffer PA, Allen AL, Pesavento P. Neoplasia in 125 donkeys (*Equus asinus*): literature review and a survey of five veterinary schools in the United States and Canada. *J Vet Diagn Invest.* 2016 Nov;28(6):662-670.

CSU VDL Pathologist Paula Schaffer contributed to this five-laboratory investigation of neoplastic diseases in donkeys. Although generally treated as horses in clinical settings, donkeys have unique characteristics that make them of physiological and medical interest. Therefore, the lack of a review of neoplastic disease in this species represented a hole in the literature.

Their study reviewed autopsy and biopsy records for all 357 donkey and burro accessions from five U.S. and Canadian veterinary schools. A total of 126 tumors was diagnosed in 125 donkeys for a neoplasia prevalence of 35% in this survey population. The mean age of donkeys with tumors was 13 years, with a range of under 1 to 30 years. The survey demonstrated commonalities as well as differences in neoplastic diseases between donkeys and horses:

- Equine sarcoid, the most common tumor, represented 72% of overall tumors, which compares to the highest reported percentage of sarcoids in horses of 44%. The high relative prevalence could be attributed to the absence of SCC and melanoma, both common skin tumors in horses.
- Sarcoids diagnosed in males outnumbered females by a factor of 1.8 to 1, with the vast majority of lesions in males occurring in the inguinal region.
- Soft-tissue sarcomas were the second most common



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skin tumor. Although donkeys and mules are considered 2.5 times more likely to develop soft-tissue sarcomas than any breed of horse, this study suggests some reported soft-tissue sarcomas and fibromas are actually sarcoids lacking the distinctive epidermal component.

- Melanocytomas were uncommon in this data set, matching reports in the literature, and were not observed in the typical locations for gray horse melanomas.
- Other reported tumors in this study included peripheral nerve sheath tumor and gastrointestinal stromal tumor.
- Lymphosarcoma, the most common malignant tumor in horses, is very rarely reported in donkeys.

SALMONELLA PREVALENCE IN DOGS AND CATS

Reimschuessel R, Grabenstein M, Guag J, et al. Multi-Laboratory survey to evaluate *Salmonella* prevalence in diarrheic and non-diarrheic dogs and cats in the USA between 2012 and 2014. *J Clin Microbiol.* 2017 Feb 15. pii: JCM.02137-16. doi: 10.1128/JCM.02137-16.

VDL Assistant Director Kristy Pabilonia represented CSU's VDL among 10 other collaborating labs working to determine the periodic prevalence of *Salmonella* in a population of dogs and cats in the United States visiting veterinary clinics. The studied 2,965 fecal samples solicited from 11 geographically dispersed veterinary testing laboratories across 36 states between January 2012 and April 2014 using a harmonized method. Findings include:

- The overall study prevalence of *Salmonella* in cats, at three in 542, was 1%. The prevalence in dogs, at 60 out of 2,422, was 2.5%.
- Diarrhea was present in only 55% of positive dogs; however, 3.8% of all diarrheic dogs were positive, compared with 1.8% of the nondiarrheic dogs.
- *Salmonella*-positive dogs were significantly more likely to have consumed raw food, to have consumed

probiotics, or to have been given antibiotics. Rural dogs were also more likely to be positive than urban or suburban dogs.

- In the 67 isolates, 27 unique serovars were identified, with three dogs having two serovars present. Antimicrobial susceptibility testing of 66 isolates revealed that only four of the isolates were resistant to one or more antibiotics. Additional characterization of the 66 isolates was done using pulsed-field gel electrophoresis and whole-genome sequencing (WGS). Sequence data compared well to resistance phenotypic data and were submitted to the National Center for Biotechnology Information (NCBI). This study suggests an overall decline in prevalence of *Salmonella*-positive dogs and cats over the last decades and identifies consumption of raw food as a major risk factor for *Salmonella* infection. Of note is that almost half of the *Salmonella*-positive animals were clinically nondiarrheic. ▲



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Get to Know the Laboratory

New Members Join the Lab Team

Laurie Stutz, no stranger to CSU, feels back at home in sample receiving support. While studying agricultural production, she worked at Ridgen Farm feedlot and swine operation and managed the calf operation at the university dairy for several years. After a 25-year hiatus, raising a family and working for an energy education company, Laura has returned to CSU. A Larimer County 4-H leader, she also milks and shows a small herd of Nigerian Dwarf dairy goats.

Brittany Ellis grew up in northern Colorado, where she and two brothers were raised in the rodeo world. After competing in rodeo and participating in 4-H through childhood she continued to compete through college and now at the amateur level. A former hourly VDL staff member, she returns as lab support.

Alysia Cozza, new sample receiving support, started to work on cattle and horse farms at a young age in her native Florida, where she found the love of horse riding. After competing at a young age in barrels and hunter/jumpers, she went on to train horses for jumpers and also went to Edison State College where she received an

associates degree in pre-vet. She has worked at Veterinary Clinics since age 16. In 2011 she received a bachelor's degree in equine science and a minor in business administration from CSU. She hopes to pursue a master's degree in equine sciences.

Carolyn Laughlin, new lab support, grew up in Southern California. Becoming a veterinarian was one of her goals since childhood. She graduated from CSU in 1990 with a bachelor's degree in biology. After graduating, family took priority for many years. Her three children are still a huge part of her life today. She enjoys traveling, volunteering, going to movies, scrapbooking and music.

Shelby Miller, new main-office administrative assistant, is a Fort Collins native who graduated from CSU two years ago and comes to the VDL from a supervisory position at Kohl's Department stores.

Krista Kirkpatrick, new main-office administrative assistant, was raised in Colorado, attended school at CSU and first started at the VDL as a student. After getting a degree in psychology, Krista continued working in the office and advanced to administrative assistant.

From left:
Laurie Stutz
Brittany Ellis
Alysia Cozza
Carolyn Laughlin
Shelby Miller
Krista Kirkpatrick



CSU College of Veterinary Medicine and Biomedical Sciences Dean Mark Stetter, Assistant VDL Director Kristy Pabilonia and VDL Director Barb Powers met with Colorado Commissioner of Agriculture Don Brown and State Veterinarian Keith Rohr in late March following the commissioner's signing of the National Animal Health Laboratory Network agreement between CSU and USDA. As one of only 11 level 1 NAHLN labs nationwide, VDL is critical in surveillance for early detection of foreign animal disease, surge testing during an outbreak, and testing samples during an outbreak recovery phase.



Lab Updates

New LIMS System Coming Soon

CSU's Veterinary Diagnostic Labs is now in phase two of a three-phase process to replace our laboratory's computer system. The new U.S. Animal Laboratory Information Management System is a comprehensive laboratory information management system designed to fit the needs of all lab staff and clients.

Our project team has worked closely with VDL section heads and subject-matter experts to streamline the accession workflow and then determine essential configuration of the new system environment. The result will be simple, intuitive online navigation that will allow users to transfer, manage and process accessions with ease. Once we complete phase three and go live, the system will offer several seamless benefits:

- A redesigned and improved web portal for you to view reports and invoices online.
- System-wide advanced search capabilities that will provide robust data management tools for our laboratories.
- Integration with CSU's Veterinary Teaching Hospital applications, our new VDL accounts-receivable system and specialized lab equipment.

— Darci Hathaway CSU College of Veterinary Medicine & Biomedical Sciences IT Services Project Manager

- Continued safe and secure technology that permits access to data across systems without sacrificing privacy.

- For our VDL staff, a new internal website for access to custom reports and statistics.

The final phase of this complex integration of the new system will include training for all personnel, development of detailed user guides, client testing and then final live release and follow-up. On behalf of the lab and the project team, I'd like to extend my thanks for the valuable involvement of all stakeholders, and I welcome any comments or suggestions you have for us as we launch our new system. ▲



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

New Western Slope Lab Director **Raye Walck** attended the CSU Annual Conference in April, along with **National Veterinary Services Laboratories** training course in **Equine Infectious Anemia**. She has been busy conducting outreach representing CSU VDL at a local middle school science night with anatomical specimens, parasites, and backyard poultry biosecurity information, as well as presenting heart and lung anatomy and bacteriology to youth science classes. She plans to conduct various veterinary clinic visits and host Colorado Mesa University/Western Colorado Community College class visits and tours of the lab. If you're interested in meeting with her, contact her at the Western Slope Lab.

In June, **Walck**, along with Rocky Ford Lab Director **Gene Niles** and VDL Lab Coordinator **Charlie Davis** attended the **Colorado Cattlemens Association** meeting in Grand Junction. **Davis** also attended the **99th Colorado Wool Growers Convention**, July 12 and 13 in Montrose. Look for **Niles** to be in attendance at **Academy of Veterinary Consultants** meetings in August in Denver and in Kansas City in December.

VDL Avian Diagnostics and BSL3 Operations Section Head **Kristy Pabilonia** will be at **American Association of**

Veterinary Laboratory Diagnosticians Accreditation and Executive committee meetings Aug. 8 through 10 in Denver.

Plan now to meet with **Pabilonia** and **Walck**, along with VDL Director **Barb Powers**, Virology Section Head **Christie Mayo**, Chemistry and Toxicology Section Head **Dwayne Hamar** and other VDL faculty at this year's annual meeting of the **American Association of Veterinary Laboratory Diagnosticians**, Oct. 12 to 18 in San Diego.

VDL Pathologist **Terry Spraker** will be on hand at the 22nd Biennial Conference of the **Society for Marine Mammologists**, Oct. 22 through 27 in Halifax, Nova Scotia.

VDL Pathologist **Sushan Han** will be presenting cases of Lobomycosis in the Atlantic bottlenose dolphin at this year's 49th annual conference of the **American Association of Zoo Veterinarians**, Sept. 23 through 29 in Dallas.

Drop by our booth at the **Colorado Veterinary Medical Association Convention**, Sept. 21 through 24 in Loveland, and give your feedback to lab faculty, including Director **Powers**, Section Head **Pabilonia**, Coordinator **Davis**, Virology Section Head **Christie Mayo** and Bacteriology Section Head **Josh Daniels**.

LAB LINES

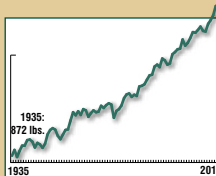
INSIDE THIS ISSUE

- LAB UPDATES p. 6
Answers about invoicing.
- WILDLIFE DIAGNOSIS p. 9
Fecal floats for pinnipeds.
- LAB UPDATES p. 14
New team additions.
- ON THE ROAD p. 15
Where and when you can meet our faculty.

BACTI SECTION HEAD p. 1
New VDL Bacteriology Section Head Josh Daniels hopes to push the boundaries.



PROTEIN DIAGNOSTICS . . . p. 2
How VDL's protein electrophoresis can pinpoint diagnostics.



FEEDING TO DEATH? p. 4
Is excessive weight gain in feedlot cattle causing cardiac remodeling?

SELENIUM TOXICOSIS . . . p. 8
In this latest VDL case study, Gene Niles looks at heart failure and selenium.



VDL'S LATEST RESEARCH p. 12
From feral swine to salmonellosis, the latest studies from VDL faculty members.



NEW LIMS SYSTEM p. 15
A preview of our state-of-the-information system.

Update from the Director

**TO CONTACT
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DLAB@colostate.edu

Welcome to this issue of *LabLines*. It is amazing that the summer is reaching its end already; time has really flown by this spring and summer. We are thrilled to have our new Bacteriology Section head on board, Josh Daniels, who comes to us after serving for the past nine years as director of the Clinical Microbiology Laboratory at The Ohio State University College of Veterinary Medicine. You'll find more about his background and his plans for the section on the cover of this issue of *LabLines*. We expect great improvements to our Bacteriology Section under his guidance.



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

Also inside this issue, look for articles that detail some of our latest research involving cattle with cardiac issues that may be linked to the trend to grow them to heavier and heavier weights, as well as work on selenium toxicity. In addition, you'll also find several of the most recent research briefs from our faculty, students and staff members.

Earlier this year, we completed our Annual Report, and you can view some graphical snapshots and highlights inside this issue. But I would encourage you not to stop there; please go online if you would like to see a complete assessment of our last fiscal and calendar year. We have

had an increase in accessions, as you will see from the Annual Report. That means we are as busy as ever, working to meet our mission of providing timely, accurate and pertinent animal disease diagnostic services and educational outreach.

To help us streamline that huge mission, some new innovations are occurring in our computerized system. For the first time in a long time, we are now taking over our own billing system, which has been an issue for many years. In October, we will release a new computer system, so you will see a change in your reports. Look for details in the next issue of *LabLines*.

Finally, of great importance, is that we renewed our agreement with USDA and the Colorado Department of Agriculture to remain a core laboratory in the National Animal Health Laboratory Network. We do surveillance for foreign animal diseases and emerging diseases and convey any reportable diseases.

We hope to see many of you at the Colorado Veterinary Medical Association Meeting in September and at the American Association of Veterinary Laboratory Diagnosticians' meeting in October.

Barbara E. Powers