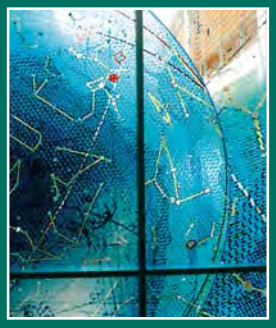
Colorado State University VETERINARY DIAGNOSTIC LABORATORIES

LAB LINES

Diagnostic news and trends from the Colorado State University Veterinary Diagnostic Laboratories
Volume 14, Number 1 Spring/Summer 2009



PLAN TO JOIN US IN DEDICATING YOUR NEW FACILITY

Save the date! On Sept. 11 we will host our official opening ceremony, including tours of the new Diagnostic Medicine Center. Plan now to join us. Watch our web site and other correspondence for more details.

New Home for CSU Veterinary Diagnostic Lab

It's Finally Here!

It has been our dream for over 10 years, and now it's come true: The new home for the Veterinary Diagnostic Laboratory at CSU officially opened in June.

The 88,000-square-foot Diagnostic Medicine Center is sited immediately north of the James L. Voss Veterinary Teaching Hospital and is linked to the VTH by an enclosed corridor. The single, beautifully designed, spacious facility centralizes and expands the 15,000 square feet of space we formerly occupied in several sites scattered around the main campus, the teaching hospital and temporary "outbuildings" on South Campus.

By mid-June, we hope to be completely moved in. The move is planned to allow for minimal disruption in our daily services. This new facility will enable us to continue and enhance our service to animals of all species, animal industries, veterinarians, public health and food-supply protection. It will also expand our role in educating the next generation of veterinarians and laboratory diagnosticians, as well as contribute to advancements in veterinary laboratory diagnostics.

Please join us in expressing our appreciation to the State of Colorado and our supporters. See Page 2 for details.

Home at Last!

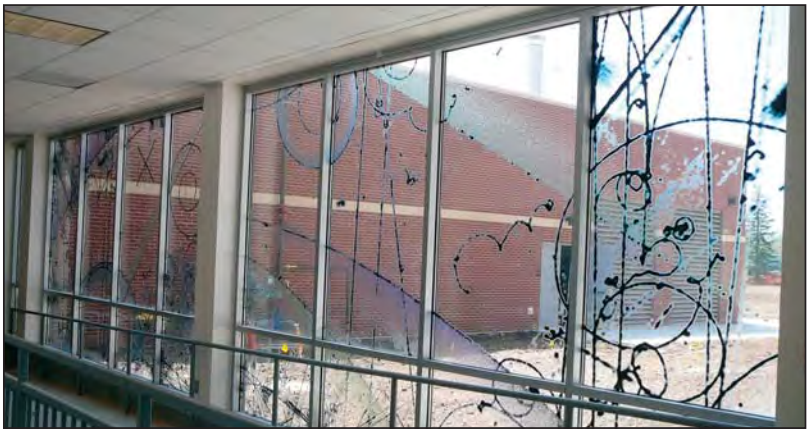
New Diagnostic Medicine Center

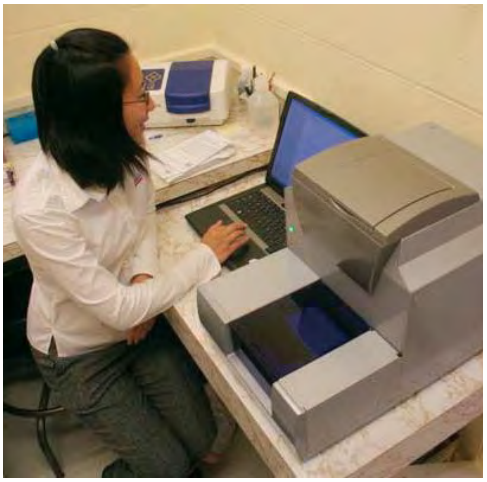
The new Diagnostic Medicine Center is a three-story, 88,000-square-foot facility scheduled for final completion in June. It is adorned with state-required mandatory artwork efficiently integrated into beautifully designed terrazzo floors and etchings on selected glass walls. A central atrium allows natural light in and encourages intellectual exchange and collaborations to occur in an otherwise busy laboratory environment.

Included in the building is 2,000 square feet of BSL3 space and 1,200 square feet of a high BSL2 necropsy facility, in addition to the 2,680 square feet of main necropsy floor. Specially designed flexible laboratory space for all laboratory sections from microbiology to chemistry to histology are on the three floors. The Veterinary Teaching Hospital Clinical Pathology and Animal Population Health Institute laboratories also have space in the building.

Please bear with us while we complete our move to the new facility, scheduled for mid-June. We have planned the task to provide as minimal interruption in our level of service as possible. For users coming to the laboratory, we have a main entrance but also a service entrance for your convenience.

To all our supporters who assisted us in making our needs known, and to the State of Colorado, which recognized those needs, we pledge this new facility will launch CSUVDL into a new era of service to animals, veterinarians, public health, education and industry. Thanks to all involved, and welcome home! ▲

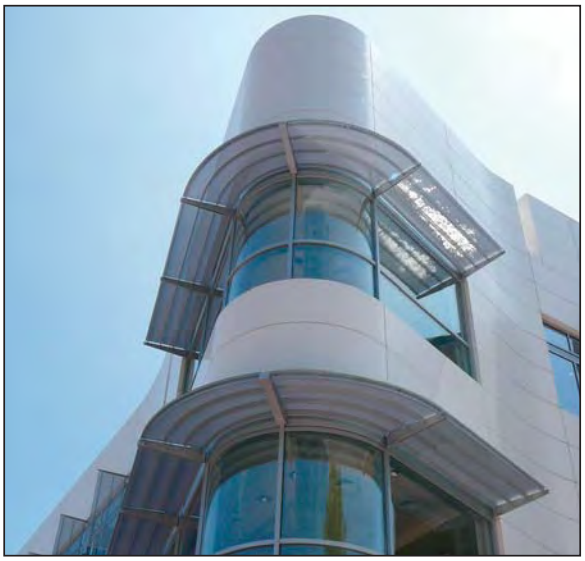




Joe Zemba, LaJunta Tribune-Democrat

Farm Credit of Southern Colorado, the Colorado Cattlemen's Association and the Colorado Livestock Association donated a QIAxcel multicapillary electrophoresis system to CSUVDL's Rocky Ford Diagnostic Laboratory. Designed to overcome the bottlenecks of gel electrophoresis, the fully automated system processes 96 samples per run, providing unrivaled resolution, speed and throughput in DNA and RNA analyses. It will be used primarily to automate and speed Bovine Virus Diarrhea and Tritrichomonas foetus testing at Rocky Ford. When coupled with other equipment received from Fort Collins, the Rocky Ford laboratory will now have a totally automated molecular diagnostics laboratory.

Qiagen representative Dorothy Mei demonstrates the new electrophoresis equipment donated to Rocky Ford by Farm Credit of Southern Colorado, Colorado Cattlemen's Association and Colorado Livestock Association.



Opposite page, clockwise from left: State-mandated artwork includes etchings on selected glass walls; main entrance (pictured) is supplemented with a service entrance; flexible lab space is supplemented with 2,080 square feet of necropsy space (not pictured); central atrium takes full advantage of natural light.

Above, clockwise from left: Three-story facility consolidates all CSUVDL sections into one center; open space and strategic use of glass encourage intellectual exchange; facility includes 2,000 square feet of BSL3 space; free-flowing curves and non-linear patterns repeated in the architectural elements mirror the biological forms of the natural world with which we deal daily.

Diagnostic Sample Quality Assurance

Packing Samples to Ensure Compliance with New Regulations

For training manuals, CDs and pamphlets, we recommend www.saftpak.com or www.iata.org. Any staff responsible for shipping dangerous goods should be trained by an approved program. Documentation should be kept on file. Please Note: It is the shipper's responsibility to ensure the package complies with all current regulations. Regulations change frequently. Shippers may be fined for violations of the transport regulations. Average fines exceed $1,200 for basic infractions.

Part 2 of our installment on diagnostic specimen shipping regulations helps you pack samples. To read Part 1, in which we discussed the different sample classifications and corresponding labeling, visit us on the web, at www.dlab.colostate.edu/webdocs/news/LablinesVol13-2.pdf

Regulations for transporting Class 6.2 Dangerous Goods have changed, and all packages containing diagnostic animal specimens transported by air should be packed to ensure compliance with the new rules.

CATEGORY A

Most clients will not ship Category A agents. However, please remember that all persons who ship category A agents should have documented training on this subject. For Category A specific packing instructions, please visit www.saftpak.com or www.iata.org.

CATEGORY B

Category B infectious substances must comply with Packing Instruction 650 and 49CFR 173.199. Packaging for samples falling into this category must consist of:

- An inner package that contains:
  - ☑ A watertight primary receptacle;
  - ☑ A watertight secondary receptacle; and,
  - ☑ Absorbent material placed between the primary and secondary receptacles; and,
- An outer, secondary packaging of sufficient strength to meet the testing standards.

No shipper's declaration is required when shipping Category B infectious substances; however, each shipment must include an itemized list of contents, placed between the secondary container and the outer packaging.

The outer package for Category B infectious substances must include:

- The proper shipping name (for PI 650, Biological substance, Category B), required only if the package contains an identified Hazardous Material;
- A UN No. 3373 diamond-on-point marking at least 50 mm by 50 mm and no larger than 100 mm by 100 mm;
- The volume in g/mL of the sample(s);
- The name and addresses of the shipper and consignee; and,
- The name and phone number of the person responsible for the shipment.

BIOLOGICAL SUBSTANCE CATEGORY B

— Christina Weller, CSUVDL Microbiologist, and Kristy Pabilonia, CSUVDL Assistant Professor and Avian Diagnostics/Select Agents Section Head

EXEMPT PATIENT SPECIMENS

Exempt Animal Specimen packaging must consist of:

- A leakproof primary receptacle(s), with absorbent material for liquid specimens;
- Leakproof secondary packaging; and,
- An outer packaging with at least one surface measuring 100 mm by 100 mm.

As with Category B infectious substance shipments, no shipper's declaration is required. The outer package must be labeled as "Exempt Animal Specimen." The label should be computer-printed and easy to read.

EMPTY PACKAGES

Per IATA 5.0.2.13.5.3, any package that has previously contained an infectious substance must be thoroughly disinfected or sterilized. Any labels or markings indicating that it contained an infectious substance must be removed or obliterated before it is reused or sent elsewhere.

DRY ICE

If dry ice is included in a shipment, additional guidelines must be followed and marking applied. Refer to www.saftpak.com or www.iata.org for these guidelines.

OVERPACKS

An "overpack" may be used to consolidate several smaller packages in a single box. All overpacks must display:

- Orientation arrows on two opposing sides, if not already printed on the overpack;
- The word "Overpack," which should be affixed as a label;
- The proper shipping name, UN number and technical name if shipping infectious substances;
- The total volume in g/mL of all the containers within the overpack,
- The dry ice proper shipping name and quantity, if applicable;
- The name and phone number of the person responsible for the shipment; and,
- Complete information on the shipper and consignee.

Each individual inner package may contain, but must not exceed, the maximum quantity allowed under the list of dangerous goods. ▲

Get to Know the Laboratory

Dr. Colleen Duncan, Anatomic Pathology

Colleen Duncan joined CSUVDL earlier this year as an assistant professor in anatomic pathology. Dr. Duncan obtained her DVM training and MSc degree at the University of Saskatchewan, where she collaborated on studies related to the emerging fungal pathogen, *Cryptococcus gattii*, in horses, wildlife, companion animals and humans on Vancouver Island. She relocated to Fort Collins to train in the PhD/anatomic pathology residency. At CSU she has continued to study epidemiology in conjunction with pathology, working on issues related to disease surveillance with emphasis on Bovine Viral Diarrhea Virus in wild cervid populations.

"CSUVDL's veterinarians and diagnosticians have diverse skillsets, and I'm excited for the opportunity to continue working with them," she says. "My personal interests in wildlife health and large animals, along with my combined training in population medicine and diagnostic pathology, can benefit our clients. Many pathologists are reductionists, with exceptional knowledge on cellular or subcellular changes that result in the observed pathology. But when I look at the animal on the necropsy floor, I wonder what's going on in the rest of the herd, and why? This population perspective can be helpful when designing diagnostic testing schemes and working with veterinarians on disease preventive protocols that are both logistically and financially feasible."▲

Contact Dr. Duncan at (970) 297-5422 or Colleen.Duncan@colostate.edu

Equine Bacteriology

Contagious Equine Metritis Testing

You may be experiencing an increase in client requests for information or testing of animals for contagious equine metritis (CEM). This increased interest is in response to animals that have tested positive for the organism *Taylorella equigenitalis* in the quarter horse industry. As *LabLines* went to press, 19 stallions and five mares had tested positive for the organism, according to the USDA Hot Issues website. Thus far, this has resulted in tracebacks to 904 additional horses exposed to the organism across 48 states.

— Doreene Hyatt, PhD, CSUVDL Bacteriology Section Head

CSUVDL is one of the 15 laboratories approved by the National Veterinary Services Laboratory (NVSL) to conduct CEM testing. The testing protocols vary depending on whether the testing is part of the traceback investigation or if the tests are for clients who want testing to assuage fear of exposure. The testing protocols follow rigorous standards and require specific media to be used for collection (Ames media with charcoal), specific timelines for both sample entry (samples must be to the laboratory within 48 hours after collection) as well as for reporting (reported after 7 days of incubation).

Samples collected as part of the traceback investigation must be collected under the supervision of a state veterinarian or veterinary medical officer. Samples from stallions involved in the traceback must be sent to the NVSL for testing. All mare samples can be tested at CSUVDL. ▲

FOR MORE INFO

www.aphis.usda.gov/newsroom/hot_issues/cem

Test cost: $9 per swab

Diagnostic Interpretation

A Positive Is a Positive but a Negative Doesn't Mean Much, Right?

QUESTIONS OR COMMENTS?

Please feel free to contact Dr. Kennedy at (719) 254-6382

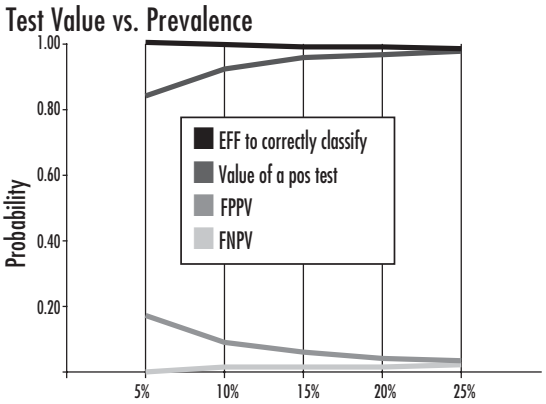
We offer a myriad of diagnostic tests, from gross necropsies to molecular diagnostics. Correct interpretation of such a variety of diagnostic tests certainly requires knowledge of the parameters of the test, e.g. sensitivity and specificity, but those are insufficient when applying the results to make an informed management decision. The application of predictive value, apparent prevalence, and diagnostic test efficiency are numerical values that help establish the significance of a diagnostic test; however, an article in the *Journal of the American Statistical Society* provides another measurement relevant to interpreting diagnostic test results.

The article, published in 1994, concerned pooling sera to detect disease in humans; however, the concept is not limited to pooled testing but is applicable to any diagnostic test.[1] In human medicine, there is equal, if not greater, concern that a positive diagnosis is truly positive. Informing a patient that they have a fatal incurable disease to only inform them at some later time that the test was in error actually may be more devastating than to be unaware of the disease. The article proposes a set of parameters that are clinically relevant in veterinary medicine to making management decisions, including euthanasia, slaughter or costly treatments. Those parameters are False Positive and False Negative Predictive Values (FPPV and FNPV, respectively). Although the terms resemble negative and positive predictive values, they are not the same nor are they complementary probabilities.

1 Litvak E, Tu XM, Pagano M. Screening for the Presence of a Disease by Pooling Sera Samples. J Am Stat Assoc. 1994 Jun; 89(426):424-434.

Prevalence	5.00%	10.00%	15.00%	20.00%	25.00%
Sensitivity	95.00	95.00	95.00	95.00	95.00
Specificity	99.00	99.00	99.00	99.00	99.00
EFF to correctly classify	0.988000	0.986000	0.984000	0.982000	0.980000
Value of a Pos Test	0.833333	0.913462	0.943709	0.959596	0.969388
Value of a Neg Test	0.997349	0.994420	0.991166	0.987531	0.983444
FPPV	0.166667	0.086538	0.056291	0.040404	0.030612
FNPV	0.002651	0.005580	0.008834	0.012469	0.016556
AP	0.057000	0.104000	0.151000	0.198000	0.245000

— Jim Kennedy, DVM, MS, Director, CSUVDL Rocky Ford Branch.



The FPPV is the probability that a test calls a sample positive when it is truly negative, a false positive, while the FNPV is the probability that a test calls a sample negative when it is truly positive, a false negative. Both parameters factor the prevalence of the disease into the calculation. These parameters become most important at the extremes of prevalence and less so in the middle ranges where disease presence is equally likely as the absence of disease. As an example a disease with a prevalence of 5 percent, a test with a sensitivity of .95 and a specificity of .99 yields the probability that a sample is incorrectly identified as a positive of .17 and the probability that it was incorrectly classified as negative of .003. Using the same test parameters but a prevalence of 25 percent the values for FPPV and FNPV are .03 and .017, respectively. Changing the basic test parameters of sensitivity and specificity to .98 and .9 will give a FPPV of .660 and an FNPV of .001 if a 5 percent prevalence is assumed and if 25 percent prevalence level .234 (FPPV) and .007 (FNPV). The table and chart at left reflect the value of various parameters as prevalence is varied.

In summary, if someone claims to have seen a zebra running freely on the plains of southeast Colorado, ask for confirmation; while if they say they saw it while on safari in Africa, there is less reason to doubt and confirmation may not be needed. If a disease is diagnosed and the history and clinical signs support it, confirming tests may not be needed, while if prior knowledge is lacking or if the disease is rare all positive diagnoses should be confirmed through further testing and observation. A positive test may not mean a positive animal and a negative test should not be ignored. Diagnostic tests do not stand alone but are parts of a bigger puzzle. ▲

What's Your Diagnosis?

Histologic Exam of Canine Lung

Histologic exam of a lung section from a 12-week-old puggle dog revealed marked expansion of bronchi, bronchioles and terminal bronchioles by large numbers of histiocytes admixed with sloughed degenerative and necrotic epithelial cells and fewer numbers of lymphocytes and plasma cells. Occasionally within sloughed degenerative epithelial cells, there were large intranuclear eosinophilic to amphophilic inclusion bodies. Fibrin, foamy histiocytes, edema and extravasated red blood cells obscured alveolar spaces of the remaining pulmonary parenchyma.

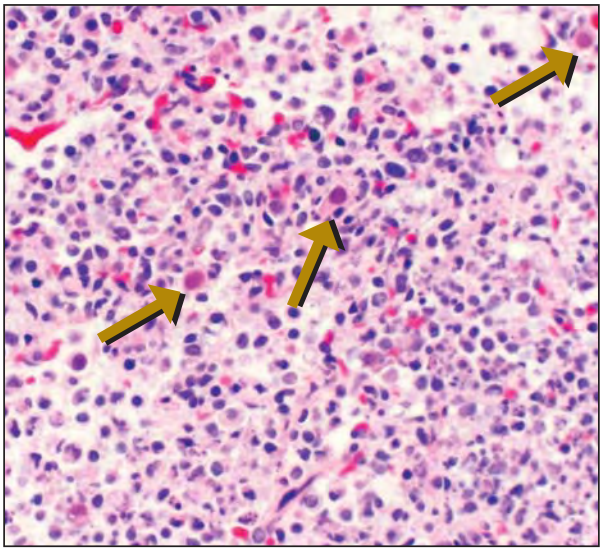
Based on histologic findings within the lung, including the intranuclear inclusion bodies, a tentative diagnosis of canine adenovirus was given. This animal also had marked thymic hypoplasia and splenic lymphofollicular hypoplasia, which is consistent with an immunocompromised state.

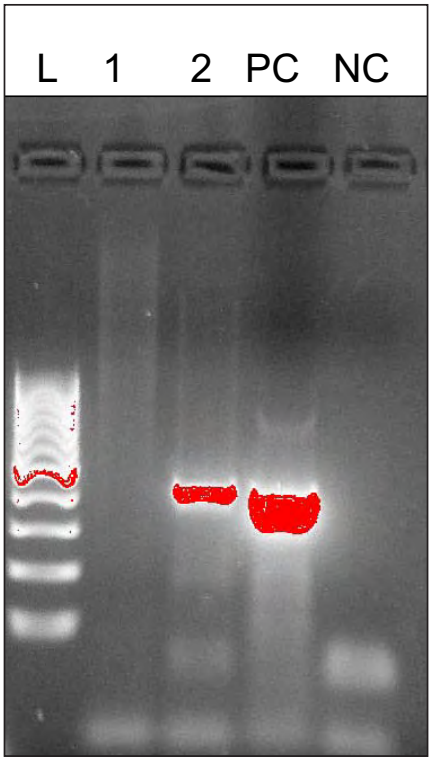
To confirm our suspicion, fresh lung samples were submitted for polymerase chain reaction (PCR) and tested for canine adenovirus, canine distemper and canine herpesvirus-1. PCR following gel electrophoresis indicated positive for adenovirus; PCR for distemper and canine herpesvirus-1 were negative.

— Monali Bera, CSUVDL Pathology Resident, and Debra Kamstock, DVM, PhD, DACVP CSUVDL Pathologist

The PCR test does not differentiate between adenovirus 1 and 2 however, as CAV-2 typically induces pulmonary lesions with a tropism for bronchiolar epithelial cells (canine adenoviral pneumonia as seen in this case) CAV-2 was considered to be the underlying etiologic agent in this puppy. Canine adenovirus type 2 is a double-stranded DNA virus, serologically related to but genetically distinct from canine adenovirus type 1 which is the cause of canine infectious hepatitis. CAV-2 can be a predisposing factor for *Bordetella bronchiseptica*, the causative agent in canine infectious tracheobronchitis (kennel cough).

Clinical signs associated with CAV-2 infection are nonspecific and include a nonproductive cough, fever, depression, anorexia, dyspnea and nasal discharge. This animal had pure adenoviral pneumonia, which is extremely rare in dogs. This dog was, however, immunocompromised, as demonstrated by severe thymic hypoplasia and splenic lymphoid depletion. The definitive cause of this underlying immunosuppression is unknown; however, contributory factors may have included stressful conditions and poor nutrition. ▲





Histologic image of a lung section from a 12-week-old puggle dog (above) shows occasional large intranuclear eosinophilic to amphophilic inclusion bodies within sloughed degenerative epithelial cells, as demonstrated by the arrows. The PCR product (right) in lane 2 (test sample) represents the adenovirus positive sample from this animal's lung (L= ladder, PC= positive control, NC= negative control).

REAL-TIME PCR FOR TRICH NOW AVAILABLE AT CSUVDL

Some states now require or accept real-time PCR for the detection of *Tritrichomonas foetus* in cattle. In response, we are pleased to announce this service is now available at CSUVDL. Sample collection or submission protocol will not change, and the price remains the same as conventional PCR, at $25. If the state to which your clients are shipping cattle requires or accepts real-time PCR, please write "real-time PCR" on the submission form.

Annual Diagnostic Summaries

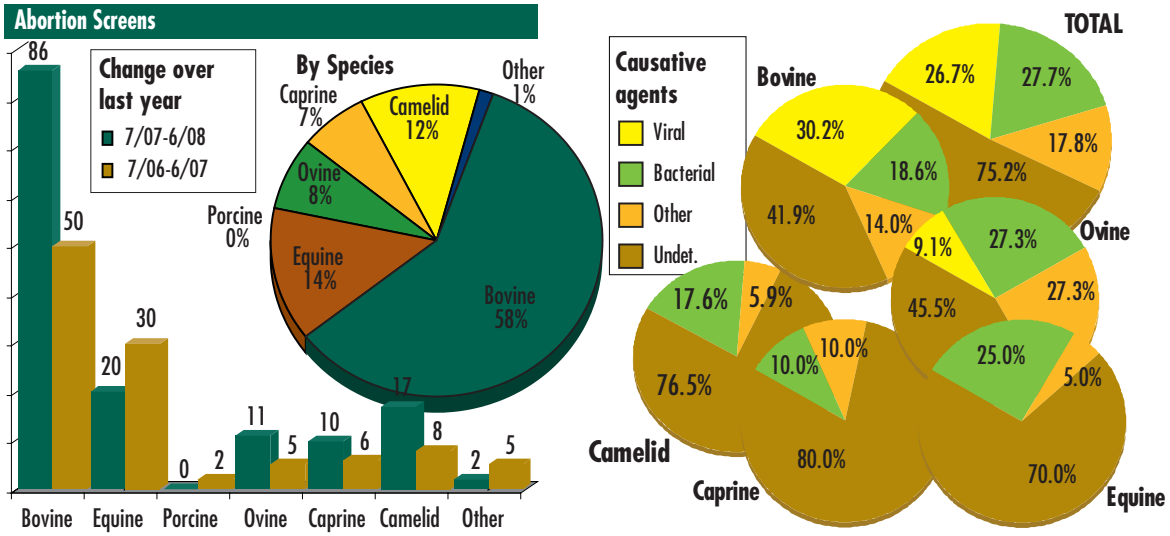
Abortion, Diarrhea Statistics

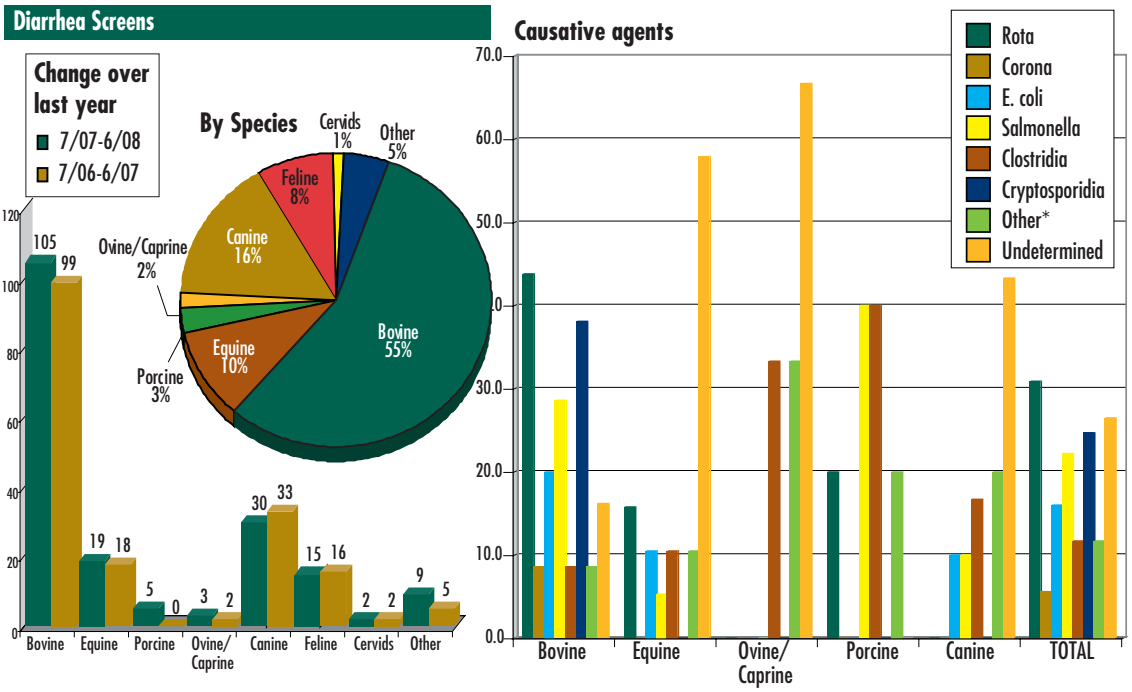
In food animals, quick and accurate diagnosis of infectious abortions calls for the herd veterinarian and a veterinary diagnostic laboratory working together, communicating, sampling and testing appropriately. Yet, still only about half of submitted fetuses result in a definitive diagnosis. Most diagnosed abortions can be traced to infections by bacterial, viral, fungal and protozoal agents.

In the spring, we see many cases of diarrhea in young animals, or scours. Most of these cases we can identify the cause of, which are usually viral, bacterial, parasitic or a combination of the above.

— Barbara Powers, DVM, PhD/DACVP, CSUVDL Director

The following charts indicate the number of abortion and diarrhea screens for the listed species for the last two fiscal years, along with the number of specific diagnoses or causative agents identified for abortions and scours in fiscal year '07-'08. ▲





Food Animal Production Medicine

IBR Abortions on the Increase

— *Chris Gates, CSU Microbiologist, Jeanette Bishop, CSUVDL Molecular Diagnostics Research Associate, Anita Schiebel, CSUVDL Microbiologist, and Hana Van Campen, DVM, PhD, DACVM, CSUVDL Virology Section Head*

The number of bovine fetuses that are positive for bovine herpesvirus-1 (BHV-1), the causative agent of IBR as determined by FA staining, virus isolation (VI) or PCR has increased dramatically since the winter of 2002-2003. A total of 37 fetuses have tested positive for BHV-1 from Oct. 21, 2008, through March 9, 2009. In contrast, CSUVDL diagnosed only one IBR abortion in 1998 and none in 1999-2001.

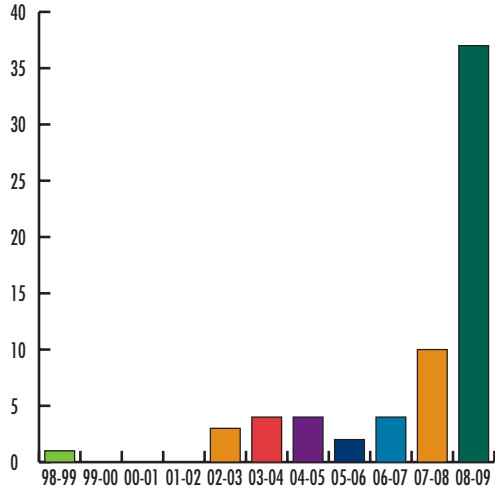
A total of 30 bovine fetuses were IBR FA-positive in 2002-2008. Fetal ages were stated by the submitter or estimated at necropsy to be 124 to 250 days of gestational age. Thirteen fetuses were noted to be moderately to severely autolyzed at necropsy, and 13 had multifocal areas of necrosis in the liver and/or lung and other organs. Three fetuses were noted to have hemorrhage or serosanguinous fluid in the thoracic and/or abdominal cavities. Intranuclear inclusions supportive of BHV-1 infection were described for only four fetuses. Eight IBR FA+ fetuses were tested for the presence of BHV-1 DNA by PCR and all eight were PCR positive. Only two BHV-1 viruses were isolated from fetal tissues. Two of 30 fetuses yielded evidence of another explanation for the abortion, such as cardiomyopathy and suppurative placentitis.

Vaccination histories are rarely given on the submission forms; however, in eight IBR FA+ fetuses, from four herds, the vaccine history included using a vaccine containing modified-live IBR in pregnant cows. In one case, the exact vaccination record of the heifers prior to breeding was unknown. Where the age of the cow was noted, all were 2- or 3-year-old heifers.

This year, from Oct. 21, 2008, until March 13, 2009, 37 IBR FA+ fetuses representing 26 herds were examined. Fetal ages were estimated to be 150 to 260 days of gestational age. A distinct change from the previous years' cases is that 35 IBR FA+ fetuses were also FA+ for BVDV antigen. Of 16 available pathology reports, seven described focal areas of necrosis in liver or placenta, one reported intranuclear inclusions and seven fetuses had lesions compatible with in utero BVDV infections including lymphoid depletion, and interstitial or bronchopneumonia. Eight IBR FA+ fetuses were also tested for BHV-1 DNA by PCR and all 8 were PCR positive. Two noncytopathic BVDVs have been isolated and are currently being characterized. To date, no BHV-1 viruses have been isolated from the 2008-2009 abortions. Two of 37 fetuses had findings compatible with an alternate diagnosis; one with neosporosis and one with salmonellosis.

For 14 fetuses from six herds, there was a history of using an MLV-IBR vaccine in pregnant cows. However, for 11 fetuses from nine herds, only inactivated IBR or no vaccines were administered to pregnant cows.

IBR Abortions 1998-2009



The histories including cow age and dates of vaccinations are not complete for the 2008-2009 cases. We will be contacting the submitting veterinarians to gather this data in order to determine factors contributing to this trend. ▲

ADVICE FOR CLIENTS

- **Read the vaccine labels carefully prior to use in pregnant cattle. Make sure cows have been vaccinated with the products listed on the label prior to vaccination during pregnancy.**
- **Keep records of the vaccines, including date of purchase, lot and serial number, and date administered. Keep sales receipts.**
- **Request vaccination records for purchased heifers.**

For additional information, please call Hana Van Campen at (970) 297-1287 or e-mail: hvancamp @lamar.colostate.edu

CSUVDL in the Field: Case Study

When Many Things Go Wrong

— *Tawfik Aboellail, BVSc, MVSc, PhD, DACVP, CSUVDL Pathologist, and John Maulsby, DVM, CSUVDL Case Coordinator*

Managing a dairy in frigid Colorado during winter isn't easy. A Colorado dairy reported 180 calves less than 6 months of age over three different pens experienced a neurological syndrome characterized mainly by ataxia, muscle stiffness, blepharospasms and blindness. More than 10 calves succumbed, irrespective of rigorous treatment with antibiotics, sulfa, dexamethasone and supportive fluid therapy. Necropsy of seven calves revealed no significant gross lesions in the central nervous system, particularly the brain, while only one animal showed mild suppurative meningitis histologically.

The list of differential diagnoses included:

- Infections, mainly Salmonella/coli septicemia and rabies
- Toxicities, mainly salt intoxication and lead
- Parasitic infestations, mainly nervous coccidiosis
- Dietary deficiencies, particularly thiamine deficiency (polioencephalomalacia) secondary to excessive sulfur/sulfate intake and copper deficiency.

Ancillary testing was negative for rabies, and insignificant bacterial isolates were cultured from the brains of all animals. Copper levels were normal, and lead levels were between 0.1 and 0.2 ppm in blood and kidney wet weight (Normal reference intervals: blood—normal < 0.2 ppm; kidney—normal < 2.00 ppm). A few to no coccidian parasites were present in different segments of the gastrointestinal tract of several animals which showed mild to moderate lymphoplasmacytic and eosinophilic enteritis. Variable numbers of *Eimeria* oocysts (1+ to 4+) were detected in the feces of those animals. Abomasitis was evident in one animal, and another calf showed suppurative enteritis. Three calves had variable degrees of crainoventral consolidation with fibrinosuppurative bronchopneumonia. Two different animals had nonspecific portal hepatitis with mild to moderate portal fibrosis. Testing for other viruses, namely BVDV, was also negative.

MANAGEMENT EVALUATED ON-SITE

Several faculty members from the Veterinary Teaching Hospital and CSUVDL went on a field visit to evaluate different aspects of management. Many calves at that point were ill-thrift, diarrheic, and some were still showing nervous manifestations. About 50 percent had been treated with NuFluor® florfenicol for respiratory disease, and many were still coughing. Some pens had insulated waterers, but two pens, where the affected heifers originated, had old concrete water troughs with several inches of ice. The dairy was keeping its bull calves as well as heifers in the same pens and the pens were generally overcrowded with a wide variation in the size, condition and health of the calves in each pen. Decoxx® decoquinate was added to check coccidiosis, and sulfa was administered in the drinking water for five days.

Necropsy of the eighth calf showed classical deep laminar cortical necrosis reminiscent of polioencephalomalcia and the brain fluoresced under UV light. Sodium level in that brain was high, measuring 8,750 ppm (dry weight). Brain adequate sodium levels are 3,200 to 5,600 ppm, toxic >7,200 ppm. Following the field visit, another calf became sick and was treated with fluids, dextrose, thiamine and dexamethasone. The calf favorably responded. Three other animals since mid-January had been successfully treated with thiamine.

MANAGEMENT-RELATED STRESSORS

This case typifies a syndrome in which management predisposed stressed calves to many disease processes involving more than one organ system. Reversal of clinical symptoms with thiamine treatment and the classical laminar necrosis found in the brains of neurologically clinical calves are unequivocal indicators of polioencephalomamalcia. The use of antibiotics could have disrupted the homeostasis of thiaminase producing bacteria or disturbed the ratio of the good and harmful sulfate/sulfur reducing bacteria precipitating the polioencephalomalacia. However, salt intoxication is not totally ruled out as some neuropathology experts argue that it could develop late in the course of the disease. Absence

JOHNE'S LIQUID CULTURE. CSUVDL has passed the individual 2009 fecal proficiency panel for *Mycobacterium avium* ssp. paratuberculosis using ESP liquid media. This allows us to conduct official testing for the National Johne's Program using this method until Dec. 31, 2010. It uses a liquid based system that allows *M. paratuberculosis* detection in an average of only 36 days, compared to 12 to 16 weeks using solid media. Using this method we can grow the organism faster and use PCR to determine if it is *M. avium* spp. paratuberculosis and not another *Mycobacterium*.

Cost for liquid culture is $25 per sample. Submit feces or tissues. Pools of up to five samples are accepted. Typical turnaround time is 30-42 days. We also remain USDA-approved to offer the slower method of solid media culture, as well.

of histological lesions from affected calves, though, and autofluorescence of brains from calves that succumbed to a documented salt intoxication/water deprivation is not typical of that condition (autofluorescence associated with water deprivation, to our knowledge, has not been previously reported in the literature).

Whether this syndrome started as water deprivation or culminated in polioencephalomalcia, the lesson is that we must ensure an ample and accessible source of water for growing calves during the freezing winter months, avoid over-crowding, and establish coccidiosis prophylaxis. Treatment of neurologic calves with thiamine should be instituted once infectious etiologies are ruled out. Last but not least, sending multiple animals for necropsy is always rewarding even if it takes eight calves to reach a definitive diagnosis. ▲

CSUVDL Establishes Endowment Fund

Your Support Matters

Join the new CSUVDL Endowment Fund and help make a significant impact on the future growth of our mission of service, teaching, research and outreach. Currently, most revenues to operate and support the CSUVDL are generated via fees for service. This limits scientific progression, expansion and education. Increased financial support through the VDL Endowment Fund will remove those limits and provide increased opportunities to pursue research, aid field investigations, support the development of new technologies for disease diagnosis and aid in the expansion of current services offered to our clients. It will provide an avenue for upgrading outdated equipment and allow for continued educational advancement for both our clients and future diagnosticians. It will further our mission of promoting and protecting animal and human health.

We invite you to join us on this mission. Please assist us in reaching our initial goal of $25,000 to establish and grow the Endowment Fund. You may contribute by completing the form below or by visiting us online at www.dlab.colostate.edu and clicking "Support the DLAB online."

We would like to extend our sincere gratitude to an inaugural donor, who has requested anonymity, for the initial contribution that has allowed us to launch this endeavor. Thank you for supporting the VDL and our mission.

HAVE QUESTIONS OR NEED MORE INFO?

Dr. Debra A. Kamstock
Assistant Professor
CSUVDL Endowment Fund Committee Chair
(970) 297-1281
Debra.Kamstock @colostate.edu

YES! I want to make my support matter…today and for the future

I would like to help the CSU Veterinary Diagnostic Lab continue and strengthen its mission of promoting and protecting animal and human health.

— Detach and Mail —

Please accept my check for a gift of $ _____
(Payable to Colorado State University Foundation Veterinary Diagnostic Laboratory)

Name _____

This gift is from ❑ Me ❑ My spouse and me ❑ My partner and me

Spouse's/Partner's Full Name _____

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Please feel free to make your gift in honor of a friend, family member, other individual, pet or organization who has inspired you to support us.

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Name on Card _____

Signature _____

Use this gift for: ❑ General purpose, to include education, training, equipment, operations
❑ No specific designation ❑ Diagnostic investigations/research only
❑ Other _____

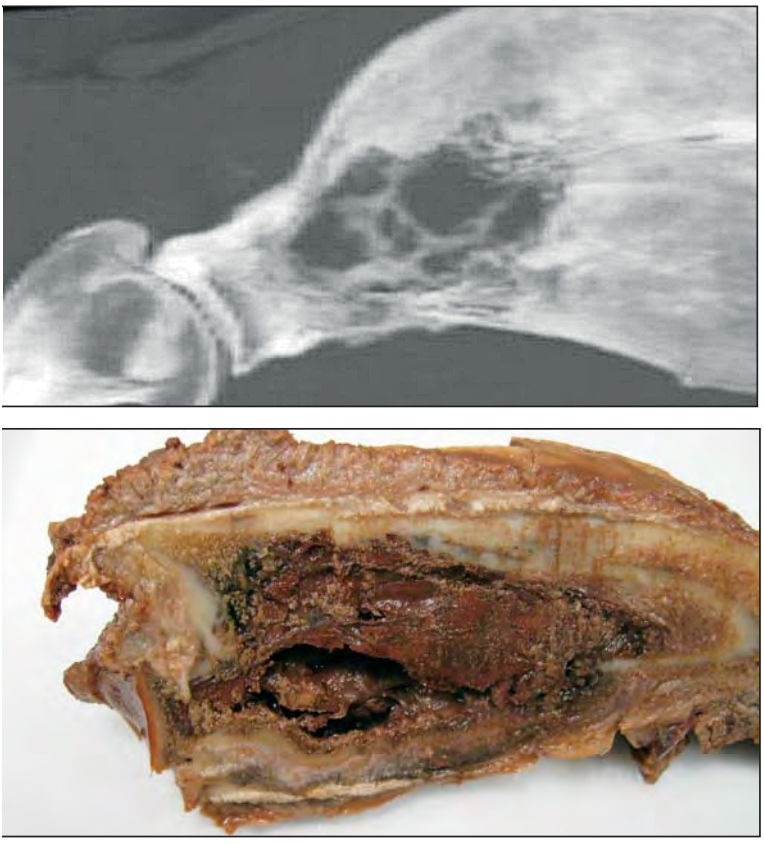
Please return this form with your gift to:
Colorado State University Foundation, P.O. Box 1870, Fort Collins, CO 80522
Or go to www.dlab.colostate.edu and click "Support the DLAB online"

* Necessary information so we can confirm receipt of your contribution and thank you for your support. By contributing to the CSUVDL you become a member of our family and a supporter of our cause. As such, you will automatically receive our biannual newsletter *LabLines* so we can keep you updated of the lab's activities, from investigations to interesting diagnostic cases to test development to comings and goings of residents, post-docs, graduate students and faculty.
❑ If you prefer not to receive this mailing please check here. All donations are charitable.

61713/V0918

CSUVDL Educational Outreach

A Picture = Thousand Words



Photos helped diagnosis by sharing (top) a CT scan showing lysis in the scapula, and (bottom) scapula after sectioning, showing extensive areas of hemorrhage.

The convenience of sharing digital images has made it easier for veterinarians in practice and veterinarians in the laboratory to share visual information and improve diagnostic services for a patient. Here's an excellent example:

A dog was presented to the veterinarian because of lameness in the front leg. A physical examination indicated the problem was in the shoulder area, but it was not clear what particular area or type of tissue was involved in the problem. Routine radiographs did not localize the lesion. However, a CT scan identified a large area of lysis within the scapula. Because the lesion was severe, amputation of the limb was performed. When the limb was submitted to the laboratory, extensive areas of hemorrhage were found in the bone and muscle, but it was not clear what areas needed to be examined histologically. In fact, the gross examination suggested the skeletal muscle was the primary problem. However, since the CT scan results could be sent to the laboratory via e-mail, the area of concern was identified and appropriate sections were prepared.

— Pat Schultheiss, DVM, PhD, DACVP, CSUVDL Pathologist

A diagnosis of telangectatic osteosarcoma was made. This type of osteosarcoma has many dilated blood channels and extensive areas of hemorrhage which make it difficult to distinguish the tumor from surrounding tissues. Without the information provided by the CT scan, many slides might have been made before finding the actual tumor.

In addition to this case that had CT scan information, there are many instances where digital images of routine radiographs are helpful to us. Also digital photographs of gross lesions, especially skin lesions, are very helpful when a pathologist is evaluating histologic lesions. Photographs submitted along with tissue are always appreciated. ▲

Food Animal Production Medicine

Enzyme Method NEFA Testing

Cathy Bedwell of the chemistry/toxicology section has recently validated an enzyme method for detection of Non-Esterified Fatty Acid (NEFA). NEFA testing is used to evaluate the level of free fatty acids in the blood, in order to evaluate the nutritional plane of dry dairy cows nearing calving.

NEFA testing may be used as a management tool to ensure parturient cows are on the correct level of nutrition. If NEFA levels are elevated above normal, the cow is metabolizing adipose tissue more than she should to maintain her condition as a parturient cow. This cow should be on a better plane of nutrition. If NEFA levels are below normal levels, the plane of nutrition could be lowered appropriately. If 40 percent of the test samples run on a dry pen of cows are above the goal levels it is considered a significant problem with pre-partum negative energy balance and excessive adipose mobilization.

— John Maulsby, DVM, CSUVDL Case Coordinator, and Dwayne Hamar, PhD, Chemistry/Toxicology Section Head

A serum sample from a red top clot tube is used to run the test. It is better to evaluate at least 10 percent to 20 percent of a herd's dry cows to get an accurate representation of the dry pen. If 10 or more samples are run, the cost of the test is $8 per sample. If fewer that 10 samples are run, the cost will be $14 per sample. ▲

SERUM NEFA TARGETS

More than two weeks before calving: <0.32 mEq/L

Between two weeks and two days prior to calving: <0.40 mEq/L

Within two days of calving: Concentrations are usually high and difficult to interpret.

Elevated concentrations in greater than 40 percent of animals: A negative energy balance and excessive adipose metabolism

Chemistry and Toxicology

Beware Fast-Acting Temik Poison

— *Tawfik Aboellail, BVSc, MVSc, PhD, DACVP, CSUVDL Pathologist, and Dwayne Hamar, PhD, CSUVDL Chemistry/ Toxicology Section Head*

Fast-acting poisons can strike nontarget species so quickly there's no time to lose. One such poison is Temik® aldicarb, a member of the carbamate pesticides which include Carbaryl,® Sevin® and Propxur.® With an LD50 of roughly 1 mg/kg, Temik falls into the category of super-toxin, those with an LD50 of < 5 mg/kg—so toxic a teaspoon can kill a fully grown rhino. In our region, Temik is used to control nematodes in potato fields and requires a permit to purchase.

Aldicarb toxicity presents a challenge to clinicians and diagnosticians. Symptoms are vague, and gross findings are nonspecific at best. Symptoms may include tremors, salivation, diarrhea, vomiting, labored and fast breathing, weakness, and even paralysis.

The poison attacks the nervous system and inhibits breathing. The mode of action targets the enzyme cholinesterase, affecting nerve impulse transmission. Acetyl cholinesterase (AChE) is found in synaptic junctions and red blood cells, as is buytryl cholinesterase (also known as pseudocholinesterase or plasma cholinesterase). Inhibition of AChE leads to accumulation of acetyl choline at muscarinic receptors (cholinergic effector cells), at nicotinic receptors (skeletal neuromuscular and autonomic ganglia) and in the CNS.

We have diagnosed Aldicarb poisoning in several dogs. The latest case was an outdoor mixed breed dog that showed progressive vomiting and diarrhea, and then died two hours later. Generalized congestion and pancreatic hemorrhage were the only gross lesions observed in the carcass, which had moderate postmortem autolysis.

Gastric contents contained many black granules the size of poppy seeds. The gastrointestinal tract, particularly the stomach, contained big chunks of lard-like bait, which suggested a malicious act. High-performance liquid chromatography (HPLC) is often the method of choice and is the basis of U.S. EPA Method 531 for detecting carbamate pesticides. Plasma or red blood cell cholinesterase testing to evaluate exposure to carbamate insecticides is available at CSUVDL. Temik poisoning was confirmed in this case by a toxicology screen for carbamates, which revealed 272 ppm of aldicarb in the gastric contents.

Temik granules found in gastric contents and on gastric mucosa.

Atropine blocks Temik's nicotinic effects, reversing the neuromuscular blockade. Two regimens for initial atropine treatment are currently suggested; in both cases the cessation of the cholinergic symptoms (salivation, bronchial secretion, sweating and bradycardia) indicates sufficient atropinization. The skin should be dry, the lungs should be clear on auscultation and the heart rate should be 80 to 100 beats per minute. Strictly avoid atropine overdose, as it can promote heart rhythm disturbances.

Regimen 1: 2-10 mg atropine IV, followed every 15 minutes by 2 mg atropine IV until symptoms cease.

Regimen 2 (preferable):

- 2 mg atropineIV, wait five minutes
- If symptoms persist: 4 mg atropine IV, wait five minutes
- If symptoms persist: 8 mg atropine IV, wait five minutes
- If symptoms persist: 16 mg atropine IV, wait five minutes
- If symptoms persist: 32 mg atropine IV, wait five minutes

Use no higher doses than needed, and wait the full five minutes after each. If further treatment is required (taking into account the relatively short effect of carbamates), it should be done by continuous application of 1 to 2 mg per hour. Treatment can cease when plasma cholinesterase level has returned to above 30 percent. ▲

Temik's fast action has reportedly made it a favorite of burglars to quickly silence guard dogs and watchdogs.

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Lab Updates

Test and Service Improvements

CHEMISTRY/TOXICOLOGY LABORATORY MOVING TO SOUTH CAMPUS

—Dwayne Hamar, PhD, CSUVDL Chemistry/Toxicology Section Head

Following more than 30 years occupying space on the third floor of the main campus' Pathology Building, the Chemistry/Toxicology Section has joined our colleagues in the new Diagnostic Medicine Center. Our new laboratory will be more than twice the size of our current space, including two chemical fume hoods, and separate rooms for analytical instruments, sample preparation and "messy" activities, such as forage grinding. These improvements will allow us to grow and expand the quality analytical services you have come to expect from us.

Moving an entire laboratory is a daunting task, regardless of the distance traveled! Refrigerators, freezers, large equipment, nonchemical supplies and samples will be moved in mid-June by movers hired expressly for this purpose, as will chemicals, by CSU's Environmental Health Services' trained personnel. Moving the analytical instrumentation poses special challenges. These instruments will be transported by specialized movers, and will need to be installed at their new location, calibrated and thoroughly checked to ensure proper functioning prior to use. We will work diligently to get the laboratory up and running quickly, but please be aware that our turnaround times will be adversely affected during this period of time.

CLINICAL ENDOCRINOLOGY LABORATORY TESTING SERVICES EXPANDED

—Michael R. Lappin, DVM, PhD, DACVIM, CSUVDL Endocrinology & Special Serology Lab Supervisor

EXPANDED THYROID PANELS AT A LOWER PRICE. The canine thyroid panel now includes assays for total T4, endogenous TSH, and anti-thyroglobulin antibody. The good news is that even though an additional assay is now offered, we have decreased the price to clients outside the Veterinary Medical Center. For the clinicians that still believe a T4 by equilibrium dialysis is needed for the case assessment, this assay can be added on for an additional fee.

This panel now approximates that achieved at Michigan State, plus we can run the assays Monday through Friday. Samples delivered to the lab by 3 p.m. can still receive results the same day, if requested, for no added charge.

ADDISON'S DISEASE SCREENING TO REDUCE COST. Several recently published manuscripts have shown that determination of a screening cortisol level can be used to exclude hypoadrenocorticism (Addison's Disease) from the list of differential diagnoses.

Dogs with either typical or atypical (normal electrolytes) hypoadrenocorticism almost always have a screening cortisol concentration of < 2; whereas, dogs with other conditions showing similar clinical signs generally have a concentration of > 2. For some cases with equivocal results, an ACTH stimulation assay will be required. However, use of the screening assay saves considerable expense for most cases, as cosyntropin is not required. If emergency treatment is required prior to sample collection, dexamethasone can be used for glucocorticoid replacement without adversely affecting the test results, if the sample is collected shortly after administering the drug.

The Clinical Endocrinology Laboratory offers the screening cortisol assay Monday through Friday. Samples delivered by 2 p.m. can still receive the results the same day, if requested, for no additional charge. Samples received after 3 p.m. can have results returned as early as 10 a.m. the next day.

SPECIALIZED INFECTIOUS DISEASES LAB UPDATES

—Michael R. Lappin, DVM/PhD/DACVIM, CSUVDL Endocrinology & Special Serology Lab Supervisor

BLOOD-BORNE DISEASE PCR ASSAYS. When screening dogs for *Ehrlichia canis* (relatively common in our region) and *Anaplasma phagocytophilum* (previously *E. equi* and rare in our region) infections, the point of care assay from IDEXX performs relatively well. However, it can miss acute cases of *E. canis* and *A. phagocytophilum* and doesn't consistently detect antibodies against *E. ewingii, E. chaffeensis, A. platys*, or *Neorickettsia risticii* (previously *E. risticii*; atypical ehrlichiosis in dogs). In addition, while feline ehrlichiosis was first documented in a cat from Boulder, there is currently no validated serological test for use with cat serum.

The *Ehrlichia* group polymerase chain reaction (PCR) used at CSUVDL is designed to amplify DNA of all known *Ehrlichia* spp., *Anaplasma* spp., and *Neorickettsia* spp. It can detect as many as seven known pathogens in dog and cat blood (0.3 ml in EDTA minimum). The result can be sequenced to determine the infective organism. The combination of *E. canis* IFA and *Ehrlichia* group PCR appears to be the most sensitive way to diagnose this infection in the dog.

CSUVDL also performs PCR assays for *Rickettsia rickettsii* (Rocky Mountain spotted fever agent) and *R. felis*. While infections with *R. rickettsii* are rare in our region now, the PCR assay can be superior to serology alone as

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Western Slope Animal Diagnostic Laboratory
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Grand Junction, CO 81501
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Fax (970) 242-0003

TAKE A VIRTUAL TOUR OF THE LAB

www.dlab.colostate.edu/webdocs/tour/endotour.htm

NEED MORE HELP?

Please contact Michael R. Lappin, at mlappin@colostate.edu, or Arianne Miller, at akmiller@colostate.edu, (970) 297-0367

the serological test also detects antibodies against four other non-pathogenic spotted fever group *Rickettsia* found in our region. Serology can be combined with the PCR assay to maximize sensitivity. It also appears that *R. felis* can cause fever in some cats and this PCR assay works well with cat blood.

Recently, our research group has documented *Bartonella henselae* and *B. vinsonii* in dogs in Colorado and Wyoming, as well as *B. henselae* and *B. clarridgeaie* in cats. The dogs had infective endocarditis and the cats had fever or uveitis. The PCR assay we use for *Bartonella* spp. can amplify the seven most common species from blood. For cats, the PCR assay can be combined with serology for maximal sensitivity. The index of suspicion for these agents is higher if there is a history of fleas.

Recently, the organisms previously known as *Haemobartonella felis* and *H. canis* were reclassified as hemotropic Mycoplasmas. Our research group has now documented *Mycoplasma hemofelis*, Candidatus *M. haemominutum*, Candidatus *M. turicensis*' *M. hemocanis*, and Candidatus *M. haematoparvum* in cats or dogs in our region. Our PCR assays can amplify each from dog or cat blood, and they should be on the differential list for acute hemolytic anemia and either acute or chronic fever.

You can elect to perform a fever panel (also great for blood donors) which includes PCR assays for the *Ehrlichia* group, *Bartonella* spp., *Rickettsia* spp. and haemoplasmas. Alternately you can pick one to four different PCR assays to match perfectly with the travel history and risk factors for your case.

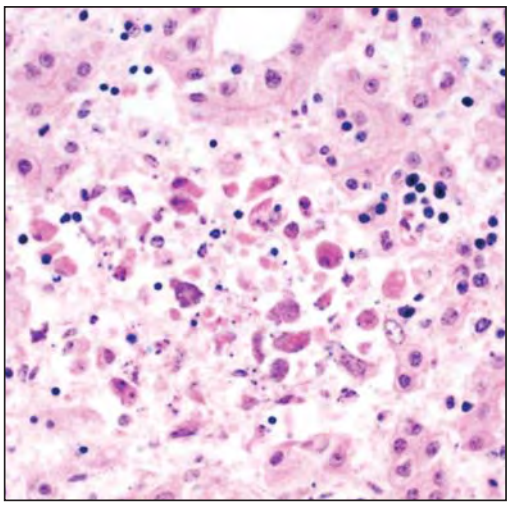
For these flea- and tick-borne diseases, it is optimal to collect the blood into EDTA (0.3 ml minimum) prior to antibiotic treatment. The DNA of these agents is very stable, so the samples can be submitted after days in the refrigerator. ▲

Case Study

Neonatal Calf Hepatitis and Mortality

— Hana Van Campen, DVM/PhD/DACVM, CSUVDL Virology Section Head, and Barbara Powers, DVM/PhD/DACVP, CSUVDL Director

A dairy herd experienced a sudden increase in illness and deaths, from 4 percent to 20 percent, in 4- to 20-day-old calves following intranasal vaccination with a modified live viral vaccine containing IBR, BVD1, BVD2, BRSV and PI_3 viruses. The calves were depressed, anorexic and had signs of respiratory disease that did not respond to antibiotic treatment. Fresh and formalin-fixed tissue samples were submitted for six calves that died. All six lung samples had lesions of bronchopneumonia or bronchointerstitial pneumonia, positive IBR FA staining and positive bovine herpesvirus 1 (BHV-1) PCR tests. Large eosinophilic intranuclear inclusions characteristic of BHV-1 infection were identified in respiratory epithelial cells and in hepatocytes. Two of the calves had significant hepatocellular necrosis. Five calves had positive FA staining for BVDV in the lung.



Multifocal areas of acute hepatic necrosis with intranuclear inclusions characteristic of BHV-1 infection.

Other findings included one calf with PI_3 and coronavirus FA staining and one calf with fungal hyphae in lung. Lymphoid hypoplasia was observed in the spleens of two calves, suggesting immunosuppression following stress or infection with a lymphotropic virus. Five calves had nephritis, and *E. coli* was cultured from one kidney sample. The calves had not been treated with either gentamicin or tetracycline, which with dehydration could contribute to the renal lesion.

The hepatic lesions, bronchopneumonia and intranuclear inclusions found in these calves were nearly identical to those previously described for 11- and 15-day-old dairy calves infected with both BHV-1 and BVDV.[1] The history, clinical disease, lesions and deaths suggest infection with both BVD1 and BVD2 viruses suppressed the immune system of these neonatal calves. As a result, the IBR virus was able to spread to the liver, replicate and cause extensive hepatic necrosis resulting in death. ▲

1 Pálfi V, Glávits R, Hornyák A. The pathology of concurrent bovine viral diarrhoea and infectious bovine rhinotracheitis virus infection in newborn calves. Acta Vet Hung. 1989;37(1-2):89-95.

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

LAB LINES

INSIDE THIS ISSUE

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Update from the Director

BARBARA POWERS, DVM/PHD/DACVP
DIRECTOR

Spring has been slow to arrive this year, with many late snowstorms in April. However, this does not dampen our excitement and enthusiasm as we prepare to move into our new building in June. See inside for the exciting details and pictures. We are very grateful for all who supported us with the long journey to make our dream a reality. We are also grateful for the equipment donation from our supporters to the Rocky Ford laboratory (see details on page 2). In January, we had our annual meeting with our external advisory committee which guides our future directions. The committee members enjoyed a tour through the new facility, although it has come along much farther since January. Our advisory members are listed inside; please contact them (or us) with any comments you have.

We are pleased to have Dr. Colleen Duncan join our pathology group this January. We were sad to say goodbye to long-time employee and phone receptionist Jennifer Swenson, who moved to California.

Inside this issue of *LabLines*, you will find many informative articles on diseases and disease testing, diagnostic services, test statistics, test interpretations and shipping regulations. On our web page, our annual report of complete test statistics, results and activities of faculty is available; or contact us for a hard copy.

The past year has been difficult for all of us in these economic times, but we are striving to continue to meet our mission. We are also excited to launch our new endowment fund and invite you all to contribute! We look forward to seeing many of you at our Grand Opening of the Diagnostic Medicine Center on Sept. 11. We further hope to see more of you at the Annual Colorado Veterinary Medical Association meeting at Keystone, Sept. 17 through 20, and the Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians in San Diego, Oct. 7 through 14. ▲

LAB NEWS

Barbara E. Powers

MISSED AN ISSUE OF *LABLINES*?

Read them all on-line, at www.dlab.colostate.edu/webdocs/news/

Read our annual report of complete test statistics, results and activities of faculty, available now at www.dlab.colostate.edu/webdocs/annualrpt.pdf Or contact us for a printed copy.

Colorado State University Veterinary Diagnostic Laboratories

LAB LINES

Diagnostic news and trends from the Colorado State University Veterinary Diagnostic Laboratories
Volume 14, Number 2 Fall/Winter 2009

Terrestrial rabies returns to Colorado

Wake-Up Call!

On Sept. 14, a diagnosis of rabies in a Colorado horse was made—the first in over 30 years. The El Paso County horse initially appeared to be lame on the left foreleg and resisted having the foot examined by its owner. By the time it was presented to the veterinarian two days later, the left forelimb signs had progressed to severe ataxia, then recumbency with the inability to rise. The horse was euthanized and the brain submitted to CSU VDL in Fort Collins. Direct fluorescent antibody staining revealed rabies viral inclusions within neurons on impression smears of the brainstem and a smaller number of inclusions in the cerebellum. Nonsuppurative encephalitis was found on histopathologic examination and Negri bodies were not evident. The brain was submitted to the CDC, which identified the rabies virus as a skunk strain.

The owner reported a skunk eating the contents of a birdfeeder and following her around the yard during daylight hours two months earlier. As of Oct. 14, eight skunks, one cow, one horse and one mountain lion have been diagnosed with rabies in El Paso County. Statewide, 32 rabid skunks and one fox, in Prowers County, have been found this year. Rabies in terrestrial animals has been a rarity in Colorado in the last three decades. However, since 2007 surveillance by the Colorado Department of Public Health and the Environment detected an increased number of rabid skunks in eastern Colorado. Skunk rabies is now considered endemic east of Interstate 25.

The case of rabies in a horse and cow serves as a wake-up call to veterinarians. Remember to:

- Check your titers every two years for pre-exposure prophylaxis.
- Ensure that staff who decapitate neurologic animals are vaccinated for rabies.
- Wear barrier personal protective equipment when handling neurologic animals. Add a face shield to protect the mucous membranes during necropsy and head or brain removal. ▲

— Hana Van Campen, DVM,PhD,DACVM, CSU VDL Virology Section Head

RABIES IN COLORADO JAN. 1 TO OCT. 14, 2009

County	Bat	Skunk	Other	Total
Adams	1			1
Arapahoe	1	4		5
Boulder	31			31
Chaffee	1			1
Denver	12			12
El Paso	2	8	3*	13
Elbert		3		3
Gilpin	1			1
Jefferson	2			2
Kiowa		1		1
Kit Carson		1		1
Larimer	6			6
Lincoln		1		1
Mesa	2			2
Montezuma	1			1
Morgan	1	7		8
Otero		2		2
Prowers		1	1**	2
Weld	1			1
Yuma	0	4		4
Total	**62**	**32**	**4**	**98**

** Horse: 1; Cow: 1; Mountain Lion: 1. ** Fox*
Source: Colorado Department of Public Health and Environment.

FOR MORE INFO

Get updated information on rabies case distribution by species and details on preventive measures for this zoonotic disease from the Colorado Department of Public Health and Environment. Call them at (303) 692-2700 or visit www.cdphe.state.co.us/dc/zoonosis/rabies.

Livestock at risk should be vaccinated annually. Find information on vaccines and protocols from the National Association of State Public Health Veterinarians, at www.nasphv.org/documentsCompendia.html, or *J Am Vet Med Assoc.* 2008 May 15;232(10):1478-86.

Clockwise from top: Attendees included representatives of academia, industry, government and agriculture; about 350 guests toured the new facility, including the stunning three-story atrium; CSU Mascot Cam the Ram greets guests; VDL Director Barb Powers, CSU President Tony Frank and CVMBS Dean Lance Perryman officially open the new facility; posters and displays detailed the history and progress of the facility's construction; CSU President Frank greets Professor Emeritus Charles Hibler.

Welcome to CSU VDL

Up and Running with a Great Start

An estimated 350 honored guests helped the Veterinary Diagnostic Lab christen its new Diagnostic Medicine Center at our open house and dedication ceremony on Sept. 11. Guests included veterinary clients; external advisory committee members; producers and producer group representatives, including the Colorado Cattlemen's Association and the Colorado Livestock Associaiton; representatives of the Colorado Veterinary Medical Association; members of the CSU System Board of Governors; and representatives of USDA, the Colorado Department of Agriculture, the governor's office and state legislators.

"I think this realization of over a decade's worth of hard work — now a centerpiece for our campus — demonstrates the value people see in what we do here every day at the VDL," said Director Barb Powers. ▲

Lab Updates

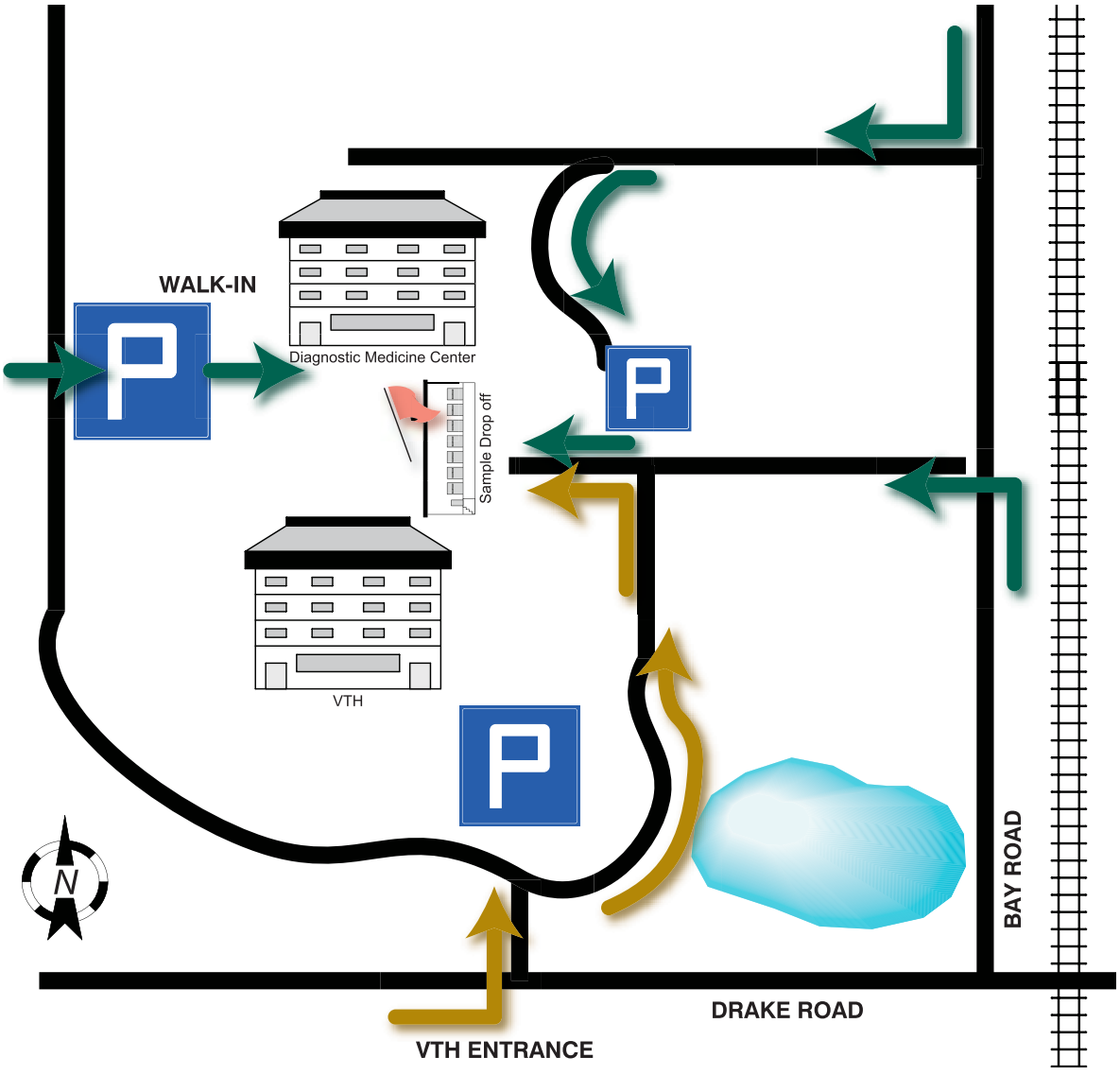
Where to Deliver Drop-off Samples

Haven't been to see the new home for the Veterinary Diagnostic Laboratory at CSU which opened in June? Why not take the ideal opportunity to visit by dropping off samples at our new 88,000-square-foot Diagnostic Medicine Center? We are conveniently located immediately north of the James L. Voss Veterinary Teaching Hospital, linked to the hospital by an enclosed corridor. Drops can be made 24 hours a day at the loading dock behind the facility. We look forward to seeing you.

Here's how to find us:

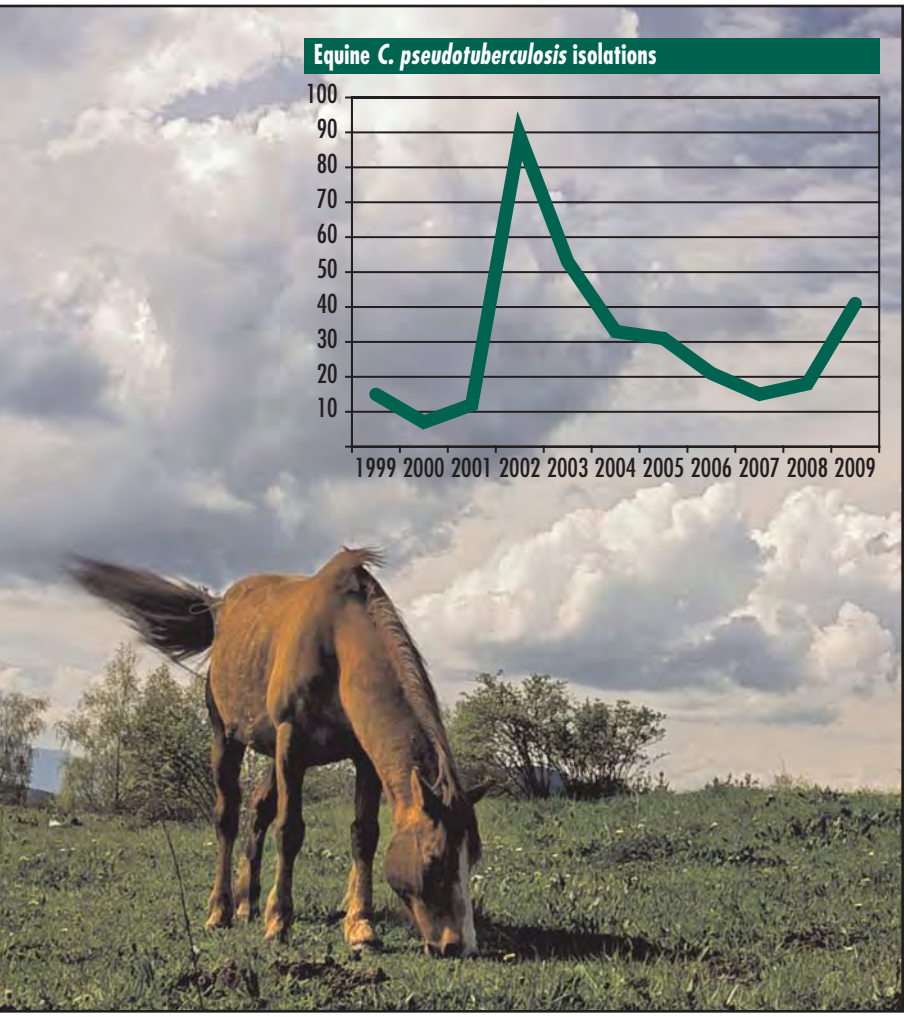
- Take Bay Road north from Drake at the Redwing intersection.
  Cross the bridge, take the first left and follow all the way to the Diagnostic Medicine Center dock.
- Enter the teaching hospital entrance off Drake; turn right at the T.
  Follow around the parking lot, through the gated area between corral and hospital, and left after the riding area to the dock. If a gate is closing this road off, please wait a few moments; animals are being moved in the area. ▲

After-hours drop-off areas with refrigerators are also available.



HOW ELSE TO GET SAMPLES TO US?

- **FedEx courier service. Fee depends on package weight and location. We have a FedEx discount; contact us for information.**
- **Direct Ship/Overnight Mail. Fee depends on package weight and location.**
- **Regular Mail/UPS Service. Fee depends on package weight. For biopsy samples, we can send you pre-filled mailers free with a $2 return fee. FedEx also offers a two-day service on 1-pound mailers for $6.50.**



Diagnostic Summary Update

Corynebacterium pseudotuberculosis in Colorado

— Doreene Hyatt, PhD, CSU VDL Bacteriology Section Head

Corynebacterium pseudotuberculosis infections cause a disease in equids commonly referred to as "pigeon fever," "pigeon breast," or "dryland distemper" and are the most common cause of ulcerative lymphangitis in equids. As mentioned in our 2000, 2001, and 2002 *LabLines*, before 1999 the CSU Veterinary Diagnostic Laboratory annually reported approximately one isolation of *C. pseudotuberculosis* from horses statewide. That changed in 2002 when we had a high of 89 isolations in the calendar year.

Whether that increase was because of an increased prevalence or because of an increased awareness of the disease is unknown, but the number of isolations has steadily decreased since 2002. Again, whether the decrease is because of an actual decrease in disease or a decrease in the number of cultures performed is unknown. Recently, there have been media reports of increased numbers of cases in Colorado. As such, updated statistics of the number of isolations of *C. pseudotuberculosis* from horses in the past 11 years are given in the graph at left. ▲

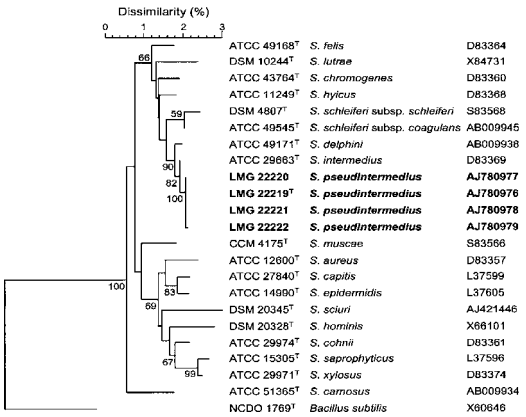
Systematic and Evolutionary Microbiology

Staph. intermedius or *pseudintermedius?*

— Doreene Hyatt, PhD, CSU VDL Bacteriology Section Head

How is one to keep up with the world of changing bacterial names, especially when a change doesn't affect treatment decisions or doesn't apply to all isolates? Such is the case of *Staphylococcus intermedius* and *pseudintermedius*.

In 2005, Belgian scientists looked genetically at *S. intermedius* isolates from a dog, cat, horse and parrot and found that there should be a new novel species, *S. pseudintermedius* (Devriese et al., 2005). Since then, it is has been reported by the same authors (Devriese, 2009) that further genetic testing is necessary to differentiate the two species and that strains from dogs that are identified by traditional culture as *S. intermedius* should be reported as *S. pseudintermedius* (but those from other hosts would need more thorough investigation). So, don't be surprised if you start seeing the new name on our reports for canine isolates. ▲



Scoure: Devriese LA, et al. Staphylococcus pseudintermedius sp. nov., a coagulase-positive species from animals. Int J Syst Evol Microbiol. 2005 Jul;55(Pt 4):1569-73.

HOW OFTEN DO YOU HEAR ABOUT PRICES DROPPING?

It isn't often in today's economy that you hear about a price dropping for testing of diagnostic samples. Here at Colorado State University's Veterinary Diagnostic Laboratory, we have added a new pricing scale for *Mycobacterium avium* ssp. paratuberculosis serology (ELISA). If you send us at least 100 serum samples, the new price will be only $4 per sample!

CSU VDL has passed the individual 2009 fecal proficiency panel using ESP liquid media that allows us to conduct official testing for the National Johne's Program using this method until Dec. 31, 2010. The liquid based system allows *M. paratuberculosis* detection in an average of only 36 days, compared to 12 to 16 weeks using solid media. Using this method we can grow the organism faster and use PCR to determine if it is *M. avium* spp. paratuberculosis and not another *Mycobacterium*.

Cost for liquid culture is $25 per sample. Submit feces or tissues. Pools of up to five samples are accepted. Typical turnaround time is 30-42 days.

Diagnostic Summary Update

Leptospirosis Serology Update

— Doreene Hyatt, PhD, CSU VDL Bacteriology Section Head

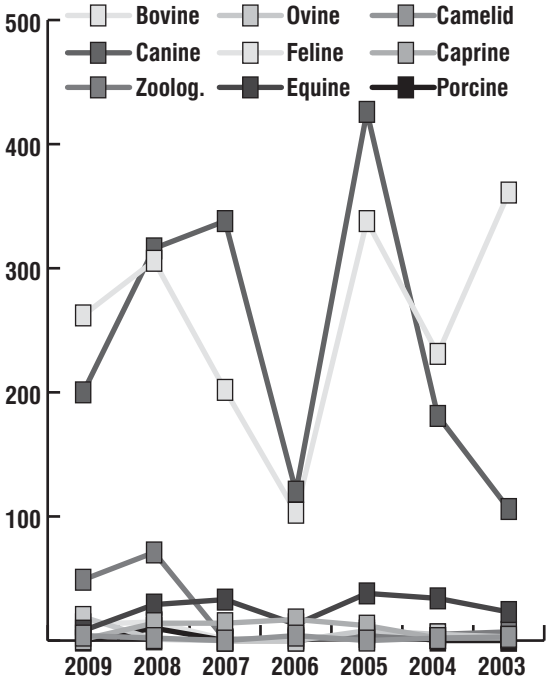
As an update of information given for the past few years, the table at right and chart below total the serum samples tested for titers to *Leptospira interrogans* from 2003 through Aug. 15, 2009.

From Jan. 1 to Aug. 15, 2009, CSU VDL performed a total of 82 tests for *L. brataslava*, with seven positives with a high of 1:1600. Additionally, we conducted 107 tests for *L. brataslava* in 2008, of which 16 were positive, with the highest titer being 1:6400. A total of 97 tests for *L. brataslava* were conducted in 2007; 16 were positive, with the highest titer being 1:3200.

Serology results for samples submitted during the past five years and year-to-date through Aug. 15 for all species are broken down. We report the total number of samples tested (N), the number of positive results (P)—defined as a titer greater than or equal to 1:100—and the highest titer reported during the year (High). ▲

	L. canicola			*L. grippo*		*L. hardjo*		*L. ictero*		*L. pomona*	
	N	P	High	P	High	P	High	P	High	P	High
2004	467	49	800	76	819,200	33	800	102	3200	87	102,400
2005	858	152	1600	135	51,200	117	1600	165	3200	123	12,800
2006	264	51	800	34	6400	21	1600	26	800	38	6400
2007	597	90	1600	105	6400	42	3200	89	3200	98	6400
2008	766	107	6400	157	6400	116	3200	149	6400	128	6400
2009	557	35	1600	80	6400	91	3200	157	3200	106	6400

Lepto positives by species



Advances in Parasitology

Which assay to detect *Tritrichomonas*?

— *Laurie A. Baeten, Kristy Pabilonia, Christina Weller, Jeanette V. Bishop, Kristin Spencer, Lora R. Ballweber*

Multiple assays exist to detect *Tritrichomonas foetus* in cattle, and significant differences in sensitivity and specificity have been reported. Here we provide data for the comparison of three different methods of detection for *T. foetus* in bovine preputial scrapings. Veterinarians collected 99 samples under field conditions for diagnostic testing in this study. Culture using the commercially available InPouch TF® (Biomed Diagnostics, San Jose, Calif.) was compared to conventional polymerase chain reaction (cPCR) and real-time polymerase chain reaction (rtPCR). Upon receipt, the InPouch TF® samples were incubated at 36.5° C for 72 hours and then evaluated by microscopy. For cPCR, genomic DNA was extracted from the In Pouch TF® samples using the ZR Fecal DNA kit (Zymo Research, Orange, Calif.) for cPCR. For rtPCR, the Ambion MagMAXTM-96 Viral RNA Isolation kit (Applied Biosystems, Foster City, Calif.) was used according to manufacturer's modifications for InPouch samples. The PCR reaction was carried out as previously described for cPCR.[1,2] For rtPCR, the newly developed VetMAXTM *T. foetus* test kit (Applied Biosystems, Foster City, Calif.) was used. This kit includes a XenoTM DNA internal control which provides an evaluation of DNA degradation during the assay.

The kappa statistics of correlation between culture and cPCR, culture and rtPCR, and cPCR and rtPCR were 0.845, 0.863 and 0.846, respectively (>0.845 very good correlation). It appears the three testing methods are similar in their ability to detect *T. foetus* in bovine preputial samples.

We also conducted a separate pilot study, designed to begin evaluation of the effect of InPouch® shipping conditions on cPCR results. The number of organisms from a stock culture was determined and dilutions made in Diamond's media. These were then inoculated into InPouch TF® samples, collected by veterinarians under field conditions for diagnostic testing, to yield 12.5 and 25 organisms per mL (less than the 50 organisms per mL reported sensitivity[2] for this cPCR). Pouches were stored vertically for approximately 48 hours, at either room temperature (21° to 23° C), refrigerated (4° C) or frozen (-20° C). Genomic DNA was extracted and cPCR conducted, as above. Six replicates of 12.5 organisms per mL and three replicates of 25 organisms per mL were evaluated.[2] For dilutions of 12.5 organisms per mL, cPCR detected *T. foetus* in five of six pouches held at each of the three temperatures. For dilutions of 25 organisms per mL, cPCR detected *T. foetus* in two of three, one of three and three of three pouches held at room temperature, 4° C and -20° C, respectively.

These early results indicate freezing may be the preferred shipment method for T. foetus when using cPCR, however additional data is needed. Similar studies to determine proper shipping techniques would be useful for rtPCR. ▲

REFERENCES

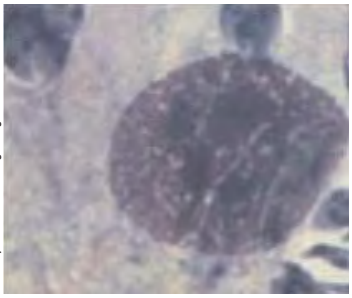
1. **Felleisen RS, Lamelet N, Bachmann P, Nicolet J, Müller N, Gottstein B. Detection of Tritrichomonas foetus by PCR and DNA enzyme immunoassay based on rRNA gene unit sequences. *J Clin Microbiol*. 1998 Feb;36(2):513-9.**
2. **Felleisen RS. Comparative sequence analysis of 5.8s rRNA genes and internal transcribed spacer (ITS) regions of trichomonadid protozoa. *Parasitology*. 1997 Aug;115 (Pt 2):111-9.**

Advances in Parasitology

Arctic foxes a definitive *N. caninum* host?

— *Stacey A. Elmore,[1] Todd M. O'Hara,[2] and Lora R. Ballweber[1]*
[1]Colorado State University, Veterinary Diagnostic Laboratory;
[2]Department of Biology and Wildlife and Institute of Arctic Biology, University of Alaska Fairbanks

Little is known about the sylvatic cycle of *Neospora caninum* in arctic and subarctic ecosystems. Serosurveys have shown exposure to the organism in caribou (*Rangifer tarandus*), musk-ox (*Ovibos moschatus*), gray wolves (*Canis lupus*) and others, but the definitive hosts in these ecosystems have not yet been identified. Domestic dogs and coyotes (*Canis latrans*) are the only confirmed definitive hosts for *N. caninum*, although there is reason to speculate that red foxes (*Vulpes vulpes*) and gray wolves may also be. Given this, coupled with the close taxonomic relationship between red and arctic foxes, it is possible arctic foxes could serve as definitive *N. caninum* hosts.



Courtesy Intervet Schering Plough

In this study, we opportunistically collected gastrointestinal tracts from 120 arctic foxes and froze them at -80° C until processing. Feces were recovered and analyzed using a nested polymerase chain reaction (PCR) protocol with the previously described primers Np6+/Np21+ and Np6/Np7, which targets the Nc5 region. PCR analysis was followed by the sequencing of amplicons. Identity was confirmed through comparisons with known isolates on GenBank. We detected *N. caninum* DNA in the feces, suggesting the voxes are exposed through hunting, scavenging or, possibly, coprophagy. Further research will address the intermediate host status of arctic canid prey species and continue arctic fox fecal surveys for evidence of natural infections. ▲

CSU VDL in the Field: Disease Updates

BVD and *T. foetus* Testing

BOVINE VIRAL DIARRHEA VIRUS UPDATE

BVD continues to be a hot topic in the beef and dairy industries. During the last fiscal year, the Colorado State University Diagnostic Laboratory system conducted 76,751 BVD tests using AC-ELISA or PCR. Test results were positive on 402 samples, or 0.5 percent. Approximately one-third of the tests were confirming previous positive results, so an estimated prevalence of BVD Persistent Infections (PI) in tested cattle for last year was 0.35 percent, a decrease over 2007. A breakdown of testing by laboratory is shown in the table below, with the previous year's data in parenthesis.

The majority of BVD PI testing is done through the use of a pooled testing strategy. Rocky Ford is the only CSU laboratory offering pooled testing. Last year, Rocky Ford performed 1,758 pooled tests for BVD (average pool size 39 samples) and found 105 positive pools. Follow-up testing of the 105 positive pools identified 287 AC-ELISA positive samples, with a calculated individual prevalence of 0.38 percent.

Once initial herd testing is accomplished and positives removed, it is extremely important to continue to monitor the herd status each year, and ***always*** test newly purchased animals before they are introduced into the herd.

— Jim Kennedy, DVM, MS, Director, CSU VDL Rocky Ford Branch.

T. FOETUS TESTING UPDATE

In the past year an increasing number of states have begun or implemented a statewide *T. foetus* testing program. All states west of a line through the eastern border of Kansas — except Kansas — now mandate some form of testing. Arkansas, Louisiana and Iowa have also either instituted mandatory testing or are in the process of developing it.

Colorado began a voluntary program in 2002 which was later replaced by a mandatory program. CSU VDL is the leader in *T. foetus* testing for the state of Colorado and provides testing for neighboring states. The table below reflects the testing performed by the CSU Laboratories — a lot of bull! If each bull we tested were assigned the duty of covering 50 cows, CSU's *T. foetus* testing was responsible for the reproductive performance of over a million cattle, or one-32nd of the entire U.S. beef-cow population. ▲

BVD TESTING 2008

Lab	Test Method	Number Tested	Number Positive
Fort Collins	AC-ELISA	1369 (2345)	37 (47)
Grand Junction	*	172 (131)	3 (3)
Rocky Ford	Pooled PCR and AC-ELISA	75,215 (72,305)	362 (436)
Total		76,751 (74,781)	402 (516)

** Grand Junction samples forwarded to Rocky Ford for testing. Numbers in parentheses represent prior year numbers.*

TRICH TESTING 2008

Lab	Test Method	Number Tested	Number Positive
Fort Collins	Culture	1254	1
	PCR	1188	104
Grand Junction	Culture	2902	18
Rocky Ford	Culture	1902	44
	PCR	3706	373
	Pooled PCR*	2710	89
Total		21617	540

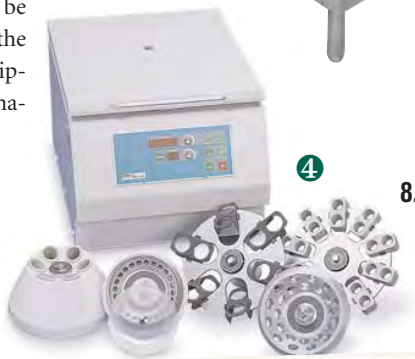
** Represents 10,665 animals.*

Lab Updates

Mission-Critical Equipment

The CSU VDL has some special equipment needs. If you are considering a gift, your support of equipment would be particularly effective in helping the VDL achieve its mission. Any of the items listed here can be sponsored in honor of someone special and will remain so named for the life of the equipment. A representative of the VDL would be pleased to discuss and explain the need for and the uses of the equipment. Or for additional information, contact:

Dr. Debra Kamstock
(970) 297-1281
Debra.kamstock@colostate.edu



1. SPECTRAFUGE 24D $1,495, non-refrigerated microfuges (2 needed).

2. TISSUE-TEK VIP 6, $100,000, vacuum infiltration processor.

3. BIRO 3334 BANDSAW, $6,750, precision pathology bonesaw.

4. Z400K CENTRIFUGE, $9,500, refigerated tabletop.

5. DAKO AUTOSTAINER, $100,000, automated horizontal IHC slide-processing system.

6. AVANTI JHC, $25,000, high-capacity, high-throughput centrifuge system.

7. ABI 7900, $80,000, fast, real-time PCR thermocycler.

8. GELDOC-IT 310, $11,000, self-contained gel imaging system.

CSU VDL Establishes Endowment Fund

Your Support Matters

Join the new CSU VDL Endowment Fund and help make a significant impact on the future growth of our mission of service, teaching, research and outreach. Currently, most revenues to operate and support the CSU VDL are generated via fees for service. This limits scientific progression, expansion and education. Increased financial support through the VDL Endowment Fund will remove those limits and provide increased opportunities to pursue research, aid field investigations, support the development of new technologies for disease diagnosis and aid in the expansion of current services offered to our clients. It will provide an avenue for upgrading outdated equipment and allow for continued educational advancement for both our clients and future diagnosticians. It will further our mission of promoting and protecting animal and human health.

We invite you to join us on this mission. Please assist us in reaching our initial goal of $25,000 to establish and grow the Endowment Fund. You may contribute by completing the form below or by visiting us online at www.dlab.colostate.edu and clicking "Support the DLAB online."

We would like to extend our sincere gratitude to an inaugural donor, who has requested anonymity, for the initial contribution that has allowed us to launch this endeavor. Thank you for supporting the VDL and our mission.

YES! I want to make my support matter…today and for the future

I would like to help the CSU Veterinary Diagnostic Lab continue and strengthen its mission of promoting and protecting animal and human health.

Detach and Mail

Please accept my check for a gift of $ _____
(Payable to Colorado State University Foundation Veterinary Diagnostic Laboratory)

Name _____

This gift is from ❑ Me ❑ My spouse and me ❑ My partner and me

Spouse's/Partner's Full Name _____

I would like to make this gift in honor of:

_____

Please feel free to make your gift in honor of a friend, family member, other individual, pet or organization who has inspired you to support us.

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Your Name

_____
Address*

_____
City, State, ZIP*

_____
Phone (with area code) E-mail

❑ Charge this gift of $ _____ to my/our
❑ VISA ❑ Mastercard ❑ American Express

Card Number _____ Expires ___/___
(mm / yy)

Name on Card _____

Signature _____

Use this gift for: ❑ General purpose, to include education, training, equipment, operations
❑ No specific designation ❑ Diagnostic investigations/research only
❑ Other _____

Please return this form with your gift to:
Colorado State University Foundation, P.O. Box 1870, Fort Collins, CO 80522
Or go to www.dlab.colostate.edu and click "Support the DLAB online"

* Necessary information so we can confirm receipt of your contribution and thank you for your support. By contributing to the CSU VDL you become a member of our family and a supporter of our cause. As such, you will automatically receive our biannual newsletter *LabLines* so we can keep you updated on the lab's activities, from investigations to interesting diagnostic cases to test development to comings and goings of residents, post-docs, graduate students and faculty.
❑ If you prefer not to receive this mailing please check here. All donations are charitable.

61713/V1013

CSU VDL In Press

A Roundup of VDL Faculty Research

Van Campen H. Epidemiology and control of BVD in the U.S. *Vet Microbiol*. 2009 In Press.

Virology Section Head Hana Van Campen reviews the incidence of Bovine Viral Diarrhea Virus in the United States, and examines the management practices that put dairy and beef herds at risk of contracting BVD disease. In general, producers appreciate little or any felt need for regional or national eradication programs, preferring to rely heavily on commercial vaccines. "The lack of a clear danger is compounded by the 'Gambler's' mentality among individual cattle producers," she writes. "The low herd prevalence validates the general belief that the application of BVDV vaccines is a 'cure-all' rather than an aid in prevention of BVDV infections."

U'ren L, Guth A, Kamstock D, Dow S. Type I interferons inhibit the generation of tumor-associated macrophages. *Cancer Immunol Immunother*. 2009 Oct 14. in Press.

Debra Kamstock, VDL Pathologist, collaborates with CSU Department of Microbiology, Immunology and Pathology members to use mouse tumor models in an investigation of effects of endogenously produced type I interferons on the generation of tumor-associated macrophages (TAM). Using immunohistochemistry and flow cytometry, the team found TAM density was significantly increased in tumors lacking the type I IFN receptor, and that increased TAM density was associated with a significant increase in tumor growth rate and angiogenesis. Their findings indicate that endogenously produced type I IFNs suppress the generation of TAM, which may in turn account for inhibition of tumor growth and angiogenesis.

Ballweber LR. Ecto- and endoparasites of new world camelids. *Vet Clin North Am Food Anim Pract*. 2009 Jul;25(2):295-310.

Although great strides have been made in understanding the biology, treatment and control of internal and external parasites of llamas, alpacas, guanacos and vicunas, new challenges have arisen, says CSU Parasitology Section Head Lora Ballweber in her review. Parasites that were unknown 15 years ago have now been described, and researchers are only beginning to figure out their implications for camelid health. New issues, including anthelmintic resistance, will require changes in approach to parasite control. Continued surveillance is critical, not only in our fight against these parasites, but to recognize the next parasitic threat for the new world camelids.

Hall JS, et al. Influenza infection in wild raccoons. *Emerg Infect Dis*. 2008 Dec;14(12):1842-8.

Raccoons can become infected, transport and potentially transmit both avian and human influenza viruses, according to a study involving Kristy Pabilonia, VDL Avian Diagnostics and BSL3 Operations Section Head. Dr. Pabilonia collaborated with USDA on its experiment which infected two raccoon cohorts with both H4N8 and H3N2 flu subtypes. Half the avian-flu group and all the human-flu group subsequently shed the virus.

Devitt JJ, Maranon DG, Ehrhart EJ, Bachand AM, Lana SE, LaRue SM. Correlations between numerical chromosomal aberrations in the tumor and peripheral blood in canine lymphoma. *Cytogenet Genome Res*. 2009;124(1):12-8.

VDL Pathologist EJ Ehrhart joins a study with CSU's Animal Cancer Center showing an alternative, less invasive method for evaluating canine lymphoma compared to the current standard tumor cell evaluation may be possible. By excising one lymph node of dogs known to have lymphoma for immunophenotyping and cytogenetic analysis, sampling and culturing a peripheral blood sample for cytogenetic analysis and using a bone marrow aspirate for staging purposes, the team showed significant correspondence between numerical aberrations in the tumor and the peripheral blood. When tumor analysis is impossible, peripheral blood offers a viable option for cytogenetic assessment. ▲

LRN™
LABORATORY RESPONSE NETWORK

CSU VDL now offers Laboratory Response Network real-time PCR testing for *Bacillus anthracis*, *Brucella* spp., *Coxiella burnetii* (Q fever), *Francisella tularensis* (tularemia) and *Yersinia pestis* (plague). LRN is an integrated national and international network of laboratories fully equipped to respond quickly to acts of terrorism, emerging infectious diseases, and other public health threats. Cost for the real-time PCR is $60 per sample.

Guardians of Public Health

Imag(in)e This: Educational Virtual Microscopy System

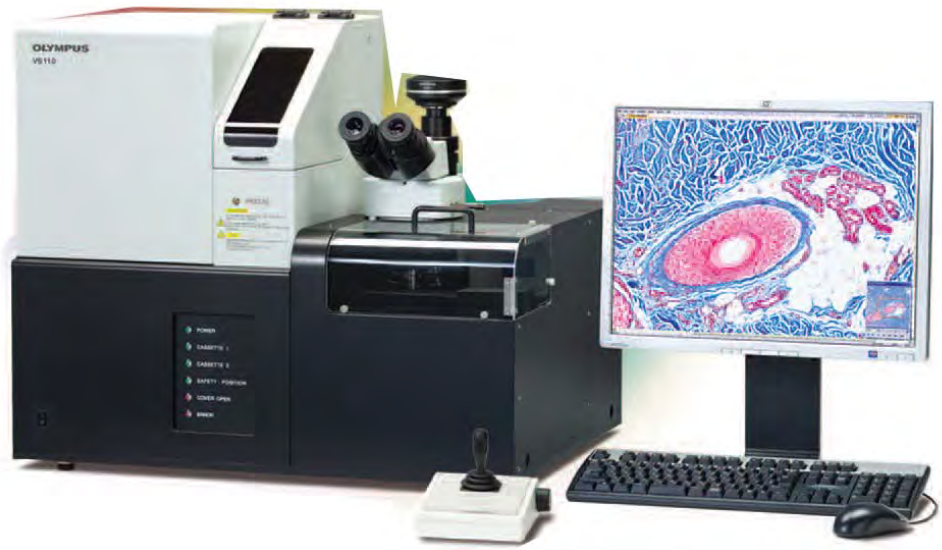
— By Lora Ballweber, DVM, MS, Parasitology Section Head

Refocusing the world's attention on the critical public health issues of vector-borne diseases, such as malaria, heartworm and Lyme diseases, to name a few, has also refocused attention on the role of veterinary parasitologists in animal and human health.

Although we will meet the current and future demands of vector-borne disease control through a variety of means, education is a key step. This education takes various forms—education of veterinary students, continuing education of practicing veterinarians, and education of both DVM and non-DVM graduate students on the latest breakthroughs to detect, treat and prevent parasitic diseases.

To help us meet the needs of that continuing education, both for practitioners and for veterinary students, CSU VDL is actively soliciting donations for the purchase of a virtual microscopy system. Acquisition of this system will allow us to create parasitology modules that will be web-based, thus allowing for distance education. The practitioner or veterinary technician can polish their skills by completing the modules in their clinic rather than traveling to distant sites. Acquisition of the system will also allow us to incorporate computer-based learning styles into the veterinary curriculum, a style that today's generation of students is very comfortable with.

If we can count on your support in this mission through a charitable contribution, please see page 9 for a giving form to join our CSU VDL Endowment Fund. ▲



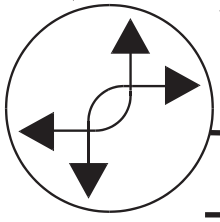
How education through virtual microscopy works

A trained laboratory technician scans slides at high resolution, including multilayer scans for thick tissue samples to create virtual 3-D images.

Virtual slides can be zoomed, viewed and otherwise manipulated in the classroom, just as if using a conventional microscope.

Large groups can observe, manipulate and discuss the same sample, simultaneously.

Images are stored digitally and then distributed through local networks and the Internet.



Clinics can access Web-based diagnostic imaging, as well as on-site education modules.

Researchers can invite colleagues to consult, whether in the same lab or across the globe.

Large numbers of slides can be assembled into databases and library information systems.

REGULAR COLUMNS

LAB LINES

INSIDE THIS ISSUE

Colorado State University
Veterinary Diagnostic Laboratories
College of Veterinary Medicine and Biomedical Sciences
Fort Collins, CO 80523-1644

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Permit Number 19

Update from the Director

Welcome to this issue of *Lab Lines*. The most exciting news since the last edition is the grand opening of our new facilities. Thanks to the support of our clients, our external advisory committee, producer groups, the Colorado Veterinary Medical Association, the CSU system board of governors and the state legislators, we are looking forward to great accomplishments to come in our new facilities. Here's why:

BARBARA POWERS, DVM/PHD/DACVP
DIRECTOR

- It permits us to maintain our accreditation from the American Association of Veterinary Laboratory Diagnosticians, which allows us to continue as one of 12 USDA National Animal Health Laboratory Network core labs with expanded BSL3 laboratory space. We can do surveillance testing to protect agriculture from BSE, Vesicular Stomatitis, Avian and Swine Influenza, Chronic Wasting Disease and others. We can now fulfill our role in CDC's Laboratory Response Network and test for zoonotic diseases, including bioterrorist agents.
- With the new flexible space, we can expand services covering a multitude of disciplines and species to meet the needs of all our clients. We now have room for more modern equipment and increased personnel to conduct testing for bacteria, viruses, parasites, toxins, cancer, nutritional deficiencies, trauma, developmental abnormalities and more.
- This facility gives us room to expand our teaching mission. We now have room to offer hands-on educational experience to address the critical shortage of veterinarians, professionals and qualified technical staff in diagnostic laboratory medicine. We can now adequately educate post-graduate veterinarians and veterinary students, as well as undergraduates. Plus, we now have room to perform research, develop and validate new diagnostic tests, investigate new and emerging diseases, and assist others in their research.

LAB NEWS

Even though we have this wonderful and beautiful new facility, the hard work, dedication and expertise of its inhabitants — our faculty, staff and students — remains key in providing quality and timely service with a smile. ▲

Barbara E. Powers

MISSED AN ISSUE OF *LABLINES*?

Read them all on-line, at www.dlab.colostate.edu/webdocs/news/

Watch for great accomplishments ahead from CSU VDL's expanded, state-of-the-art facilities.

Colorado State University Veterinary Diagnostic Laboratories

LAB LINES

Diagnostic news and trends from the Colorado State University Veterinary Diagnostic Laboratories
Volume 18, Number 1 Spring/Summer 2013

Lab Updates

An Export Testing Success Story. How the Lab Enhances Opportunity

Colorado State University's Veterinary Diagnostic Lab system performs several hundred thousand regulatory tests each year to facilitate the intrastate, interstate and international movement of livestock. But the recent record number of animal tests moved through the VDL's Rocky Ford Lab, in conjunction with the Fort Collins lab and the Rocky Mountain Regional Animal Health Laboratory in Denver, has eclipsed the scope of what we have accomplished in the past. During the past two years, Rocky Ford has tested 55,422 head of breeding cattle purchased from cattlemen throughout the western United States for export to Russia. To the best of our knowledge, the November 2012 shipment on which Rocky Ford processed the regulatory testing was the largest single shipload of live cattle ever exported from the United States. The numbers are staggering:

Cattle	2011-12	2012-13
■ Bovine Leukemia Virus ELISA	54683	57887
■ Johne's ELISA	22311	32911
■ Campylobacter Cultures	877	3415
■ T. foetus PCR, pooled	176	650
Horses:		
■ EIA AGID		96
■ Equine Viral Arteritis Virus SN		24

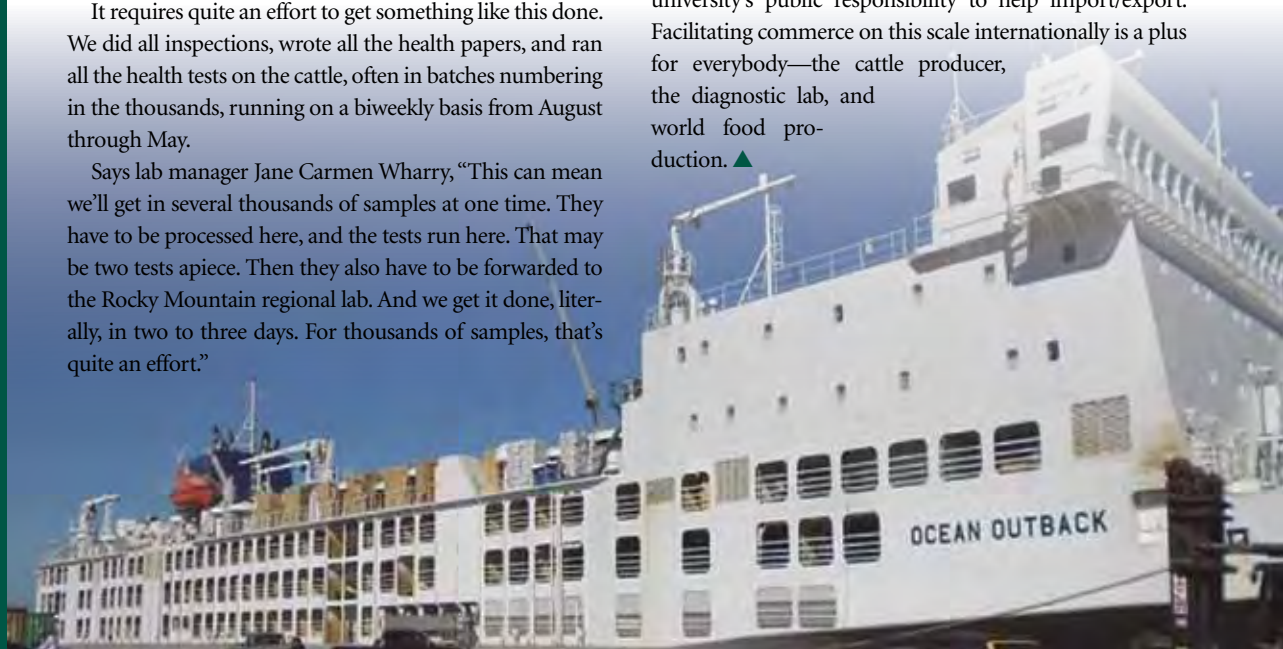
It requires quite an effort to get something like this done. We did all inspections, wrote all the health papers, and ran all the health tests on the cattle, often in batches numbering in the thousands, running on a biweekly basis from August through May.

Says lab manager Jane Carmen Wharry, "This can mean we'll get in several thousands of samples at one time. They have to be processed here, and the tests run here. That may be two tests apiece. Then they also have to be forwarded to the Rocky Mountain regional lab. And we get it done, literally, in two to three days. For thousands of samples, that's quite an effort."

— Gene Niles, DVM, Director of Rocky Ford Veterinary Diagnostic Lab

The testing generated much needed income for the 2012-2013 fiscal year, but more importantly, the work demonstrates the valuable role the public diagnostic lab plays in facilitating private enterprise in the important animal agriculture sector.

In addition to wearing our hat as a diagnostic facility, we also wear a hat in a regulatory function. This fulfills the university's public responsibility to help import/export. Facilitating commerce on this scale internationally is a plus for everybody—the cattle producer, the diagnostic lab, and world food production. ▲



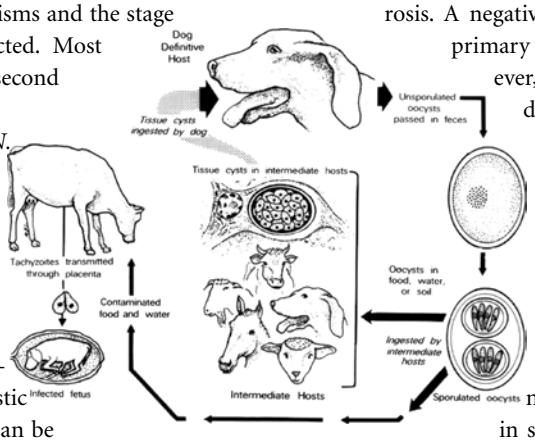
Diagnostic Parasitology

Decoding *Neospora* Diagnostics

— Ashley Malmlov, MS, CSU Microbiology, Immunology and Pathology Postdoctoral Fellow; and Lora R. Ballweber, DVM, MS, CSU VDL Parasitology Section Head

Although the worldwide protozoan *Neospora caninum* infects both dogs and cattle, canids are the definitive host, responsible for excretion of oocysts into the environment. Cattle are an intermediate host and become infected through one of two routes: by consuming oocysts in contaminated feed or water, or by transplacental transmission. Although transmission by oocysts does occur, vertical transmission is associated with the primary clinical issue, abortion. During pregnancy, replication can increase drastically; the parasite invades the placentome and multiplies. It then enters the fetal bloodstream, with subsequent invasion of fetal tissues, particularly those of the central nervous system. The consequences of infection vary with the number and strain of organisms and the stage at which the fetus is infected. Most abortions occur during the second trimester.

Determining whether *N. caninum* is the cause of a particular abortion event is problematic. If available, submission of the aborted fetus is best. The brain is the preferred tissue, followed by heart, liver and placenta. Finding the characteristic lesions in an aborted fetus can be supportive of a diagnosis. Even better is demonstration of the organisms through immunohistochemical staining. Fetal peritoneal fluid or blood can be tested with an ELISA. This test provides only a yes/no answer — whether the animal does or doesn't have IgG antibodies against the parasite.



A positive result indicates the fetus was infected and made antibodies to the organism; therefore, the likelihood of an *N. caninum*-induced abortion increases. Unfortunately, a negative result is not very informative. In young fetuses, the immune system may not yet be mature enough to mount an immune response. If the fetus is old enough, the infection may be in the acute phase and not yet producing IgG. Blood from the dam can also be tested by ELISA. A positive result, however, only raises the index of suspicion for neosporosis. A negative result may imply another primary cause for the abortion; however, antibody levels do fluctuate during pregnancy and may drop below detection limits.

Thus, a single test on an aborting cow is not helpful. A better approach is to evaluate the herd. Identify two sets of animals: those that have aborted and a second set of pregnant animals matched to the first set in stage of gestation and animal age. If the prevalence of *N. caninum* antibodes of the aborting group is higher than the control group, then neosporosis is the probable cause of abortions. However, if the prevalences are similar in both groupings of animals , then *N. caninum* is probably not the cause of abortion. ▲

Further reading

Dubey JP, Schares G. Neosporosis in animals – The last five years. *Vet Parasitol*. 2011 Aug 4;180(1-2):90-108.

NEOPSPORA TESTING THROUGH CSU VDL

ELISA SEROLOGY

- **Submit 1-2ml serum on ice**
- **Cost: $10 each for 1-10 tests $7 each for >10 tests**

FETAL TESTING

- **Submit entire fetus or tissue.**
- **Cost: $60 includes complete work-up for other abortifactive agents**

CSU VDL in the Field: Case Study

Sampling Hooves to Confirm Selenium Toxicity

— *Dwayne Hamar, PhD, Chemistry and Toxicology Section Head; Cathy Bedwell, Chemist; and Charlie Davis, DVM, CSU VDL Lab Coordinator*

During autumn 2012, 24 performance horses that were not being used were grazing a summer pasture northwest of Ft. Collins. In late August, one horse was lame and removed from the pasture. By the end of September, X-rays revealed a severe rotation of the coffin bone. The animal was euthanized and necropsied at the CSU Veterinary Diagnostic Lab. At that time, various tissues were sampled and frozen.

CAREFUL TARGETING IS CRITICAL

The first week in October, two additional horses were lame. Both animals exhibited horizontal hoof separation about 2 cm from the hair line and severe tail hair loss. Blood samples for selenium analysis were obtained from the two affected horses and three clinically normal horses from the herd. Hoof samples for selenium analysis were obtained at the area of the hoof separation from the two affected horses and from the hoof of the horse that was euthanized. The blood selenium concentrations averaged 0.41 ppm, with a range of 0.39 to 0.47 ppm. Normal equine blood selenium concentrations range from 0.17 to 0.25 ppm. Blood selenium concentrations greater than 1.10 ppm are considered toxic. The hoof selenium concentrations averaged 5.64 ppm, with a range of 4.78 to 6.82 ppm. Normal equine hoof selenium concentrations range from 0.60 to 1.20 ppm, with concentrations greater than 5.00 ppm considered toxic.

These results clearly demonstrate the importance of carefully targeting the area of the hoof that is sampled for selenium analysis. When the hoof is sampled too far above or below the horizontal separation, the ability to confirm selenium toxicity is impeded.

We recommend submitting whole blood (GTT or PTT) and hoof taken from the separation line to confirm suspected cases of selenosis. ▲

- **Selenium is an essential micronutrient.**
- **The major known function is it incorporation into glutathione peroxidase.**
- **Selenium can exist in tissues as either the inorganic or organic form.**
- **In the organic form, it may replace sulfur in amino acids. Because seleno-amino acids don't form disulfide bonds, excess selenium decreases the mechanical strength of hair and hooves.**

The hoof of the euthanized horse. The sample for selenium analysis was taken at the horizontal separation line.

The hoof of the euthanized animal with three core samples removed for selenium analysis. The Se concentrations from the top, middle and bottom cores were 2.13, 6.30 and 0.52 ppm, respectively.

SELENIUM TESTING THROUGH CSU VDL

- **Submit submit 1 to 2 mL whole blood or hoof tissue**
- **Cost: $20**

Food Animal Production Medicine

Our Dairy Production Focus Grows

The preface to the 2011 edition of *Dairy Production Medicine* recognizes the reality today's veterinarian faces when serving modern, intensive animal operations: "During the last 30 years, the role of veterinarians working with dairy cattle has changed.... Dairy production medicine...is multidisciplinary and includes clinical medicine, economics, epidemiology, food safety, genetics, human resource management, nutrition, preventive medicine, and reproduction. These specialities must work in concert to harmonize management of the individual dairy farm in order to obtain a profit...."

— Dave Van Metre, DVM, DACVIM, Professor, CSU Animal Population Health Institute

To fulfill its role, CSU's Department of Clinical Sciences and Veterinary Diagnostic Lab now offer access to the services of two new dairy production medicine specialists in the Diary Population Health Management program. They are on-site to work through local veterinarians to address herd-level issues that might be aided by their skills in records analysis, cow mortality, epidemiology, transition-cow management and other skills. ▲

Jessica McArt, assistant professor in Dairy Population Health Management, received her DVM in 2007 from Cornell, where she spent two years as an intern and resident in the school's Ambulatory and Production Medicine Clinic. While there, she investigated the efficacy of assessing corpus luteum function through ultrasonography to improve reproductive synchronization programs. She received her PhD in December 2012, focusing on the epidemiology, economics and treatment of early lactation subclinical ketosis and postpartum immune function. She hopes to continue her work in transition-cow wellbeing and management, herd-level risk factors for excessive negative energy balance, and preventive measures during the dry and early postpartum periods.

Craig McConnel, a Washington State 2002 DVM, interned in ruminant medicine at University of Sydney before completing a clinical studies masters investigating pinkeye and *Moraxella bovis* vaccinal antigens. At CSU, he completed a PhD exploring epidemiology of dairy cow mortality, examining management and pathologic aspects and record-keeping options regarding culls. As former lecturer in ruminant health at Australia's Charles Stuart University, he was involved in research quantifying E. coli O157:H7 shedding patterns. Now an assistant professor in Dairy Population Health Management, he will continue that research, as well as pursue his interests in mortality and dairy-cow welfare that began with the Colorado Dairy Health Management Survey initiated within the Integrated Livestock Management program.

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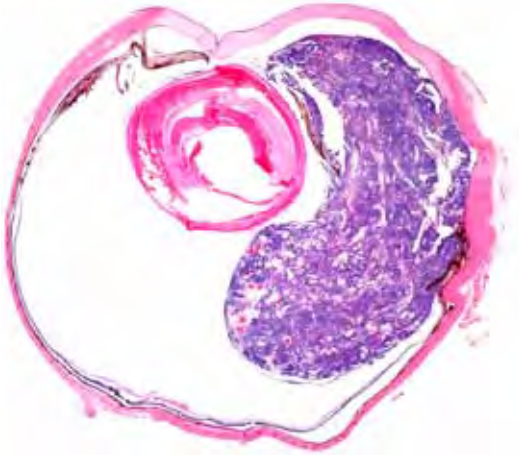
Canine Oncology Innovations

Neuroectodermal Retinal Tumors

A study in a special July ocular pathology issue of *Veterinary Ophthalmology* describes the clinical, histological and immunohistochemical features of primary intraocular primitive neuroectodermal tumors in eight dogs. Four of eight tumors exhibited histological features similar to human retinoblastomas characterized by Flexner-Wintersteiner rosettes and fleurettes, and demonstrated variable immunoreactivity for retinal markers opsin, S-antigen (S-Ag) and interphotoreceptor retinoid binding protein (IRBP). All dogs with tumors displaying histological and immunohistochemical features of retinal differentiation were 2 years old or younger. All four tumors diagnosed as medulloepitheliomas did not display histological and immunohistochemical features of retinal differentiation, and were present in dogs 7 years or older. Age of onset, in conjunction with immunohistochemistry for opsin, S-Ag and IRBP, is an important aid in the differentiation of primary, primitive neuroectodermal tumors arising within the canine ciliary body and retina. ▲

— Daniel P. Regan, DVM, PhD, CSU Microbiology, Immunology and Pathology Postdoctoral Fellow



Tumors evaluated in the study arose from either the ciliary body or retina and consisted of a large, nodular expansile mass which partially filled the anterior and posterior segments, frequently displacing the iris and lens.

Regan DP, Dubielzig RR, Zeiss CJ, Charles B, Hoy SS, Ehrhart EJ. Primary primitive neuroectodermal tumors of the retina and ciliary body in dogs. *Vet Ophthalmol*. 2013 Jul;16 Suppl s1:87–93.

Exotics Medicine

Metastatic Mineralization in Sloths

Metastatic mineralization was diagnosed in 16 captive two-toed sloths ranging in age from 2 months to 26 years old (mean 11.9 years), housed at facilities throughout the United States. Gross and histological lesions were characterized as well as concurrent disease processes. Gross mineralization was detectable at necropsy in five of 16 sloths, and was most prominent in the great vessels, particularly the aorta. Affected vessels were diffusely dilated, firm and brittle with tan plaques that partially occluded the lumen.

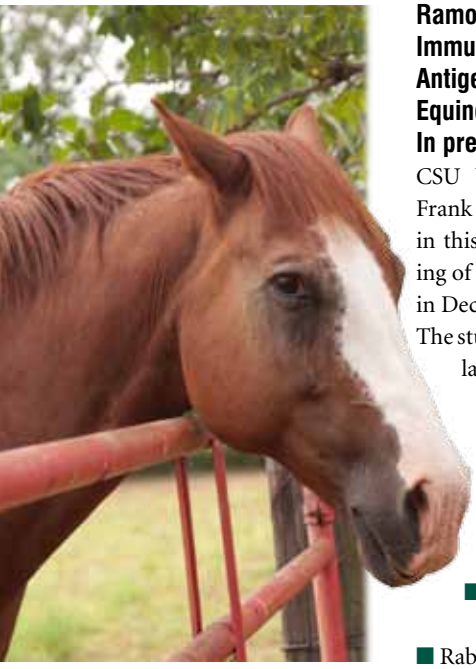
Histologically, vascular mineralization was detected in nine of 16 sloths and varied from moderate to severe, including osseous metaplasia, smooth muscle hyperplasia and degeneration consistent with arteriosclerosis. Mineralization was detected throughout viscera, most commonly in the stomach mucosa (13 of 16 sloths), kidneys (12 of 16 sloths) and lungs (six of 15 sloths), and was associated with mononuclear inflammation and local tissue destruction. Fourteen of 16 sloths had significant renal disease, including two treated for clinical renal failure prior to death. Nine of 12 had mild to severe cardiomyopathy, possibly secondary to severe vascular compromise. Metastatic mineralization is caused by prolonged hypercalcemia. Common causes include high calcium diets, dietary or metabolic calcium-to-phosphorus imbalance, hypervitaminosis D, and primary or secondary hyperparathyroidism. Severe vascular mineralization occurred in sloths in the absence of renal disease. ▲

— Sushan Han DVM, PhD, DACVP, CSU VDL Pathologist; and Michael M. Garner, DVM, Dipl ACVP, Northwest ZooPath

"Hoffmann's two-toed sloth," © 2011 Geoff Gallice, via Creative Commons Attribution license

CSU VDL In Press

A Roundup of VDL Faculty Research



Ramos-Vara JA, Frank CB, Dusold D, Miller MA. Immunohistochemical Expression of Melanocytic Antigen PNL2, Melan A, S100, and PGP 9.5 in Equine Melanocytic Neoplasms. *Vet Pathol*. 2013 In press.

CSU Veterinary Diagnostic Lab Pathologist Chad Frank cooperated with a team of Purdue pathologists in this study, first reported at the 2012 annual meeting of the American College of Veterinary Pathologists in December and now in press at *Veterinary Pathology*. The study searched both Purdue's and CSU's diagnostic lab databases for cases of either poorly pigmented or unpigmented excisional biopsies of equine melanocytic neoplasms. The team eventually identified 50, which were then tested against four human and canine markers:

- Mouse monoclonal antibody antimelanoma marker clone PNL2
- Mouse monoclonal antibody anti–Melan A, clone A 103m
- Rabbit polyclonal antibody anti-S100 protein
- Rabbit polyclonal antibody anti–PGP 9.5

The study followed published procedures validated in canine tissues, and semiquantitatively evaluated immunoreactivity to evaluate possible cross-reactivity of monoclonal antibody PNL2, normal tissues and various nonmelanocytic tumors. They also evaluated immunoreactivity for all four markers based on cell phenotype and whether the cell location was superficial vs. deep.

They found PNL2, PGP 9.5, and S100 protein were detected in all 50 neoplasms. PNL2 was not expressed in 62 nonmelanocytic tumors (equine sarcoids, schwannomas, carcinomas, sarcomas, endocrine tumors, sex-cord stromal tumors, germ cell tumors, and leukocytic tumors) or in normal tissues other than epidermis. They showed antibody PNL2 to be a sensitive marker and more specific than S100 protein or PGP 9.5. In contrast, none of the neoplasms expressed Melan A.

Aboellail TA. Pathologic and immunophenotypic characterization of 26 camelid malignant round cell tumors. *J Vet Diagn Invest*. 2013 Jan;25(1):168-72.

VDL Pathologist Tawfik Aboellail's study of 20 alpaca and 6 llama lymphoma or leukemia cases from the VDL database over a seven-year period employed immunohistochemistry using four markers:

- A T-cell marker (cluster of differentiation [CD]3)
- A B-cell marker (paired box protein [PAX]-5)
- A leukocyte integrin beta-2 marker (CD18)
- A neuroendocrine marker (synaptophysin)

Aboellail broadly categorized clinical disease and postmortem findings in the animals into two syndromes in each species:

- T-cell juvenile disseminate in alpacas, in 40 percent
- Adult multicentric lymphomas in alpacas, in 60 percent
- Adult multicentric lymphomas in llamas, in 83 percent
- Adult epitheliotrophic lymphomas in llamas, in 17 percent.

He suggests further work using human markers to continue refining classification of camelid lymphoma and leukemia, especially the lymphoblast non–B-cell, non–T-cell lymphomas, using CD7, B-NKL, M/NKL, and CD4+ and CD56+.

Frank CB, Valentin SY, Scott-Moncrieff JC, Miller MA. Correlation of Inflammation with Adrenocortical Atrophy in Canine Adrenalitis. J Comp Pathol. 2013 In press.

VDL's Chad Frank leads another study with his former residency colleagues at Purdue, which compared sections of adrenal glands from 33 dogs with adrenalitis against those of 37 dogs without adrenal lesions. The affected dogs were classified clinically as having hypoadrenocorticism, or Addison's disease (AD), suspected of having AD, not having AD or unclassified. The adrenal inflammation was lymphoplasmacytic in 17 dogs, lymphocytic in four, lymphohistiocytic in one, granulomatous in three and neutrophilic in eight cases.

Adrenal glands from control dogs lacked leucocyte infiltration and had a cortical-to-medullary area ratio of 1.1-to-7.2. All three dogs with AD, eight of the 17 dogs with suspected AD and one of the 11 dogs without AD had a cortical-to-medullary area ratio less than 1.1. Because the area ratio was correlated ($r = 0.94$) with a linear cortical-to-medullary thickness ratio, a thickness ratio of less than 1.1 could also indicate severe adrenocortical atrophy. Severe adrenocortical atrophy was associated typically with lymphoplasmacytic infiltration and nearly complete loss of cortical cells; however, the zona glomerulosa was partially spared in three dogs with lymphoplasmacytic adrenalitis and severe cortical atrophy. In contrast, non-lymphoid inflammation was generally part of systemic disease, multifocal and was unaccompanied by severe adrenocortical atrophy.

Mcleland S, Duncan C, Spraker T, Wheeler E, Lockhart SR, Gulland F. Cryptococcus albidus infection in a California sea lion (Zalophus californianus). J Wildl Dis. 2012 Oct;48(4):1030-4.

VDL Pathology Resident Shannon Mcleland and Pathologists Colleen Duncan and Terry Spraker help detail this case of an approximately 1-year-old male California sea lion found in a canal adjacent to San Francisco Bay and brought to the Marine Mammal Center in Sausalito, where it was examined and treated supportively with oral fluids by stomach tube and IM penicillin G before ultimately dying three days after admission. *Cryptococcus albidus*, a ubiquitous fungal species not typically considered to be pathogenic, was recovered. Yeast morphologically consistent with a *Cryptococcus* spp. was identified histologically in a lymph node and *C. albidus* was identified by an rDNA sequence from the lung. Infection with *C. albidus* was thought to have contributed to mortality in this sea lion, along with concurrent bacterial pneumonia.

To the best of the authors' knowledge, this is the first report of cryptococcosis in pinnipeds, more specifically in a California sea lion, and the first report of *C. albidus* infection in any species of marine mammal. Animals have served as good sentinels of cryptococcal disease in the current emergence of *C. gattii* in the Pacific Northwest and British Columbia. Morbidity and mortality of cetaceans recently contributed to the recognition of *C. gattii* infection within these regions. Surveillance for infectious organisms in marine mammals is logistically challenging; however, even novel fungal organisms should be considered a potential primary, or at least contributing, pathogen. Given the current emergence of *C. gattii* in the region where the sea lion was found, without careful mycologic examination this case might have been attributed to *C. gattii*, and *C. albidus* may not have been recognized as a potential pathogen of pinnipeds. Thus, culture is always recommended to confirm organisms that are identified histologically, and *C. albidus* should be considered as a potential pathogen with a role in marine mammal morbidity and mortality.

Cole PA. Association of canine splenic hemangiosarcomas and hematomas with nodular lymphoid hyperplasia or siderotic nodules. J Vet Diagn Invest. 2012 Jul;24(4):759-62.

Lab Pathologist Pat Cole evaluated histologic sections from 120 cases of splenic hemangiosarcoma and 100 cases of splenic hematoma, along with accompanying information about age, sex, breed and clinical history provided by the submitting veterinarian. Cases were collected from a 30-week period and were selected in consecutive order. Hemorrhagic lesions caused by splenic torsion, trauma or other types of tumors were not included in the study, as well as splenic masses interpreted as hemangiomas, because this diagnosis represented only about 2 percent of the canine splenic masses evaluated at CSU's VDL during the year of the study. The submissions included either the whole spleen or portions selected by the submitting veterinarian. Information about gross appearance was not available for all cases, but many hemangiosarcomas were a single nodule, and some of the hematomas were over 6 cm in diameter.

Lymphoid hyperplasia was present in none of the hemangiosarcoma cases and in 27 percent of the hematoma cases. Siderotic nodules in the capsule or trabeculae were present in 25 percent of hemangiosarcoma cases and in 36 percent of hematoma cases. Hemoabdomen was noted in the clinical history of 54 percent of hemangiosarcoma cases and in 22 percent of hematoma cases. The average age (10.3 and 9.6 years, respectively), sex ratios (slightly more males), and most common breeds (Labrador Retriever, Golden Retriever, and German Shepherd Dog) were similar for the hemangiosarcoma and hematoma cases.

Since lymphoid hyperplasia is much more common in cases of hematoma, the presence of this feature lends support to a diagnosis of hematoma rather than hemangiosarcoma. Signalment, history of hemoabdomen, and presence of siderotic nodules do not point to one diagnosis over the other. In the present series of cases, lymphoid hyperplasia was commonly associated with splenic hematoma but was not seen in hemangiosarcoma cases. This suggests that the presence of lymphoid hyperplasia is supportive of a diagnosis of hematoma, although not diagnostic in itself. Of the factors reviewed, only the presence of lymphoid hyperplasia appeared useful for increasing confidence that a lesion is truly a hematoma and that hemangiosarcoma hadn't been overlooked.

Schaffer PA, Wobeser B, Martin LE, Dennis MM, Duncan CG. Cutaneous neoplastic lesions of equids in the central United States and Canada: 3,351 biopsy specimens from 3,272 equids (2000-2010). J Am Vet Med Assoc. 2013 Jan 1;242(1):99-104.

VDL Pathology post-doctoral fellow Paula Schaffer and Pathologist Colleen Duncan searched the computerized records of CSU's VDL and the teaching hospital at University of Saskatchewan, for 5,141 reports of equine cutaneous biopsy specimens over a 10-year period. A total 3,351 showed histologic evidence of neoplastic disease. They found sarcoid, squamous cell carcinoma, and melanoma were the most common tumors diagnosed. Tumors associated with UV radiation were 2.3 times as common in biopsy specimens received by CSU. Appaloosas and Paints, respectively, were 7.2 and 4.4 times as likely as other breeds to have tumors associated with UV radiation. Thoroughbreds were predisposed to cutaneous lymphoma; Arabians were more likely to have melanomas. Draft and pony breeds were 3.1 times as likely as other breeds to have benign soft tissue tumors. Morgans and pony breeds more commonly had basal cell tumors. Tumors in the perianal region were significantly more likely to be SCC or melanoma while tumors on the limbs were more likely to be giant cell tumor of soft parts. ▲

Diagnostic Sample Quality Assurance

Laboratory Reminders and Updates

Check this quick update on a few quality-assurance reminders from your team at CSU's Veterinary Diagnostic Laboratory:

- When submitting swabs for **PCR testing**, please keep these guidelines in mind:
  - ✓ Use sterile polyester (Dacron) tipped swabs with a plastic shaft. Wooden shafts may inhibit PCR.
  - ✓ Do not use bacterial transport media (ex - Culturette tube).
  - ✓ Send the swab in a sterile red-top tube or other sterile capped tube.
  - ✓ Consult CSU VDL website for detailed submission information, including information on transport media to use for PCR samples.
  - ✓ Please submit samples as soon as possible and ship overnight; degradation can affect results.
  - ✓ Keep samples refrigerated before shipment.
  - ✓ Keep samples cold during transport, with the exception of *Tritrichomonas*.

The CSU Veterinary Diagnostic Laboratory welcomes all questions regarding sample submission to ensure the most accurate results for your testing needs. Please call with any questions or if you require further information at (970) 297-1233, or visit our website at csu-cvmbs.colostate.edu/vdl.

- Both the Fort Collins and Rocky Ford laboratories are approved to receive submissions of Equine Infectious Anemia (Coggins) test forms and report the results back to veterinarians electronically, via either the Global Vet Links or USDA's Veterinary Services Process Streamlining system. However, it's important that you have all your information submitted to the system at the time your sample is submitted to the lab. Many times the tests are completed, but we are unable to post results to the system because the information is not yet there.

- A new name has again been adapted for the bacterium *Arcanobacterium pyogenes*. The proposed new name, *Trueperella pyogenes*, has been introduced because the genus *Arcanobacterium* is not monophyletic. *Trueperella* has been proposed in honor of the German microbiologist Hans Georg Trüper. *Trueperella pyogenes,* also formerly referred to as *Corynebacterium pyogenes* and *Actinomyces pyogenes*, may cause abscesses, mastitis and pneumonia in ruminants and in pigs. ▲

Get to Know the Laboratory

New Members Join the Lab Team

Amy Boyd joins the CSU VDL histopathology lab, where she will be working toward full certification as a histopathology technician upon completion of her training and certification testing.

A native of Greeley, she came to Fort Collins to attend CSU, graduating with a bachelor of science degree in zoology in 2004. She and her husband live in Wellington, where they enjoy all Colorado has to offer, spending their free time snowmobiling, snowboarding, hiking, camping, jet skiing and touring the mountains by motorcycle.

Cassy Grothe joined the lab in September in a lab support position, working in both sample receiving and the virology lab. She earned a Bachelor of Science degree in biology from the University of Texas at Austin and moved to Colorado from Texas in 2008.

Lisa Jackson joined the VDL in March as an administrative assistant II—veterinary transcriptionist. Before recently relocating to Colorado, Lisa worked as an office specialist with the Clark County Office of the Coroner and Medical Examiner in Las Vegas for six years. She has done medical transcription for the past 28 years and brings a wealth of knowledge and experience to the job. She is excited to be here in Colorado and working with the team.

Phil Buxton has a bachelor of science degree from CSU in biology. He has previously worked in two biotech labs in the field of molecular diagnostics, as well as a toxicology lab where he was the quality-assurance officer. He now works for VDL in sample receiving and tissue trimming. He lives in Wellington with his wife, son and daughter.

Wendy Hart joined the VDL in February in lab support, where she handles sample receiving. Originally a native of Palo Alto, Calif., she was raised in Loveland, and eventually began her veterinary career as a veterinary technician at a small animal hospital for five years. She has one daughter who lives in Yuma, who shares her interest in raising and showing American Paint horses. She enjoys working with the people of the lab and the variety of activities she experiences in her daily work.

Lisa Snelling joined the Veterinary Diagnostic Lab in March in a lab support position, working with all of the individual laboratory sections. Lisa is a Colorado native from Kersey. She earned both a bachelor of science as well as a master of science degree in microbiology from the University of Colorado. Prior to joining the VDL, she worked as the biology lab director and instructor for six years at the University of Denver department of biological sciences. ▲

The CSU VDL Advisory Board met in early January to discuss the AAVLD site visit report, future directions, suggestions for new test offerings, suggestions to improve our website, results reporting and billing. Overall they were very complimentary of the service we are providing. Members included:
(Front, from left) Marv Hamann, Dwayne Hamar, Kenny Rogers, Keith Roehr, Sunny Geiser-Novotny, Ed Hansen, Larry Mackey, Steve Wheeler, Joan Bowen, Norm Brown, Tim Hackett and Barb Powers.
(Back, from left) Gene Niles, Del Miles, Leesa McCue, Don Kitchen, Charlie Davis, Kellee Mitchell, Connie Heighes, Jan Carroll, Kristy Pabilonia, Bob Davies and Gary Mason. Also in attendance, but not pictured: Gregg Dean, Mike Gotchey, Pete Hellyer, Ron Kollers, Elisabeth Lawaczeck and Chris Orton.

Guardians of Public Health

Farm Bill Provision Would Help Assure Continued NAHLN Resources

As of presstime, Colorado Senator Michael Bennet was urging the U.S. House of Representatives to find a path forward on a full five-year reauthorization of the Senate-passed Farm Bill, which included his provision to provide a more stable flow of resources for the National Animal Health Laboratory Network (NAHLN). NAHLN monitors animal-borne illnesses that pose significant threats to animal and public health, such as mad cow disease and foot-and-mouth disease. Bennet's provision, first introduced as the Animal and Public Health Protection Act, creates a funding authorization for NAHLN that protects it against the uncertainty of Congress' yearly budgeting process.

"The proper funding authorization for the NAHLN is critical," said Barb Powers, director of CSU's Veterinary Diagnostic Laboratories, "so states, including Colorado with its NAHLN member laboratory, Colorado State University Veterinary Diagnostic Laboratories, can protect their agriculture industries from emerging and foreign animal diseases that can cause economic devastation, and also address our nation's need for a safe, stable and nutritious food supply by protection of not only animal health, but public health."

"Livestock production sits at the heart of Colorado's $40 billion agriculture sector," said Bennet, a member of the Senate Agriculture Committee. "Labs, like the ones at CSU, help support the economic vitality of our livestock industry and protect the public by identifying diseases early and preventing the consequences of potentially devastating outbreaks. This commonsense, yet vital, research yields tremendous economic and public health benefits to Colorado and the entire country."

CSU's VDL is one of the core member laboratories in the NAHLN. State and university laboratories in the NAHLN perform animal-disease diagnostic tests as well as targeted surveillance and response testing for foreign animal diseases. Network labs share information with other labs and public health officials. ▲

CSU VDL International Outreach

VDL Faculty Visit Egypt

In January, CSU Veterinary Diagnostic Lab Pathologist Tawfik Aboellail and VDL Avian Diagnostics and BSL3 Operations Section Head Kristy Pabilonia escorted three veterinary students to Cairo. Aboellail coordinated the experience with Cairo University, his alma mater. Pabilonia serves as the faculty advisor of the International Veterinary Student Association at CSU. The veterinary students spent time learning about veterinary medicine in Egypt, which included meetings with the faculty of Cairo University and tours of dairy, camel and Arabian horse production facilities.

Clockwise, from top left:

- Veterinary students April Zander, Jenn Perez and Edwina Gutierrez visit Cairo University
- Cairo University faculty member Alaa Eldiin Eissa, Tawfik Aboellail, Edwina Gutierrez and Jenn Perez at the Alexandria library
- Kristy Pabilonia at the Great Pyramid of Giza

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

Look for CSU VDL pathologists ***Colleen Duncan***, ***Tawfik Aboellail*** and ***EJ Ehrhart*** attending the ***American College of Veterinary Pathologists*** annual meeting, Nov. 16-20 in Montreal.

VDL pathologist ***EJ Ehrhart*** just returned from a week guest lecturing at New Zealand's ***Massey University***.

VDL Western Slope Laboratory Director ***Don Kitchen***, Rocky Ford Laboratory Director ***Gene Niles***, Pathologist ***Tawfik Aboellail***, Avian Diagnostics and BSL3 Operations Section Head ***Kristy Pabilonia***, VDL Director ***Barb Powers*** and others will be in attendance at the annual meeting of the ***American Association of Veterinary Laboratory Diagnosticians***, Oct. 17-23, in San Diego. ***Aboellail*** will offer an oral presentation on an investigative pathology study, Pathologic lesions and pathogenesis of percutaneous infection of CD-1 mice with western equine encephalitis virus (WEEV)

Pabilonia attended a ***National Poultry Improvement Plan*** contact representative meeting, June 18-20 in Athens, Ga., and will be at a workshop on ***Regional Surveillance and Research for Wildlife-Borne Diseases***, Aug. 6-8 in Fort Collins, and a conference on ***Options for the Control of Influenza***, Sept 4-10 in Cape Town, South Africa.

VDL Chemistry and Toxicology Section Head ***Dwayne Hamar*** will present on his work regarding selenium at this year's ***Colorado Veterinary Medical Association*** annual meeting, Sept. 19-22 in Loveland. Also in attendance will be ***Kristy Pabilonia and*** VDL Lab Coordinator ***Charlie Davis.*** Stop by the booth to meet him.

Davis attended the ***Western States Livestock Health Association*** meeting, March 20-21 in Salt Lake City, where new animal ID requirements dominated discussion of regulatory issues at the meeting. He also represented the lab and its services with a booth at the ***Colorado Livestock Association***/***Colorado Cattlemen's Association*** annual meeting, June 17-19 in Breckenridge, and at the summer meeting of the ***Colorado Wool Growers Association***, July 16-18 in Montrose. VDL Director ***Powers*** also attended the CCA meeting.

Davis and ***VDL Virology Section Head Hana Van Campen*** hosted a University of Nebraska animal science student group with a guided tour of the lab on May 15.

VDL Pathologists ***Colleen Duncan*** and ***Terry Spraker*** traveled to Saint Paul Island, Alaska, to conduct research on northern fur seals. ***Duncan*** will also return to Anchorage in August for polar bear research, and along with ***Pabilonia*** will travel to Hawaii for a reproductive disease study in Hawaiian monk seals.

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INSIDE THIS ISSUE

Update from the Director

BARBARA POWERS, DVM, PHD, DACVP
DIRECTOR

I hope summer is going well for all of you. Please find within this issue some exciting news on export testing that has been done at Rocky Ford over the last year or two, truly an amazing feat for this branch laboratory to complete these high volumes of tests under tight, short deadlines. Also you will find interesting articles on cattle *Neospora* and equine selenium toxicosis. A new section in *LabLines* will highlight cases of exotic animal conditions and diseases. Also included, some interesting articles on retinal tumors in dogs and a summary of a variety of research lab residents and faculty have published.

We have new additions to the laboratories, and these new staff members are listed on page six. A number of the staff previously in these positions have either retired or moved out of state for other opportunities.

We are also very excited to announce that early this year, two new clinicians in Clinical Sciences Dairy Population Health Management joined the college. Although in the Department of Clinical Sciences, we will work closely with them to meet dairy diagnostic needs. Our previous *LabLines* highlighted some of the new tests we offer.

On the federal level, our own Colorado Senator Michael Bennet introduced a bill that was added to the Farm Bill authorizing the National Animal Health Laboratory Network. CSU VDL has been a core laboratory in this network since 2002, but federal funding has been inconsistent and difficult, especially in the last few years. We are extremely pleased that Senator Bennet was able to introduce this bill into the Farm Bill, which the Senate eventually passed. This helps not only the State of Colorado, but also the entire nation to protect our food supply. Earlier in 2013, we met with our Advisory Committee, who offered many useful suggestions for further improvement. Please feel free to contact them or us at any time if you have suggestions for our continued improvement.

We hope to see many of you at the Colorado Veterinary Medical Association Meeting in September or at the American Association of Veterinary Laboratory Diagnosticians Meeting in San Diego in October. I hope your summer and fall goes well.

Sincerely,

Barbara E. Powers

Our thanks to the Colorado Cattlemens Association and the Racing Associates of Colorado for the generous donation of a new microplate absorbance reader for the lab. The reader will help improve the speed and efficiency or our ELISA testing services.