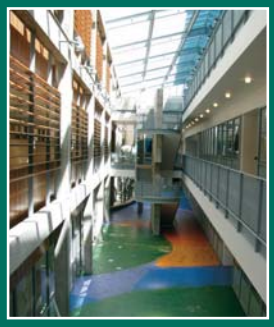
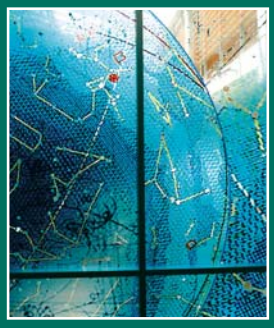
Colorado State University
VETERINARY DIAGNOSTIC LABORATORIES

LAB LINES

Diagnostic news and trends from the Colorado State U[illegible]agnostic Laboratories
Volume 14, Number 1 [illegible]ng/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!

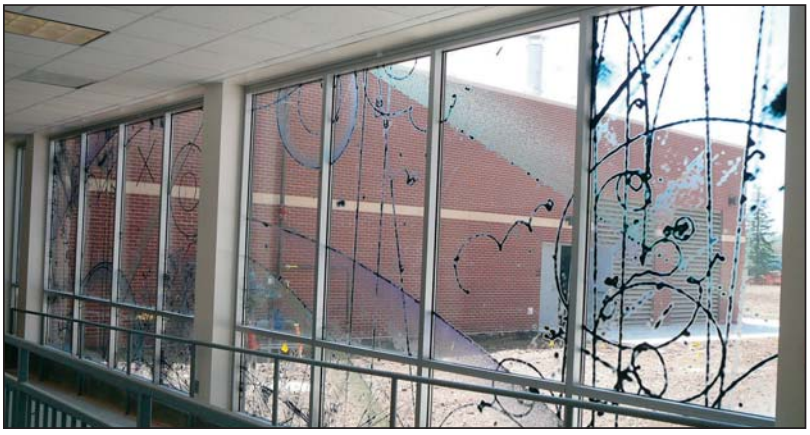
New Diagnostic Medicine Center

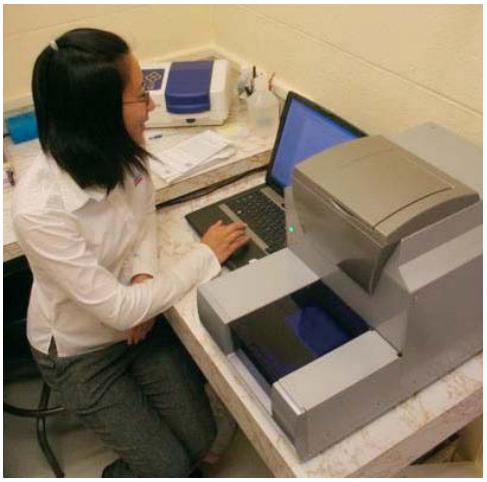
The new Diagnostic Medicine Center is a three-story, 88,000-square-foot facility scheduled for final completion in June. It is adorned with state-required mandatory artwork efficiently integrated into beautifully designed terrazzo floors and etchings on selected glass walls. A central atrium allows natural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

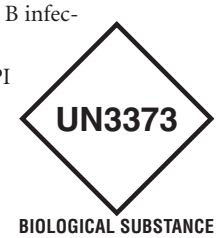
Category B infectious substances must comply with Packing Instruction 650 and 49CFR 173.199. Packaging for samples falling into this category must consist of:

- 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

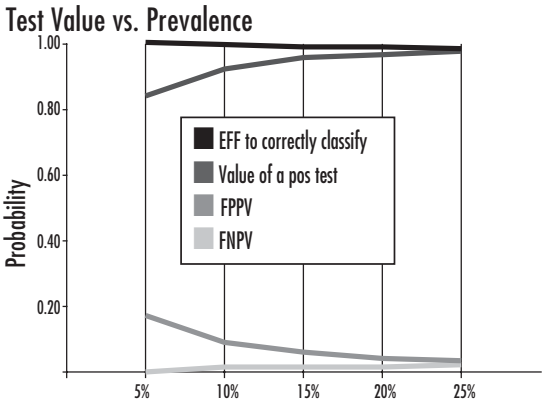
We offer a myriad of diagnostic tests, from gross necropsies to molecular diagnostics. Correct interpretation of such a variety of diagnostic tests certainly requires knowledge of the parameters of the test, e.g. sensitivity and specificity, but those are insufficient when applying the results to make 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.

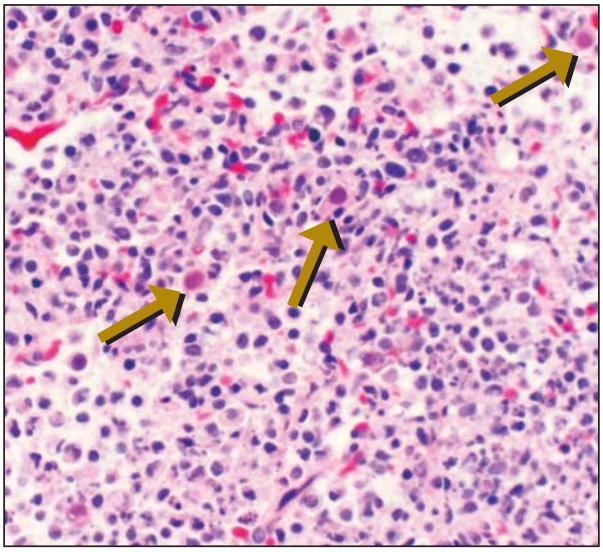
Based on histologic findings within the lung, including the intranuclear inclusion bodies, a tentative diagnosis of canine adenovirus was given. This animal also had marked thymic hypoplasia and splenic lymphofollicular hypoplasia, which is consistent with an immunocompromised state.

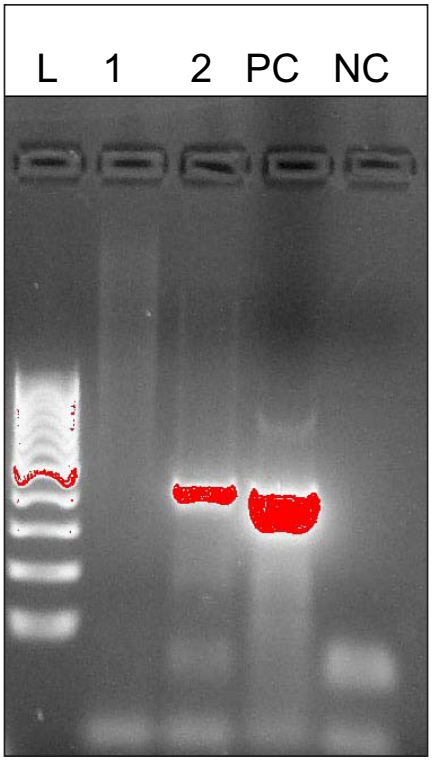
To confirm our suspicion, fresh lung samples were submitted for polymerase chain reaction (PCR) and tested for canine adenovirus, canine distemper and canine herpesvirus-1. PCR following gel electrophoresis indicated positive for adenovirus; PCR for distemper and canine herpesvirus-1 were negative.

— 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

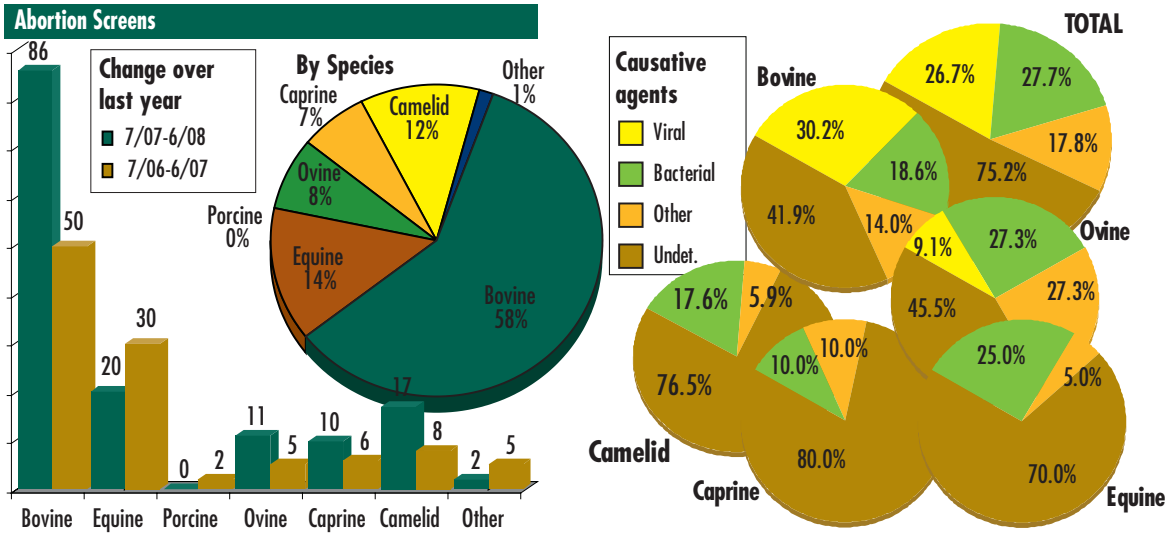
Abortion, Diarrhea Statistics

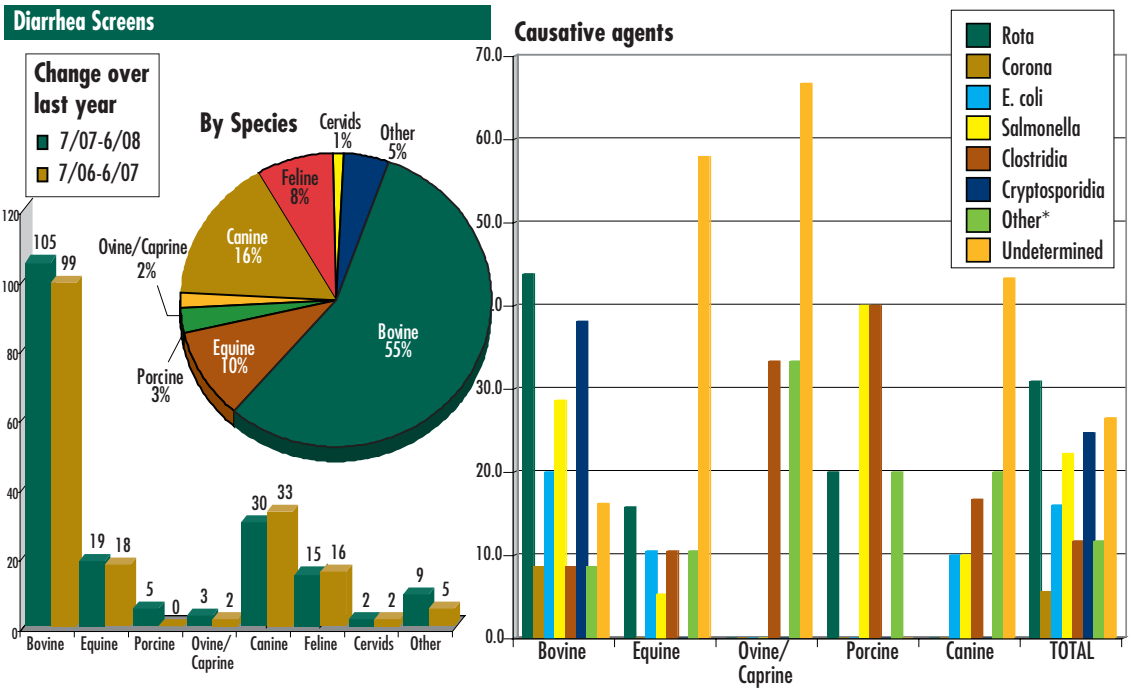
In food animals, quick and accurate diagnosis of infectious abortions calls for the herd veterinarian and a veterinary diagnostic laboratory working together, communicating, sampling and testing appropriately. Yet, still only about half of submitted fetuses result in a definitive diagnosis. Most diagnosed 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.

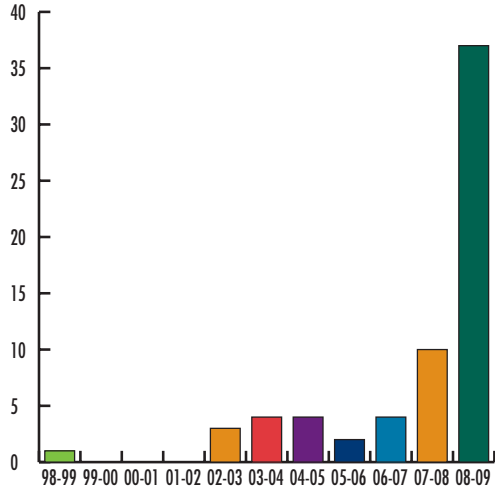
A total of 30 bovine fetuses were IBR FA-positive in 2002-2008. Fetal ages were stated by the submitter or estimated at necropsy to be 124 to 250 days of gestational age. Thirteen fetuses were noted to be moderately to severely autolyzed at necropsy, and 13 had multifocal areas of necrosis in the liver 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.

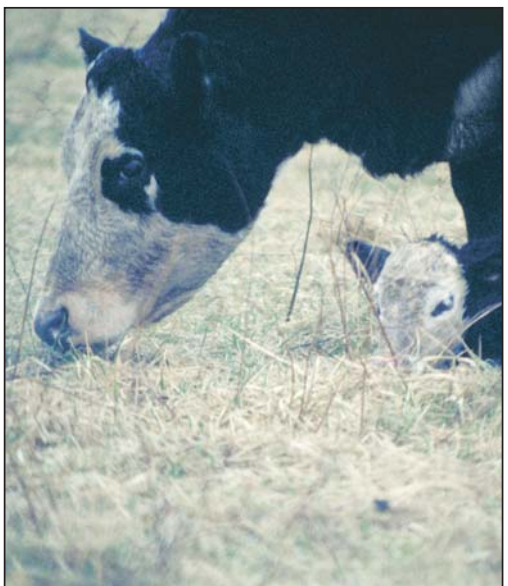
This year, from Oct. 21, 2008, until March 13, 2009, 37 IBR FA+ fetuses representing 26 herds were examined. Fetal ages were estimated to be 150 to 260 days of gestational age. A distinct change from the previous years' cases is that 35 IBR FA+ fetuses were also FA+ for BVDV antigen. Of 16 available 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

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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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❑ Charge this gift of $ _____ to my/our
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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.

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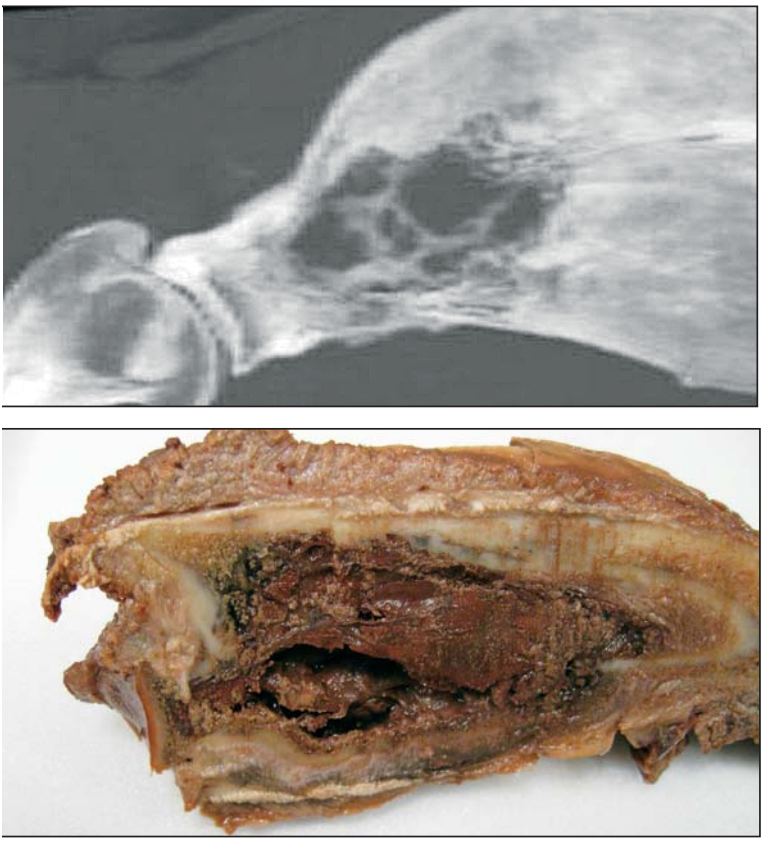
CSUVDL Educational Outreach

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*

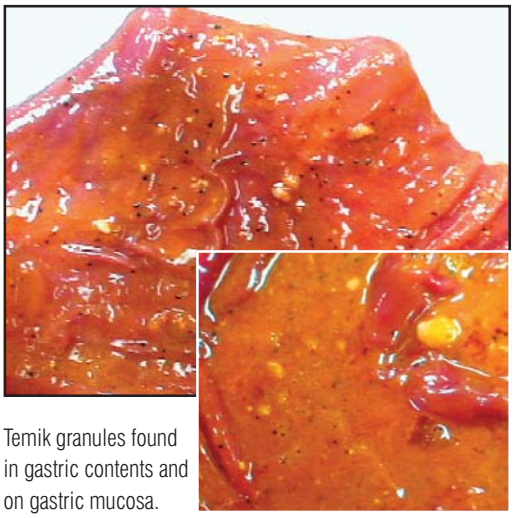
Fast-acting poisons can strike nontarget species so quickly there's no time to lose. One such poison is Temik® aldicarb, a member of the carbamate pesticides which include Carbaryl,® Sevin® and Propxur.® With an LD50 of roughly 1 mg/kg, Temik falls into the category of super-toxin, those with an LD50 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.

EXTERNAL ADVISORY COMMITTEE MEMBERS

Dr. Doug Anderson
Poultry
16634 WCR 33
Platteville, CO 80651

Bob Davies
Wildlife
Colorado Department of Wildlife
6060 Broadway
Denver, CO 80216

Dr. Joan Bowen
Small Ruminant
5036 ECR 60
Wellington, CO 80549

Norm Brown
Equine
8167 NCR 11
Wellington, CO 80549

Terry Fankhauser
Executive Director
Colorado Cattlemen's Ass'n.
8833 Ralston Road
Arvada, CO 80002

Dr. Mike Gotchey
Equine
1878 Lincoln Avenue
Steamboat Springs, CO

Dr. Marv Hamann
Mixed Practice
183 Domingo Drive
Pueblo West, CO 81007

Ed Hansen
Beef Cattle
4554 CR 74E
Livermore, CO 80636

Dr. Dean Hendrickson
Veterinary Teaching Hospital Director
CSU Veterinary
Teaching Hospital
Fort Collins, CO 80523

Dr. Ron Kollers
Small Animal
PO Box 1387
Fort Collins, CO 80522

Dr. Paul Lunn
Clinical Science Dept Head
VTH Dept of Clinical Sciences
Fort Collins, CO 80523

Dr. Larry Mackey
Large Animal
PO Box 336204
Greeley, CO 80632

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

HOW TO CONTACT US

Colorado State University Diagnostic Laboratories
300 West Drake
Fort Collins, CO 80523
Phone (970) 297-1281
Fax (970) 297-0320
E-mail: dlab@colostate.edu

Arkansas Valley Animal Disease Diagnostic Laboratory
27847 Road 21
Rocky Ford, CO 81067
Phone (719) 254-6382
Fax (719) 254-6055

Western Slope Animal Diagnostic Laboratory
425-29 Road
Grand Junction, CO 81501
Phone (970) 243-0673
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.

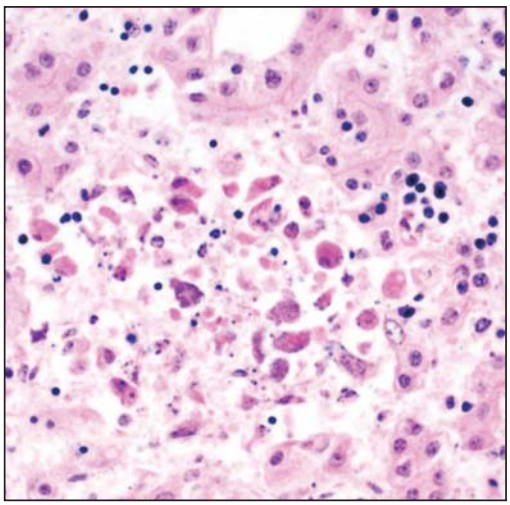
For these flea- and tick-borne diseases, it is optimal to collect the blood into EDTA (0.3 ml minimum) prior to antibiotic treatment. The DNA of these agents is very stable, so the samples can be submitted after days in the refrigerator. ▲

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.

Dr. Chris Malherbe
Dairy Cattle
22990 WCR 30
Hudson, CO 80642

Dr. Keith Rohrer
Acting State Veterinarian
Colorado Dept of Agriculture
Denver, CO 80215

Dr. Del Miles
Beef Cattle
5626 W. 19th Str, Suite A
Greeley, CO 80634

Dr. Mike Miller
Wildlife
Colorado Dept of Wildlife
317 W. Prospect
Fort Collins, CO 80526

Dr. Roger Perkins
USDA/AVIC/APHIS
755 Parfet, Suite 136
Lakewood, CO 80215

Kenny Rogers
Beef Cattle
5151 CR 34
Yuma, CO 80759

Dr. Leesa McCue
Mixed Practice
PO Box 388
Limon, CO 80828

Dr. Steve Wheeler
Small Animal
1336 W. Elizabeth
Fort Collins, CO 80521

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.

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

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Update from the 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.

BARBARA POWERS, DVM/PHD/DACVP DIRECTOR

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.