

# LAB LINES

Diagnostic news and trends from the Colorado State University Veterinary Diagnostic Laboratories  
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SPECIAL  
ISSUE  
INSIDE:  
FOCUS  
ON  
PCR



## CLINICAL TIPS

Sensitivity, specificity, positive predictive value and negative predictive value vary with each PCR assay. Many of the infectious disease agents encountered in practice colonize normal animals as well as induce disease in some individuals. Thus, the PPV of many PCR assays can be low. Veterinarians generally need to use a combination of findings to help diagnose infectious disease:

- Appropriate signalment and history for the infectious agent suspected
- Clinical signs referable to the agent
- Detection of the agent (cytology, culture, antigen assay, PCR assay) or antibodies against the agent
- Exclusion of other causes of the clinical syndrome
- Response to an appropriate treatment

When these criteria are met, the suspected infectious agent might have been the cause of the clinical disease. However, it is always possible that the disease process resolved in spite of the therapy prescribed.

## Advances in Molecular Diagnostics

# PCR detection of infectious diseases

Diagnosticians use two primary methods to detect infectious diseases: detect the organism or detect antibodies against the organism. Most frequently, they detect infectious agents in biological specimens by culture, cytology, fecal examination, histopathology, immunological techniques and nucleic acid amplification techniques.

The polymerase chain reaction (PCR) assay amplifies DNA. When a reverse transcriptase step is incorporated, it can convert RNA to DNA and then amplify it (RT-PCR). These are the most commonly used nucleic acid amplification techniques. Detection of the organism gives the most information supporting a clinical diagnosis of an infectious disease, but these assays aren't available nor optimal for all agents. Thus, antibody detection is still commonly used to diagnose infectious diseases. In some situations, the combination of organism detection and antibody detection assays is indicated. *Bartonella* spp. infection in cats and *Ehrlichia* spp. infections in dogs, for example. For these organisms, PCR-positive results can occur prior to seroconversion, so the PCR can be beneficial to prove current infection in the acute cases. In contrast, the PCR assays can become negative later in the course of disease as the immune responses develop; however, serum antibodies are generally detectable at that time. This situation may occur in many other infectious diseases, as well.

### MANAGING FOR PREDICTABILITY

When evaluating infectious disease diagnostic test results, the analytical sensitivity defines the minimum detectable amount of the substance in question that can accurately be measured; the analytical specificity defines whether the substance detected cross-reacts. The diagnostic sensitivity is the proportion of positive test results from known infected animals; the diagnostic specificity is the proportion of negative test results from known uninfected animals. The predictive value of a positive test, or PPV, is the probability that a test positive animal is diseased; the predictive value of a negative test, or NPV, is the probability that a negative animal is normal. The lower the prevalence of

Michael R. Lappin, DVM, PhD, DACVIM,  
CSU VDL Endocrinology and Special Serology Lab Supervisor

disease the lower the predictive value of a positive. Disease prevalence has little effect on negative predictive values.

Depending on the infectious agent in question, PCR and RT-PCR assays can be more sensitive than other available assays. In addition, PCR assay results can often be returned within 24 hours of sample submission, which is generally quicker than culture. However, because special equipment is required, the assays must be shipped to a diagnostic lab. If the organism in question is difficult to culture, can't be cultured or takes a long time to culture, such as some viruses, *Leptospira*, *Ehrlichia* or mycobacteria, PCR assays are of particular benefit. In contrast, some PCR assays are less sensitive than other currently available organism demonstration techniques. For example, *Giardia* spp. IFA or antigen tests are both more sensitive than *Giardia* spp. PCR assays, probably because of PCR inhibitors in feces.

Specificity of PCR assays can be high, depending on the primers used in the reaction. For example, primers can be designed to detect one pathogen genus but not others. Primers can also be designed to identify only one species. For example, a PCR assay has been developed to detect all *Trichomonas* spp. or just one species, such as *T. foetus*.

### QUALITY CONTROL IS CRITICAL

PCR assays are prone to false-positive results if sample contamination occurs during collection, transportation or testing. Some PCR assay results may also be affected by administration of anti-microbial drugs prior to sample collection. For example, haemoplasma or *Bartonella* spp.

Continues on page 2



## PCR DETECTION OF INFECTIOUS DISEASES

*Continued from page 1*

PCR results can be transiently negative during antibiotic treatment even though infection persists. Acute infections generally have higher DNA copies in samples than chronic infections, because in chronic infections the immune response has attenuated the organism. Thus, the optimal PCR assay sample is usually one collected during the acute phase prior to antimicrobial treatment.

While many commercial laboratories offer nucleic acid amplification assays, they may not be standardized. In addition, some labs offer little external quality control. For example, samples from cats with and without FIV infection were sent to four different laboratories offering FIV PCR assay.<sup>1</sup> While the lab with the best performance was right on 90 percent of the samples, two fell below 60 percent. For this reason, CSU VDL maintains stringent quality control and standardized protocols for all PCR assays.

While PCR assays are very sensitive, the PPV of many assays can be very low. For example, because the technique detects DNA or RNA of both live and dead organisms, positive test results may be achieved even if the infection has been controlled. When the organism being tested for commonly infects the background population of healthy pets, interpretation of results for a single animal can be difficult. For example, healthy cats commonly carry FHV-1, and modified live vaccine strains colonize cats. Thus, although PCR is a sensitive way to document FHV-1 infection, its FHV-1 PPV is actually very low.<sup>2-4</sup> In one study of cats with and without conjunctivitis,<sup>2</sup> more FHV-1 positive tests were detected in the healthy control group than the group with conjunctivitis. The same problems exist with feline calicivirus. The available RT-PCR assays can't distinguish between regular calicivirus, vaccine strains of calicivirus and virulent systemic calicivirus. This situation may occur with herpesvirus and other viral diseases in other species, such as cattle and dogs. Consider these realities when interpreting test results.

Real-time PCR, or fluorogenic PCR, can be more sensitive than conventional PCR for some organisms. In addition, this assay can be used to

determine the amount of microbial DNA in a sample. When used quantitatively, this technique can be used to monitor response to drug treatment.<sup>5</sup> It is possible the DNA or RNA load in a sample will correlate to the presence of disease for some agents.

### A VALUABLE TOOL...WHEN APPLIED RIGHT

Depending on the lab, PCR assays can be offered as individual tests or in panels. CSU VDL generally offers individual assays that can be coupled as indicated by the individual case. For example, it has been documented that haemoplasmas, but not *Bartonella* spp. or *Rickettsia* spp., are associated with hemolytic anemia

in cats. Thus, for cats with hemolytic anemia, only hemoplasma PCR is needed. In contrast, all three genera are associated with fever of unknown origin, and the assays can be selected alone or in appropriate combinations.

It's important practitioners carefully assess the predictive values of available PCR and the expertise and reliability of the lab. New PCR assays are being developed almost daily. At CSU VDL, each PCR assay is optimized prior to being offered commercially, to ensure quality control is stringently maintained. In addition, an appropriate specialist in bacteriology, virology or parasitology oversees the performance of each assay and is available for consultation. ▲

**'At CSU VDL, an appropriate faculty specialist in bacteriology, virology or parasitology oversees the performance of each assay and is available for consultation.'**

### REFERENCES

- 1 Crawford PC, Slater MR, Levy JK. Accuracy of polymerase chain reaction assays for diagnosis of feline immunodeficiency virus infection in cats. *J Am Vet Med Assoc.* 2005 May 1;226(9):1503-7.
- 2 Burgesser KM, et al. Comparison of PCR, virus isolation, and indirect fluorescent antibody staining in the detection of naturally occurring feline herpesvirus infections. *J Vet Diagn Invest.* 1999 Mar;11(2):122-6.
- 3 Low HC, et al. Prevalence of feline herpesvirus 1, Chlamydomphila felis, and Mycoplasma spp DNA in conjunctival cells collected from cats with and without conjunctivitis. *Am J Vet Res.* 2007 Jun;68(6):643-8.
- 4 Maggs DJ, Clarke HE. Relative sensitivity of polymerase chain reaction assays used for detection of feline herpesvirus type 1 DNA in clinical samples and commercial vaccines. *Am J Vet Res.* 2005 Sep;66(9):1550-5.
- 5 Tasker S, et al. Effect of chronic feline immunodeficiency infection, and efficacy of marbofloxacin treatment, on 'Candidatus Mycoplasma haemominutum' infection. *Microbes Infect.* 2006 Mar;8(3):653-61.
- 6 Clark M, et al. A commercially available Giardia spp. antigen assay detects the assemblages isolated from dogs. Proceedings of the ACVIM Forum, 2008.



## Innovative PCR Applications

# Help target IMA case management

Measuring antibodies against erythrocytes has classically relied upon spherocyte estimation, presence of agglutination in saline or direct Coomb's test. However, erythrocyte antibodies can also develop during infection by blood borne agents, notably *Babesia* spp., *Bartonella* spp., *Ehrlichia* spp., *Mycoplasma haemocanis* (previously *Haemobartonella canis*) and *M. haematoparvum*. Because those classic tests are only semi-quantitative, they haven't proved accurate in distinguishing primary immune-mediated anemia (IMA) from hemolytic anemia induced by infectious agents.

Flow cytometry can be used to quantitatively determine the percentage of erythrocytes that have IgM or

■ Dogs with up to 10 percent IgM or IgG binding can have either IMA or infectious hemolytic anemia.

■ Dogs with greater than 10 percent binding are likely to have IMA.

Flow cytometry used on acutely anemic dogs sampled before treatment, combined with appropriate PCR assays to amplify species-

specific DNA, offers clinics high positive and negative predictive values. The protocol can be used to help determine whether to emphasize immune suppression or anti-microbial therapy in case management. ▲

## PCR ASSAY SELECTION RECOMMENDATIONS

**Babesia** spp. Neither *B. canis* nor *B. gibsoni* infections are common in Colorado dogs. However, PCR for these agents are indicated if the dog with hemolytic anemia is a greyhound (*B. canis*), a pitbull or pitbull cross (*B. gibsoni*), has been bitten by a pitbull terrier (*B. gibsoni*), or has a recent history of Rhipicephalus ticks (*B. canis*).

**Bartonella** spp. Recently, *B. henselae* and *B. vinsonii* have been detected on dogs in Colorado and Wyoming. These agents are uncommon causes of hemolytic anemia but have been associated with cases presumed to have primary immune mediated disease that then fail to respond to immune suppression. All dogs with suspected infectious endocarditis should be screened with this PCR.

**Ehrlichia** group. *Ehrlichia* spp., *Anaplasma* spp., and *Neorickettsia* are uncommon causes of hemolytic anemia. However, if a dog is seropositive or has recent known exposure to Rhipicephalus ticks, the assay may be indicated. These agents are more commonly associated with thrombocytopenia. However, in acute cases with fever, epistaxis, polyarthritis or thrombocytopenia, *Ehrlichia* group PCR assay is indicated, particularly if antibody tests are negative.

**Hemoplasmas.** All dogs suspected to have immune or infectious hemolytic anemia should be screened for *Mycoplasma haemocanis* and *M. haematoparvum* DNA by PCR assay. These agents are commonly negative by cytology, and there is no antibody assay.

## SAMPLE

- 1 ml of EDTA blood for flow alone
- 2 ml if combining with the PCR assays

## Shipping

Deliver on a cold pack to arrive within 48 hours of collection and before noon Fridays. Samples that arrive after noon Friday will not be assayed by flow cytometry, but the PCR assays will still be completed.

## Schedule

Monday through Friday with results returned the next day

## Prices

- Flow cytometry alone \$50
- Flow cytometry...
- Plus one PCR assay \$75
- Plus two PCR assays \$100
- Plus three PCR assays \$125
- Plus four PCR assays \$150

## CANINE

IgG antibodies bound to the surface. Experiments performed over the last several years at Colorado State University have determined:

- The assay is accurate for up to 72 hours after the sample is collected. Shipping is no longer a problem.
- Dogs with 0 percent IgM and IgG binding are unlikely to have IMA.



## Canine Virology

# PCR to refine differential diagnosis

We are often asked by a referring DVM or an owner: "Hi Doc. What killed my puppy (or puppies) and what is the best diagnostic protocol?"

Among the numerous potential causes of stillbirth or post-natal death, including toxic milk syndrome, mycoplasmosis, neonatal septicemias and canine brucellosis, an investigative necropsy should include investigation for canine herpes virus.

CHV, also known as "fading puppy syndrome," is a viral infection of the adult reproductive and respiratory tracts. While adult dogs with latent infections may remain asymptomatic for a long period of time, they usually pass the virus in their vaginal secretions or as a droplet during sneezing. Puppies contracting the infection during the first three weeks of life are the most vulnerable.

An entire litter may succumb to the infection within a 24-hour period. Death may occur abruptly without any premonitory signs.

Diagnosis of CHV infection in puppies is usually made at necropsy. Characteristic gross lesions are evident in the kidneys as petechial to ecchymotic hemorrhage and necrosis producing "speckled" kid-

Tawfik Aboellail, BVSc, MVSc, PhD, DACVP,  
CSU VDL Pathologist

neys. Also, necrosis and hemorrhage can occur in the liver and lungs.

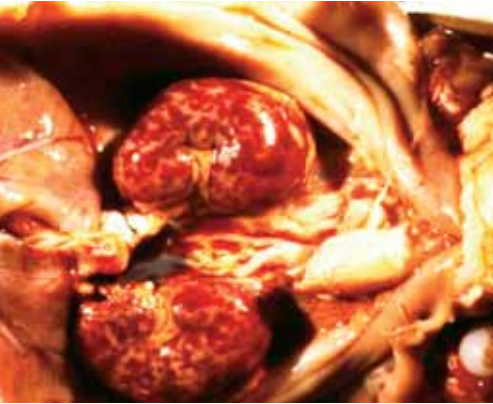
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### MOLECULAR DIAGNOSIS CAN HELP

Because the causative CHV virus is quite labile and is shed only occasionally and in small amounts, routine rapid diagnosis of CHV infections by conventional viral culture or serology is becoming more difficult and unreliable. Antibody levels are often very low and sometimes undetectable.

Polymerase chain reaction offers a highly sensitive, rapid and specific method to identify the virus in dead puppies or clinical samples from a symptomatic or asymptomatic bitch or stud. Genital, nasopharyngeal, conjunctival or throat swab can be sufficient for diagnosis. Also a small amount of blood, tracheal wash, and frozen or fresh tissue can prove recent exposure to the virus.

CSU VDL offers a reasonably priced molecular diagnosis to help you quickly evaluate whether the rest of the litter is at risk of CHV. ▲



"Turkey-egg" appearance of kidneys is due to hemorrhage and tissue necrosis. Diagnosis was confirmed in less than 24 hours by PCR.

## Molecular Parasitology

# Need the ability to rule something out?

We often think of using the PCR technique to confirm that a particular organism is present. But what about using it to determine that an organism is not?

Such was the case with a wolf shedding taeniid tapeworm eggs. This animal had been rescued from a property in the upper midwest and brought to Colorado. Fecal flotation indicated the animal was shedding taeniid tapeworm eggs. These types of eggs are usually thought to be a species of the genus *Taenia*, a common tapeworm of canids throughout the United States. However, this particular animal came from an area in which wolves harbor both *Taenia* and another tapeworm: *Echinococcus multilocularis*. While most species of *Taenia* found in canids are not zoonotic, *E. multilocularis* is. Plus, this parasite is not known to exist in Colorado. Transporting animals into new areas or reintroducing

Lora R. Ballweber, DVM, MS,  
CSU VDL Parasitology Section Head

them into areas where they once thrived is one factor responsible for the spread of many parasites. Thus, it was imperative that the true identity of these tapeworm eggs be determined.

Eggs of these species are quite difficult to tell apart based just on morphology. Therefore, CSU VDL employed molecular techniques. After extracting the DNA, amplifying it through PCR and then sequencing the resulting amplicons, it could be determined that these eggs were not *E. multilocularis*, but were *Taenia krabbei*, a common species of tapeworm found in wolves and other wild canids. Transporting this animal from an *E. multilocularis*-endemic area into a non-endemic area does not appear to have spread this particular parasite. ▲



A carrier or not? CSU VDL used PCR to rule out the presence of a potentially zoonotic tapeworm species in a transplanted wolf, ensuring the animal wasn't introducing the parasite into Colorado.



## Canine Oncology Innovations

# New canine cutaneous mast cell tumor profile helps guide therapy

In 1999, a group of researchers at Harvard Medical School led by a veterinary oncologist discovered an important clue to the pathogenesis of mast cell tumors in dogs.<sup>1</sup> Eventually, the discovery of that mutation and its effects on mast cells led to the development of a new drug, Palladia, a tyrosine kinase inhibitor, specifically targeted to treating canine mast cell tumors. Testing now available through the Diagnostic Laboratory, as a component of the new Canine Cutaneous Mast Cell Tumor Profile, can identify this mutation and help guide therapeutic decisions.

## IDENTIFYING GROWTH DRIVERS

Mast cell growth is driven in part by the binding of a growth factor (stem cell factor) to a tyrosine kinase receptor (c-kit) expressed on the surface of mast cells. When the growth factor binds, the intracellular portion of c-kit becomes phosphorylated, initiating a signaling cascade that eventually leads to mast cell division. The Harvard researchers found about 20 percent of canine mast cell tumors carried a mutation in the c-kit gene called an internal tandem duplication, or ITC; part of the gene is duplicated. The biological consequence of this duplication was a change in the protein structure of c-kit, which causes it to be permanently phosphorylated and constitutively active, even when the growth factor is not present. The unchecked growth we see in mast cell tumors results.

The new tyrosine kinase inhibitor Palladia inhibits c-kit phosphorylation, and therefore effectively inhibits mast cell growth. Recent clinical trials have demonstrated the effectiveness of Palladia as a therapy for mast cell tumors that have recurred after excision.<sup>2</sup> Palladia was most effective in patients whose tumors carried the c-kit mutation, at 60 percent to 70 percent effective, compared to patients whose tumors did not carry the mutation, at only 30 percent to 40 percent response. Detecting the c-kit mutation can help guide a decision to use Palladia over other chemotherapeutic agents such as vinblastine.

In addition to the evaluation for a c-kit gene mutation, the new canine cutaneous mast cell tumor (MCT) profile also assesses the proliferation index of the tumor via a combination of mitotic index and

Anne Avery, DVM, PhD, CSU Microbiology, Immunology and Pathology Assistant Professor; EJ Ehrhart, DVM, PhD, DACVP, CSU VDL Pathologist; and Debra Kamstock, PhD, DVM, DACVP, CSU VDL Pathologist

Ki67 expression, a marker expressed during all stages of active cell division. Plus, it evaluates expression and localization of the c-kit receptor/protein.

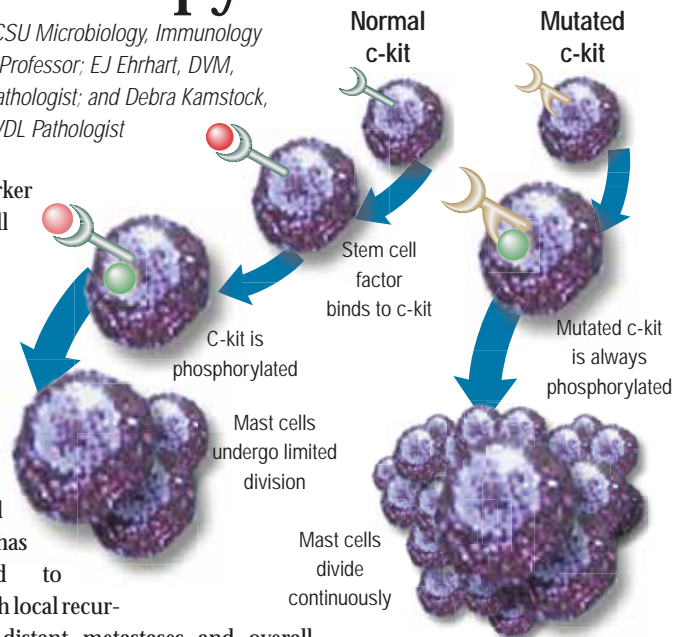
Proliferation index, both through mitotic index<sup>3,4</sup> and Ki67 expression,<sup>5</sup> has been demonstrated to inversely correlate with local recurrence, incidence of distant metastases and overall survival. Additionally, localization of the c-kit protein—specifically, aberrant cytoplasmic expression—has also been demonstrated to correlate with local recurrence and overall survival times, where Type II and III localization (focal and diffuse cytoplasmic, respectively) are associated with increased local recurrence and decreased survival times.<sup>6</sup>

## PUTTING THE ASSAY TO WORK

CSU VDL's DNA assay can be run on air-dried stained or unstained aspirates, as well as on biopsy formalin fixed tissues; however at least 10 percent of the cells in the aspirate or biopsy must be mast cells in order for the mutation to be detected. The proliferative index and IHC stain for Kit localization are carried out on biopsy samples. All tests can be run independent of the MCT profile, but the combined information obtained via the complete profile provides optimal prognostic and therapeutic guidance.

For information on this new MCT profile, including how to request one and when the test is run, visit the website [dlab.colostate.edu/webdocs/services/MCTProfileInformation.pdf](http://dlab.colostate.edu/webdocs/services/MCTProfileInformation.pdf) or call (970) 297-1281. Anne Avery, at [anne.avery@colostate.edu](mailto:anne.avery@colostate.edu), can answer questions on the c-kit mutation assay, and Brad Charles, at (970) 297-4087 can provide more information about the mast cell tumor profile. ▲

## Mast cell growth process



- London CA, Galli SJ, Yuuki T, et al. Spontaneous canine mast cell tumors express tandem duplications in the proto-oncogene c-kit. *Exp Hematol.* 1999 Apr;27(4):689-97.
- London CA, Malpas PB, Wood-Follis SL, et al. Multi-center, placebo-controlled, double-blind, randomized study of oral toceranib phosphate (SU11654), a receptor tyrosine kinase inhibitor, for the treatment of dogs with recurrent (either local or distant) mast cell tumor following surgical excision. *Clin Cancer Res.* 2009 Jun 1;15(11):3856-65.
- Romansik EM, Reilly CM, Kass PH, Moore PF, London CA. Mitotic index is predictive for survival for canine cutaneous mast cell tumors. *Vet Pathol.* 2007 May;44(3):335-41.
- Elston L, Sueiro FA, Cavalcanti JN, Metzger. The importance of the mitotic index as a prognostic factor for canine cutaneous mast cell tumors - a validation study. *Vet Pathol.* 2009 Mar;46(2):362-364.
- Webster JD, Yuzbasiyan-Gurkan V, Miller RA, et al. Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. *Vet Pathol.* 2007 May;44(3):298-308.
- Webster JD, Yuzbasiyan-Gurkan V, Thamm DH, et al. Evaluation of prognostic markers for canine mast cell tumors treated with vinblastine and prednisone. *BMC Vet Res.* 2008 Aug 13;4:32.

FOCUS  
ON PCR

## Guardians of Public Health

## Real-time influenza PCR testing

Contact Kristy Pabilonia at (970) 297-4109 if you have questions about influenza testing



CSU VDL's cooperative Avian Disease Surveillance Program helps monitor the growing urban poultry movement for early warnings of emergent pandemic influenza.

As a member of the National Animal Health Laboratory Network, CSU VDL is approved to perform influenza real-time reverse transcription PCR. Five assays are available to NAHLN labs, and CSU VDL performs all five: influenza A viruses (matrix gene), H5 avian influenza viruses, H7 avian influenza viruses and the pandemic H1N1 influenza virus (matrix gene assay and N1 assay). All positive samples are sent to the National Veterinary Services Lab in Ames, Iowa, for confirmatory testing.

In 2004, CSU VDL and the Colorado Department of Agriculture started the Colorado Avian Disease Surveillance Program. Funded by USDA and housed at VDL, the program provides free avian influenza surveillance testing for commercial flocks, backyard flocks and live bird markets. The program also provides free disease investigation services for backyard flock owners experiencing disease and mortality. In addition, the CSU VDL has also participated in the national USDA-Wildlife Services wild bird avian influenza surveillance program.

Since 2004, more than 30,000 Colorado wild and domestic bird samples have been tested for avian influ-

*Kristy Pabilonia, DVM, DACVM, CSU VDL Avian Diagnostics and BSL3 Operations Section Head; and Christina Weller, CSU VDL Avian Diagnostics and BSL3 Operations Microbiologist*

enza virus. We have detected low pathogenic avian influenza viruses, primarily in wild bird samples. No highly pathogenic avian influenza viruses have been identified.

In 2009, with the emergence of pandemic H1N1 influenza virus, the NAHLN released a modified influenza A matrix real-time PCR protocol, as well as a real-time PCR protocol targeting the 2009 N1 subtype. While the primary use for these assays is to test swine, we were granted permission to use them to screen companion animals.

To date, we have tested 14 samples from swine, cats and dogs. All swine and canine samples have tested negative. CSU VDL diagnosed two cases of H1N1 pandemic influenza virus in domestic cats in November 2009. These cases were some of the first cases diagnosed in companion animals. Both cats presented to veterinarians with respiratory abnormalities and experienced a prolonged period of illness. Information on these two cases is in preparation for publication. ▲

## Case Study

## Infectious hepatitis: Forgotten, not gone

A young adult, mixed-breed bitch whelped a litter of 11 puppies while in a rescue facility. Several became ill at 5 weeks old. One died and was diagnosed with infectious canine hepatitis (ICH). Two unrelated 4-month-old puppies were exposed.

Urine samples were submitted from nine remaining, 8-week-old puppies, the dam and the two exposed dogs for PCR canine adenovirus-1 (CAV-1) testing. All nine puppy sample were positive for CAV-1 by PCR, while the dam and exposed puppy samples were negative.

CAV-1 is the etiologic agent for ICH. The clinical signs in the acute phase of ICH—fever, anorexia, lethargy, vomiting and diarrhea—are non-specific and may be mistaken for parvovirus-1 or distemper. Frequently, ICH is suspected only when some dogs develop corneal edema or “blue-eye” in the subacute phase, at three to four weeks after infection. The disease is more severe in younger dogs, and peracute death may occur in puppies.

*Jeanette Bishop, MS, CSU VDL Molecular Diagnostics Research Associate, and Hana Van Campen, DVM, PhD, DACVM, CSU VDL Virology Section Head*

The virus targets the lymphoid tissues, including the tonsils and Peyer's patches; the liver, causing hepatitis; and the kidneys. Although the immune response clears the virus in 10 to 14 days, the virus persists in the kidneys of survivors and is shed in urine for weeks to months.

In addition to dogs, CAV-1 infects foxes, coyotes, wolves, raccoons, ferrets, skunks and bears. Immunization with CAV-2 containing modified live viral vaccines has been effective in preventing ICH in domestic dogs. While uncommon in vaccinated populations, ICH occasionally crops up in unvaccinated dogs in rescue and humane shelters. In suspect cases, whole EDTA blood may be submitted for PCR. Tissues including liver, spleen, kidney and lymph nodes are suitable post-mortem samples. Urine samples should be tested for shedding. The cost of the PCR is \$35. ▲



Lab Update

# TREKking Johne's for faster results

CSU VDL's Bacteriology Section offers a more rapid test for the detection of *Mycobacterium avium* subspecies paratuberculosis. Instead of using the old method of culturing the sample onto solid



media and checking for growth over a period of 112 days, bacteriology now performs a liquid

culture using the TREK para-JEM ESP Culture System II, which checks for bacterial growth over a period of 45 days.

The TREK system works by continuously monitoring the liquid culture for bacterial growth, notifying the technician of positive growth with a light. Just because there is growth does not necessarily mean the sample contains *M. avium* paratuberculosis. The sample is put through a decontamination process, but some organisms, such as other mycobacteria species, are hardy enough to survive the process and will grow in the liquid culture, giving a false positive. To ensure no false positives are reported, we perform a real-time PCR detecting *M. avium* paratuberculosis after growth in the liquid culture. If the sample is PCR-positive, the client will receive an *M. avium* paratuberculosis-positive report. If the sample is PCR-negative, the liquid culture will be put back onto the TREK system for the remainder of the 45

Mike Russell, CSU VDL Microbiologist, and Doreene Hyatt, PhD, CSU VDL Bacteriology Section Head

days. The PCR then will be performed again on day 45 to ensure the sample is still negative. ▲

## VDL NOW NVSL-APPROVED FOR JOHNE'S DIRECT PCR DETECTION

VDL is pleased to inform you it has passed the individual 2010 Johne's Proficiency Panel using direct PCR. This update will permit us to conduct official testing for USDA beginning immediately through the end of 2011.

The real-time PCR detection of *M. avium* paratuberculosis directly from a fecal sample will allow clients to receive a result many times on the same day we receive the sample.

We have also passed our proficiency test for liquid culture, solid culture and serum ELISA for Johne's disease and are approved by USDA through the end of 2010.

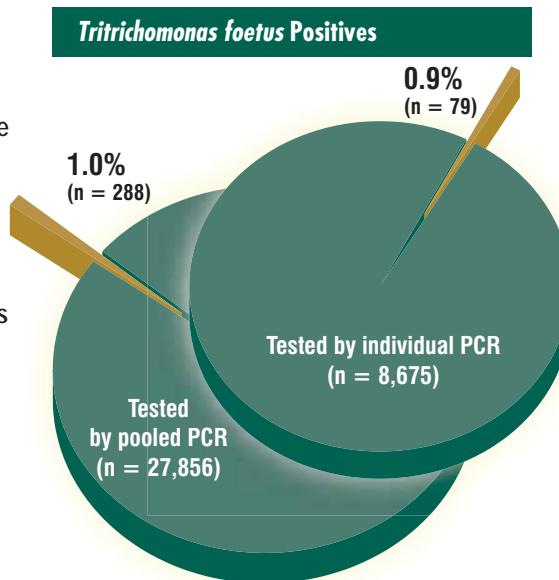


## ATTENTION: NEW LIMS!

Starting July 1 we switched to a new laboratory information management system (LIMS), call STARLIMS. You will see new and improved formats for your results. Please call with questions and comments. Please be patient with as we go through this transition.

### CSU ROCKY FORD DEMONSTRATES POOLED TRICH SAMPLE RELIABILITY

From Jan. 1, 2007, to May 5, 2010, the CSU VDL at Rocky Ford has tested 27,856 bulls by pooled PCR for the presence of *T. foetus* and found 288 positive individuals. During the same period, the lab has tested 8,675 by individual PCR and found 79 positives. The number of positive by pooling is 1 percent, while the number by individual PCR it is 0.9 percent. Statistical analysis of the test results show no significant statistical difference in the number of positives detected by pools versus the number of positives detected by individual PCR. Pooling can increase the cost-effectiveness of Trich testing without compromising diagnostic reliability.



Real-time PCR testing for *T. foetus* is now available at the Fort Collins lab. Quicker results with no change in sample collection or increase in price compared to conventional testing. Email [dlab@colostate.edu](mailto:dlab@colostate.edu) or call (970) 297-1281 for details.



## Reportable Disease Update

## Colorado horse tests EP-positive



USDA has now authorized CSU VDL to perform the cELISA tests for *Theileria equi* and *Babesia caballi* for the intra- and interstate movement of equids not displaying clinical signs of piroplasmosis. Tests are run each Tuesday and Friday. Submit 1 ml of serum and be sure to include horse's identification. Cost is \$16 per sample.

As was reported in April, a horse in Colorado tested positive for equine piroplasmosis. Although it showed no clinical signs of equine piroplasmosis, the horse tested positive to *Theileria equi* on the cELISA test. The serum sample had been submitted by a Colorado private practitioner. The positive horse has since been euthanized.

Arapahoe Park Racetrack in Aurora instituted EP testing requirements for all horses entering the track facilities—the reason the EP-positive sample was submitted. Horses must be *T. equi*- and *Babe-*

*sia caballi*-negative within 30 days of admission to Arapahoe Park. The piroplasmosis test-positive horse was in a stable with about twenty horses. None of those horses are considered positive at this time. Further testing will be done in approximately 30 days from the initial tests. If they are negative, the quarantine will be released.

Equine piroplasmosis is a reportable disease and leads to regulatory consequences. A quarantine and subsequent disease-control plan for any test-positive horses is determined by the State Veterinarian of Colorado, USDA-APHIS-VS, along with input from the owners. Currently, there is no vaccine or approved treatment for EP in the United States.

USDA-APHIS-VS' Equine Piroplasmosis Info Sheet, written in English and Spanish, provides further information for you and your clients. You can access it on the Web at [www.aphis.usda.gov/animal\\_health/animal\\_diseases/piroplasmosis/index.shtml](http://www.aphis.usda.gov/animal_health/animal_diseases/piroplasmosis/index.shtml). ▲

Questions? Call the State Veterinarian's Office at (303) 239-4161

## Bovine Virology Case Study

## An unusual case of BRSV in a calf

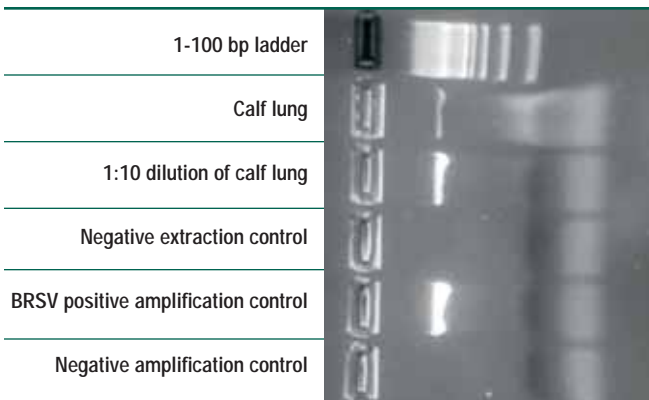
A sample of lung from a 4-month old dairy calf was submitted to the diagnostic laboratory for histopathology and FA tests. Entire lobules were positively stained for bovine respiratory syncytial virus by FA test. The pattern of

Lung tissue was also assayed for BRSV using a reverse transcriptase PCR test. It was strongly positive for BRSV genomic RNA.

Due to the histologic findings and the extensive FA staining in the lung, vaccination history of the calf was obtained. Calves were inoculated by intranasal route with a modified live BRSV vaccine labeled for intramuscular administration. Six calves became ill, five responded to treatment and one died.

Attenuated live viral IM vaccines may result in disease when administered by the intranasal route. Unforeseen environmental factors, such as immune status, and other infectious agents may tip the balance of health in calves inoculated intranasally with BRSV. Recent reports suggest intranasal vaccination for BRSV in young calves may not fully protect against subsequent viral challenge due to the presence of maternal antibodies.<sup>1</sup> ▲

Christina Gates, CSU VDL Microbiologist; Jeanette Bishop, MS, CSU VDL Molecular Diagnostics Research Associate; Barb Powers, DVM, PhD, DACVP, CSU VDL Director; and Hana Van Campen, DVM, PhD, DACVM, CSU VDL Virology Section Head



<sup>1</sup> Ellis JA, Gow SP, Goji N. Response to experimentally induced infection with bovine respiratory syncytial virus following intranasal vaccination of seropositive and seronegative calves. *J Am Vet Med Assoc.* 2010 May 1;236(9):991-9.

BRSV staining is unusual compared to that observed in bovine pneumonias. Histologically, we observed a severe interstitial pneumonia with multinucleated syncytial cells.





## Gaurdians of Public Health

# Meeting the challenge of Q fever

Q fever is a zoonotic disease that results from infection with the organism *Coxiella burnetii*. This obligate intracellular Gram-negative bacterium is distributed worldwide. Cattle, sheep and goats are the primary reservoir of the organism. The organism is shed in milk, urine, feces, amniotic fluid, birth fluids and placenta of infected animals. The organism can survive for prolonged periods in the environment and exposure to only a few organisms can result in infection. Animal infections are often asymptomatic. The primary clinical manifestations observed in animal populations are aborted fetuses and birth of weak or nonviable neonates.

## ZOONOTIC POTENTIAL

Most human cases results from contact with cattle, sheep and goats, especially during parturition. Human cases can also potentially result from consumption of unpasteurized milk. Infection is acquired through inhalation or ingestion of the organism. Companion animals are also susceptible and have been documented as the source of human infections. Q fever can result in acute and chronic cases in humans. In acute cases, symptoms include fever, vomiting, diarrhea, atypical pneumonia, headache, hepatitis, skin rash, myalgia and general malaise. In chronic cases, the disease persists for six months or longer and can result in severe disease and endocarditis. Infection during pregnancy may result in abortion, fetal death, premature delivery and death. Q fever is a reportable disease, but because approximately 60 percent of human cases are asymptomatic, it is often under-reported.

In 2009, the Netherlands diagnosed more than 2,300 human cases and a large scale effort was instituted to control prevalence of the organism in small ruminants, with the hope that this would decrease incidence of human infection. Control measures include culling of tens of thousands of pregnant goats on affected farms and mass vaccination. The impact of these actions continues to be evaluated by the scientific community and it is hoped will provide insight on the best way to handle this type of situation.

## DIAGNOSTIC CHALLENGES

Because infected animals do not always shed organisms, diagnostic testing for Q fever can be difficult. A negative PCR result does not guarantee that the animal or population is not infected. Repeated sampling and testing should be used before an animal or farm is determined to be Q-fever negative. Antibody

*Kristy Pablonia, DVM, DACVM, CSU VDL Avian Diagnostics and BSL3 Section Head; and Christina Weller, CSU VDL Microbiologist*

testing should be interpreted with care. Current serology techniques lack desired sensitivity and specificity, and seropositivity can be difficult to interpret as it is difficult to differentiate between acute infections and past exposure.

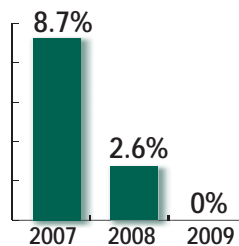
## CSU VDL MONITORING UPDATE

Since we began offering a real-time PCR assay for *C. burnetii*, the number of submissions has grown from 21 in 2007 to more than 300 in 2009. Submissions have been received from bovine, camelid, canine, caprine and ovine species. We receive samples for diagnostic testing for animals with clinical manifestations of Q fever and from animals on premises with Q-fever positive owners. In addition, we receive submissions for screening research animals and for export testing.

As the overall number of submissions has grown, the prevalence of positive samples has decreased. In 2007, 8.7 percent of the total samples tested positive; however, this amounted to only two samples out of a total of 21 tested. In 2008, 2.6 percent of samples tested positive, with no confirmed positives in 2009.

We have recently started offering the CDC Laboratory Response Network's real-time PCR assay for Q Fever. This assay incorporates three primer/probe sets, each of which targets a different sequence of the *C. burnetii* genome. This PCR will allow us to provide a higher level of diagnostic sensitivity. However, the increase in the number of target sequences from one to three means that there is an increased test cost. The cost of this test is \$60 per sample. *C. burnetii* organisms can be found in the lungs, spleen and liver, as well as milk and blood, so these samples are best for testing in non-abortion cases. In abortion cases, placenta is the best sample for diagnostic testing. Tissues, swabs and milk samples from multiple animals may be pooled for testing, but this may decrease overall sensitivity. In order to minimize exposure of our laboratory personnel to this agent, we perform this test in our new biosafety level 3 laboratory. ▲

### Q Fever Positives



Contact Kristy Pablonia at (970) 297-4109 if you have questions about Q fever testing



CSU VDL In Press

## A roundup of VDL faculty research

**Spraker TR, et al. Detection of the Abnormal Isoform of the Prion Protein Associated with Chronic Wasting Disease in the Optic Pathways of the Brain and Retina of Rocky Mountain Elk. *Vet Pathol.* 2010 May;47(3):536-46.**

CSU VDL Pathologist Terry Spraker examined the eyes and nuclei of the visual pathways in the brains of 30 Rocky Mountain elk (*Cervus elaphus nelsoni*) for the presence of the abnormal isoform of the prion protein associated with chronic wasting disease, PrP<sup>CWD</sup>. The research team found the prion in the retina of 89 percent of the elk showing an obex score higher than 7 (ranging from a disease-free 0 to a terminal 10), compared to no detection in disease-free elk or those with an obex below 6. The elk with an obex score of less than 6 had no evidence of PrPCWD immunoreactivity in the optic nerve or retina but did have PrPCWD immunoreactivity in neural regions of the visual pathway in the brain. The data demonstrate detectable PrP<sup>CWD</sup> immunoreactivity first accumulates in the visual pathways of the brain, before the retina.

**McCoy AM, Hackett ES, Callan RJ, Powers BE. Alimentary-associated carcinomas in five Vietnamese potbellied pigs. *J Am Vet Med Assoc.* 2009 Dec 1;235(11):1336-41.**

VDL Director Barb Powers assisted a CSU Department of Clinical Sciences team in documenting the cases of five geriatric Vietnamese potbellied pigs presenting to the CSU Veterinary Hospital with complaints of abdominal distress that had not responded to medical treatment, in the case of four, and a draining tract of the cranial abdomen of unknown duration, in the fifth. Although neoplasia of gastrointestinal or hepatobiliary origin in pigs is infrequently reported—and never yet reported in Vietnamese potbellied pigs—the study team tracked the cases of the five pigs with primary intestinal tumors (in three), gastric carcinoma (in one), and undifferentiated carcinoma of biliary origin (in one).

Neoplasia is generally considered rare in pigs, but it shouldn't be overlooked in the differential diagnosis of generalized abdominal distress in middle-aged and older potbellied pigs. As was evident in the report, tumors can potentially arise from multiple components of the gastrointestinal and hepatobiliary tracts. If neoplasia is suspected, tumor

excision may provide a good outcome: All three pigs that were treated surgically survived at least nine months after surgery with good quality of life.

**Ballweber LR. Taenia crassiceps subcutaneous cysticercosis in an adult dog. *Vet Rec.* 2009 Dec 5;165(23):693-4.**

Lora Ballweber, CSU VDL Parasitology Section head, details the case of an eight-year-old adult blue heeler cross presented to the referring emergency clinic with an abscess on the left hindlimb. A large, fluctuant swelling was present on the medial left thigh; cytology revealed numerous degenerative neutrophils and macrophages



Ballweber documents the budding cysticerci of *Taenia crassiceps* from a swelling on the leg of a dog (left) and a squash mount of an inverted scolex showing typical rostellar hooks.

with no other abnormalities. Ovoid parasites containing clear watery fluid with a single invaginated scolex identified upon diagnostics were identified as *T. crassiceps cystice*. *T. crassiceps* cysticercosis in dogs as been reported only six times previously in Europe and only once in the United States. *T. crassiceps* not only poses a fatal threat for dogs and cats, it is a clear zoonotic risk as well. Ballweber reminds veterinarians they should remember dogs are definitive hosts for multiple species of *Taenia*, including *crassiceps*. Comprehensive pet parasite control should continue to be a priority.

**Ballweber LR, et al. Giardiasis in dogs and cats: update on epidemiology and public health significance. *Trends in Parasitology* 2010 Apr;26(4):180-9.**

Ballweber updates the status of molecular identification of the seven genetic assemblages of *Giardia duodenalis*, named A to G. Humans are infected with assemblages A and B, dogs primarily with C and D, and cats with F. Recently, small numbers of dogs and cats have been reported to also carry assemblages A-I or B. Although the role of dogs and cats as a source of human giardiasis remains unresolved, the potential role of pets can't be conclusively excluded, she cautions. ▲







### NEW DIAGNOSTIC SCREENS

CSU VDL has introduced new diagnostic respiratory, diarrhea and abortion screens. These species-specific screens combine tests for the most commonly diagnosed viral, bacterial, parasitic and toxicological agents of respiratory, diarrhea and abortion cases. In most cases, histology is also included. The costs of these panels vary depending

on the species and type of panel, but all are offered at a discount from the cost of running all the included tests individually. Look for these new tests on Page 2 of the General Submission Form. Detailed descriptions of these panels can be viewed at [dlab.colostate.edu](http://dlab.colostate.edu). Scroll down to the In the News section and click on Panels and Screens.

### Veterinary Community Outreach

## External advisory committee

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.

- Dr. Joan Bowen, Small Ruminant Wellington
- Mr. Norm Brown, Equine Wellington
- Dr. Keith Roehr, State Vet CO Dept of Agric, Denver
- Mr. Terry Fankhauser, Executive Director, CCA Arvada
- Dr. Mike Gotchey, Equine Steamboat Springs
- Dr. Bob Davies, Wildlife Colorado Dept of Wildlife Denver
- Dr. Marv Hamann, Mixed Practice Pueblo West
- Mr. Ed Hansen, Beef Cattle Livermore
- Dr. Dean Hendrickson, Director CSU Veterinary Teaching Hospital Fort Collins
- Dr. Ron Kollers, Small Animal Fort Collins
- Dr. Elisabeth Lawaczek Colo. Dept of Public Health and Environment Denver
- Dr. Larry Mackey, Large Animal Greeley
- Dr. Leesa McCue, Mixed Animal Limon
- Dr. Del Miles, Beef Cattle Greeley
- Dr. Mike Miller, Wildlife Colorado Dept of Wildlife Fort Collins
- Dr. Roger Perkins USDA/AVIC/APHIS Lakewood
- Mr. Kenny Rogers, CCA Yuma
- Dr. Steve Wheeler, Small Animal Englewood
- Dr. Deb Young, CSU Extension Fort Collins

### WIN A \$50 CREDIT ON LAB WORK

Take a short five-minute survey to tell us how to improve LabLines and we'll enter you in a drawing to win a \$50 credit on CSU VDL services.

To begin the survey, use your Internet browser to go to: [FoodChainCommunications.com/Lablines](http://FoodChainCommunications.com/Lablines)

*Some restrictions apply. See website for details.*



JOE MCDOWELL

Craig, Colo., native and University of Northern Colorado grad Joe McDowell joined the VDL in February. Joe will help VDL maintain its commitment to quality assurance and customer service through various lab support functions. Welcome, Joe.

We would like to extend our sincere gratitude to our initial donors to the new CSU VDL Endowment. Thank you for supporting the VDL and our mission. We invite you to join us

on this mission to establish and grow the Endowment Fund. Contribute by visiting us online at [www.dlab.colostate.edu](http://www.dlab.colostate.edu) and clicking "Support the DLAB online."

#### 2008

- Barbara E. Powers, DVM

#### APRIL 2009

- Hana Van Campen

#### MAY 2009

- James A. Kennedy on behalf of Ms. Kathy Gilbert

#### AUGUST 2009

- Kristy L. Pabilonia, DVM

#### NOVEMBER 2009

- Barbara E. Powers, DVM
- Fisher's Peak Veterinary Clinic on behalf of William C. Aaroe, DVM

#### DECEMBER 2009

- Foothills Animal Hospital on behalf of Roger A. Liehr, DVM
- Constance M. Hvass, PhD, in honor of Dr. Linda Sue Wiest

#### MARCH 2010

- Amanda Hosny, CVT, in honor of Dr. Jenna Burton
- Ferris Veterinary Services on behalf of Martha A Ferris, DVM
- Colorado Cattlemen's Association

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Canine mast cell profile.
- **PUBLIC HEALTH** p. 6  
Real-time influenza PCR.
- **DIAGNOSTIC UPDATE** p. 7  
Johne's testing additions.
- **VDL IN PRESS** p. 10  
The latest research roundup from the VDL faculty.
- **MEET THE LAB** p. 11

- FOCUS ON PCR** . . . . . p. 1  
How PCR improves the detection and management of infectious diseases.
- IMA MANAGEMENT** . . . . . p. 3  
How CSU VDL's PCR can help clinics better manage immune-mediated anemia.
- WHAT KILLED MY PUP?** . . . p. 4  
PCR improves differential diagnosis of neonatal deaths.



- EP TESTING UPDATE** . . . . . p. 8  
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- AVIAN FLU** . . . . . p. 6  
Employing PCR to monitor for avian influenza.
- Q FEVER REVIEW** . . . . . p. 9  
VDL's experts detail what we know about this zoonotic pathogen.



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## Update from the Director

**M**olecular Diagnostics, specifically Polymerase Chain Reaction (PCR), is certainly not new to our laboratory or laboratories across the country. However, in the recent years, requests for this technology have dramatically increased. In general, PCR technology is accepted to be a very sensitive and specific testing procedure. It has replaced many of the older diagnostic techniques, which were either less sensitive or took a long time to complete. Does a positive PCR test always conclusively show the animal has disease? Likewise, does a negative PCR test always indicate the animal does not have disease? This issue of LabLines explores these questions and demonstrate the proper use of PCR technology to either make or confirm a diagnosis of a suspected disease. The lead article by Dr. Mike Lappin also details some of the issues regarding interpretation of PCR diagnostics. As always, a practitioner and diagnostician, working as a team, is critical in interpreting the final result of this—and any—test. That's why CSU VDL always includes close oversight and consultation with an appropriate faculty specialist in bacteriology, virology or parasitology for each assay



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

In other news, our 2009 Annual Report is available on our website or as hard copy upon request. In January, we met with our External Advisory Committee, which as always provided us with guidance on moving the laboratory forward. Our laboratory, as well as other diagnostic laboratories, veterinary clinics and people across the country have struggled in these economic times. But recently, we have seen an increase in laboratory use, signaling the economy is truly on its way to recovery.

We hope you all have an enjoyable

summer and enjoy the warm weather which has finally just arrived to Colorado after a very long, cold winter. We look forward to seeing you this fall at the Colorado Veterinary Medical Association meeting in Loveland and the Annual American Association of Veterinary Laboratory Diagnosticians meeting in Minnesota.

LAB  
NEWS

*Barbara E. Powers*



**FOLLOW OUR  
ANNUAL PROGRESS**

Check out our 2009 annual report, at [www.dlab.colostate.edu/webdocs/news/](http://www.dlab.colostate.edu/webdocs/news/)