

# Colorado State University Veterinary Diagnostic Laboratories



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## O Letter from the Director

We are already into the fall season with winter rapidly approaching. As always, we have many changes to report at the Diagnostic Laboratory. We ended our 1998-1999 fiscal year with a 23 percent increase in accessions compared to the previous year. We are constantly working on improving our diagnostic testing ability, frequently by using molecular techniques. Some of these new advances are detailed in this issue of LabLines. We remain committed to providing you with efficient, accurate, and timely results.

We have a number of new faculty and staff who have joined the Diagnostic Laboratory. Randy Basaraba, DVM/PhD, joined us in July coming from the diagnostic laboratory at Kansas State University. He is a board-certified pathologist with a specialty in food animal pathology and will be working closely with the Integrated Livestock Management program. Doreene Hyatt, PhD, also joined us in July, from Kansas State University. She is our new section head of Bacteriology. Her area of interest is bacterial pathogens of food animals and food safety issues. We are thrilled to have these two highly qualified individuals join us. On a sad note, Dr. Jim Collins will be leaving us after 17 years of service to become a department head at the University of Arizona. We soon will begin a search for his replacement. Wayne Cunningham, DVM/MS, Assistant State Veterinarian, has accepted a joint appointment with Cooperative Extension. He will be working more closely with us, helping to protect animal health in our state and provide outreach education. We also have added Erica James to the office area and Gail Miller to the virology section. Our new pathology residents are Dr. Debra Kamstock (anatomic pathology) and Dr. Randy Wilson (clinical pathology).

We enjoyed visiting with those of you who attended the Colorado Veterinary Medical Association meeting at Vail in September. We hope to see many more of you at Colorado State University's Annual Conference in January!

*Barbara E. Powers*

**Barb Powers, DVM/PhD**

**Colorado State University  
Diagnostic Laboratories  
300 West Drake  
Fort Collins, CO 80523  
Phone 970/491-1281  
Fax 970/491-0320  
email: [jkammerz@vth.colostate.edu](mailto:jkammerz@vth.colostate.edu)  
<http://dxlab.cvmb.colostate.edu/dab>**

**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**

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## HOW SHOULD I DIAGNOSE HYPOTHYROIDISM IN DOGS?

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Deborah Greco

The usual etiology of primary canine hypothyroidism is lymphocytic thyroiditis or idiopathic thyroid atrophy. Neoplasia accounts for fewer than 1 percent of the cases. Congenital hypothyroidism may be caused by thyroid dysgenesis, dyshormonogenesis, T4 transport defects, goitrogens or, rarely, by iodine deficiency.

The signalment of hypothyroid dogs carries a distinct breed predisposition, with high-risk breeds presenting as early as 2-3 years of age. Low risk breeds present at a slightly older (4-6) age. Breeds predisposed to hypothyroidism include golden retrievers, Doberman pinschers, dachshunds, Irish setters, miniature schnauzers, Great Danes, miniature poodles, boxers, Shetland sheepdogs, Newfoundlands, chow chows, English bulldogs, airedales, cocker spaniels, Irish wolfhounds, giant schnauzers, Scottish deerhounds, and Afghan hounds.

Clinical signs of hypothyroidism are gradual and subtle in onset, with lethargy and obesity being most common. Owners are often not aware of the onset of signs and think that their dog is just becoming "older." Dermatologic evidence of hypothyroidism is the most common clinical finding other than lethargy and obesity. Symmetric trunkal or tailhead alopecia is a classic finding. The skin is often thickened because of myxedematous accumulations in the dermis. Common haircoat changes seen in the hypothyroid dog include dull, dry hair, the blond "frizzies," poor hair regrowth following clipping and the presence or retention of puppy hair. Hyperkeratosis, hyperpigmentation, secondary pyodermas and demodicosis also are observed. Seborrhea, either sicca or oleosa, can be a clinical feature of canine hypothyroidism; ceruminous otitis also is common.

Cardiovascular signs of hypothyroidism, though uncommon, include bradycardia, decreased cardiac contractility, and atherosclerosis. Neuromuscular signs, such as myopathies and megaesophagus, also are uncommon manifestations. Neuropathies, including bilateral or unilateral facial nerve paralysis, vestibular disease, and lower motor neuron disorders, are seen occasionally. Myxedema coma is an unusual finding in hypothyroid dogs. It manifests as stupor and coma secondary to myxedematous fluid accumulations in the brain, and severe hyponatremia. Less common signs of hypothyroidism include reproductive disorders in female dogs such as prolonged interestrus intervals, silent heat, and weak or stillborn puppies. Corneal lipid deposits and gastrointestinal problems such as constipation are observed occasionally.

Clinicopathologic findings such as normocytic normochromic anemia resulting from erythropoietin deficiency, decreased bone marrow activity, and decreased serum iron and iron binding capacity are observed in about

25-30 percent of hypothyroid dogs. Hypercholesterolemia is seen in approximately 75 percent of affected dogs because of altered lipid metabolism, decreased fecal excretion of cholesterol, and decreased conversion of lipids to bile acids. Hyponatremia, a common finding in human beings with hypothyroidism, was observed as a mild decrease in serum sodium in about 30 percent of hypothyroid dogs in one study. Hyponatremia is caused by an increase in total body water due to impaired renal excretion of water and by retention of water by hydrophilic deposits in tissues. An unusual clinicopathologic feature is increased serum CPK possibly as a result of hypothyroid myopathy.

Diagnosis is based on measurement of serum basal total thyroxine (TT4) and triiodothyronine (T3) concentrations, serum free T4 (FT4) and T3 concentrations, endogenous canine serum TSH levels and/or dynamic thyroid function tests including the TRH and TSH stimulation tests. Variables that affect T4 are many and include age, breed, environmental and body temperature, diurnal rhythm, obesity, and malnutrition. Specifically, greyhounds have approximately half the normal TT4 and FT4 concentrations compared with concentrations in normal dogs. Obese dogs have mild increases in serum TT4 concentrations. Puppies exhibit serum TT4 concentration 2-5 times greater than adult dogs. Furthermore, there is an age-related decline in serum TT4 concentrations and response to TSH stimulation in dogs. "Euthyroid-sick" syndrome is characterized by a decrease in serum TT4 and increase in reverse T3. Concurrent illnesses such as diabetes mellitus, chronic renal failure, hepatic insufficiency, and infections can cause euthyroid-sick syndrome, resulting in decreases in serum TT4 concentrations. Drugs such as anesthetics, phenobarbital, primidone, diazepam, trimethoprim-sulfas, quinidine, phenylbutazone, salicylates and glucocorticoids also can decrease serum basal TT4 concentrations.

Free thyroid hormone concentrations are used in human medicine to differentiate between "euthyroid sick" syndrome and true hypothyroidism. In humans, the diagnostic accuracy of a single FT4 measurement is approximately 90 percent. Measurement of FT4 concentrations is achieved by equilibrium dialysis (gold standard) or analogue immunoassays. Theoretically, FT4 is not subject to spontaneous or drug-induced changes that occur with TT4. Early studies, classifying dogs as hypothyroid based on TSH stimulation tests, indicated that FT4 by equilibrium dialysis was 90 percent accurate while other FT4 assays (analogue assays) were not better than TT4. Glucocorticoids will decrease both the FT4 fraction and TT4 in dogs.

With the advent of the endogenous canine TSH (eTSH) assay, veterinarians now have a method of assessing the thyroid-pituitary axis in dogs without dynamic testing. With thyroid gland failure, decreases in serum FT4 and TT4 are sensed by the pituitary gland resulting in an increase in serum eTSH concentration. Initial studies in experimentally-induced hypothyroid dogs have been very encouraging. In human beings, when endogenous TSH is increased and FT4 is

decreased, diagnostic accuracy for primary hypothyroidism approaches 100 percent. As FT4 concentration falls, there is a logarithmic increase in serum eTSH concentration making it the most sensitive test for the detection of early hypothyroidism. However, non-thyroidal disease can affect eTSH concentrations as well as FT4 and TT4 concentrations. Because of this, the use of eTSH **alone** is not recommended as a method of assessing thyroid function.

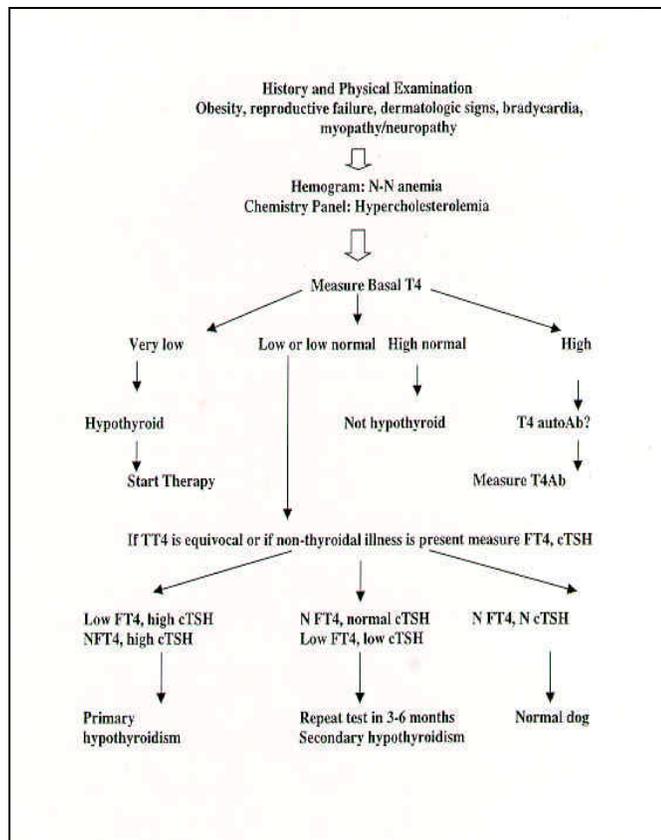
The anti-thyroglobulin autoantibody test (ATAA) has become recently available and appears promising based on initial studies. The presence of anti-thyroglobulin antibodies theoretically presages the onset of hypothyroidism in dogs with autoimmune thyroiditis. We hope this test will identify dogs with hereditary thyroid disease prior to breeding. However, no large studies of dogs with naturally-occurring thyroid disease have been performed to evaluate this assay.

For many years, the TSH stimulation test was considered the “gold standard” for diagnosis of hypothyroidism in dogs. Unfortunately, this test does not differentiate between early hypothyroid dogs and those with “euthyroid-sick” syndrome,

nor does it identify dogs with secondary or tertiary hypothyroidism. In addition, exogenous bovine TSH is no longer commercially available. Other thyroid function tests include the TRH stimulation test, thyroid scan, and thyroid biopsy. However, each of these tests has the drawback of expense, inaccuracy or invasiveness.

In summary, diagnosis of hypothyroidism is based on signalment, historical findings, physical examination, clinicopathologic features, and confirmation with a battery of thyroid function tests. We use TT4, FT4 (analogue, chemiluminescent) and eTSH. If all three are abnormal, the dog is hypothyroid. If two of the three are abnormal, secondary hypothyroidism (low FT4, low TSH) or early primary hypothyroidism is possible (high TSH, low FT4). If only one of the three thyroid tests is positive, the dog should be re-evaluated in 3-6 months. An algorithm for the diagnosis of hypothyroidism in dogs is shown below, to the left.

Thyroid panel: Submit 1ml serum, Fee=\$50.



## DIAGNOSTIC LABORATORY TSE SURVEILLANCE ACTIVITIES

Dan Gould

The transmissible spongiform encephalopathies (TSEs) are a group of progressive, neurodegenerative diseases caused by a unique agent. Examples of these diseases are the prototype, scrapie of sheep, bovine spongiform encephalopathy (BSE, known informally as mad cow disease), chronic wasting disease (CWD) of deer and elk, and Creutzfeldt Jacob disease (CJD) of humans. It is thought that BSE has been transmitted to humans in the United Kingdom. Attention has focused on these diseases because of their food safety, animal and human health, and world trade implications. At the present time, these diseases can be definitively diagnosed only by post-mortem examination of appropriate sites in the brain and demonstrating characteristic microscopic lesions and immunohistochemical staining patterns.

**BSE Surveillance**—Several federal and state agencies are involved in this surveillance program. The surveillance samples are derived from a number of sources. These include field cases of cattle exhibiting signs of neurologic disease, cattle condemned at slaughter with signs of neurologic disease, cattle submitted to public health laboratories for rabies diagnosis, neurologic cases submitted to veterinary teaching hospitals and diagnostic laboratories, and random sampling of “downer cattle” at slaughter. As of June 30, 1999, more than 8,094 brains have been examined for BSE or another form of a TSE in cattle, and there has been no evidence of either condition.

**CWD Surveillance**—The Colorado Division of Wildlife, using our laboratory, has conducted surveys of CWD in free-ranging populations of deer and elk. These studies have been especially extensive in the last three years, involving more than 2,000

animals per year. About 5-6 percent of free-roaming deer in areas of Larimer County are affected. Because of the diagnosis of CWD in farmed elk in some states, including Colorado, a surveillance program for farmed elk is also in place in Colorado. In addition, 258 cattle in CWD endemic areas are also under study. Such an investigation is only possible with the active support and participation of livestock producers in endemic areas. Funding has been provided in part by producer organizations such as the Colorado Beef Council and Colorado Cattleman's Association. Although all phases of these studies are not complete, preliminary examination of these brains has failed to reveal evidence of a TSE in this population of cattle.

In order to exclude a diagnosis of TSE, it is necessary to histologically examine areas of the brain where the microscopic lesions characteristically occur. Therefore, careful brain removal and preservation without damage to the obex/brain stem is necessary. Please contact laboratory personnel to learn more about submission requirements and needs.

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CWD Surveillance: Submit fresh and fixed brain, Fee=\$35.

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## CHRONIC WASTING DISEASE DIAGNOSED IN A COLORADO FARMED ELK

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Wayne Cunningham

An adult bull elk from a captive farm premise in Colorado was diagnosed with Chronic Wasting Disease (CWD) on September 15, 1999. This is the first positive diagnosed case of CWD in farmed elk (Alternative Livestock) in Colorado. However, two CWD cases in Nebraska were traced to a Colorado farm in both 1998 and 1999.

We diagnosed CWD in the adult bull elk after the elk was submitted for a necropsy by a veterinary practitioner. The elk had been non-responsive to antibiotic therapy for pneumonia one month prior to his demise. At necropsy, the bull was found to have a severe bronchopneumonia. We also found the hallmark histopathological signs of CWD in the brain along with a positive immunohistochemical staining, confirming the diagnosis of CWD. Elk affected with CWD on South Dakota captive elk farms were commonly diagnosed with pneumonia. The rationale for CWD-affected animals presenting with pneumonia is that the hypoglossal nucleus often is affected by CWD. This compromises the affected animals' ability to swallow, resulting in inhalation of food material and saliva into the lungs.

Currently, the index farm and the farm that sold the bull to the index farm are under state quarantine. The index farm herd is comprised of 10 animals, and the owner has chosen

to depopulate his herd and receive indemnity from the Alternative Livestock Fund. This is a fund all captive cervidae producers pay into annually for indemnification for diseased cervidae, as determined by the Alternative Livestock Board. The trace back farm will remain under quarantine until a physical animal inventory is performed, records reconciled for CWD specimen submission for all mortalities, and the epidemiology is completed.

Currently, the Colorado State Veterinarian's Office has a rule mandating that all cervidae producers licensed under the Colorado Department of Agriculture must submit appropriate brain specimens for CWD examination to an approved laboratory from all mortalities. Mortalities include natural, slaughter, and hunt-harvested cervidae that are 15 months or older. The CWD Surveillance Program was implemented to preserve the marketability of Colorado farmed elk.

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## CALCULI ANALYSIS

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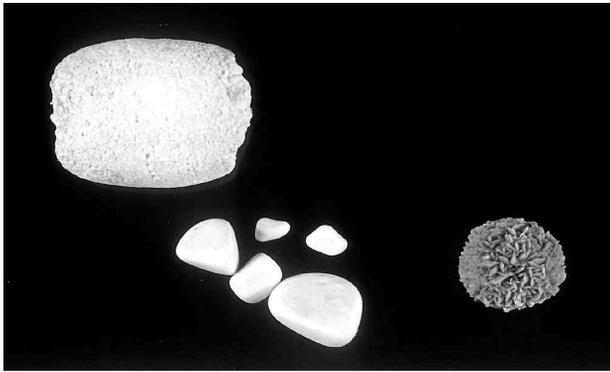
Dwayne Hamar/Cathy Bedwell/Marty Fettman

For the past two years, we have conducted a study comparing our qualitative calculi analysis with the results obtained by the Minnesota Urolith Center. We reported the qualitative results to you as soon as our analyses were completed. In order to improve the accuracy of our qualitative analysis, we also quantitated the calcium to magnesium ratio. If an adequate amount of canine specimen was available, we forwarded the sample to the Minnesota Urolith Center for analysis. Once we received the Center's reports, we forwarded results to you. We have compared our qualitative and quantitative results for 113 calculi with the results from the Minnesota Urolith Center.

If we assume the Minnesota Urolith Center results are the "gold standard" then, using our qualitative results only, we have misdiagnosed eight of 113 calculi. However, if we use the calcium to magnesium ratio determined by our quantitative analyses, we have only **one** outlier. From the time calculi are received in our laboratory, results are normally reported to you by fax or e-mail within three to five days. When sending calculi to the Minnesota Urolith Center, we received their results two to three weeks after the samples were sent. The one piece of information you receive from the Minnesota Urolith Center that we cannot provide with our method, is whether the calculi have differing major components in the nidus, shell, or surface.

We have decided to continue to provide calculi analysis for the following reasons:

- our qualitative results coupled with the quantitative calcium to magnesium ratio are accurate,
- to provide you with faster turn around time and convenience, and
- the Minnesota Urolith Center does not accept calculi from any species beside canine, feline, and exotics.



Urinary calculi from three different dogs.

The Minnesota Urolith Center does provide quantitative analysis at no charge, if in return you supply a good history of diet, etc. If you would like to take advantage of the Minnesota Urolith Center's no-charge service, they can be contacted at: Minnesota Urolith Center, University of Minnesota, College of Veterinary Medicine, Department of Small Animal Clinical Sciences, 1352 Boyd Avenue, St. Paul, MN 55108, phone 612-625-4221, or fax 612-624-0751.

Calculi analysis (including quantitation)—Submit calculi in clean container, Fee=\$15.

**MALIGNANT CATARRHAL FEVER: A New, Accurate Diagnostic Test for the Viral Agent Using PCR**

**Jim Collins**

**M**alignant catarrhal fever (MCF) is an acute lymphoproliferative disease of cattle, bison, deer, and exotic ruminants. The disease is characterized by high fever, corneal opacity, generalized lymphadenopathy, widespread vasculitis, and severe inflammatory and degenerative lesions in the mucosa of the upper respiratory tract and/or gastrointestinal tract. There are two recognized epidemiologic forms of MCF disease – 1/Wildebeest-derived MCF (WD-MCF), found in Africa and in zoological parks of North America and Europe; and 2/Sheep-associated MCF (SA-MCF), which has been reported worldwide.

Ovine herpesvirus 2 (OHV-2) is considered to be the cause of SA-MCF, but the virus has been refractory to isolation in culture. However, sequences from a single region of the OHV-2 genome with partial sequence homology to the WD-MCF virus (Alcelaphine herpesvirus-1) have been identified and used to establish a polymerase chain reaction (PCR) to detect OHV-2. We have applied the PCR to detect OHV-2 DNA in peripheral blood leukocytes and in tissues from terminal cases of SA-MCF in bison and cattle.

Previous investigations of SA-MCF resulted in the detection of herpesviruses and retroviruses. Therefore, we analyzed cases of SA-MCF in cattle and bison retrospectively for OHV-2 and for other persistent bovine herpesviruses, retroviruses, and pestivirus. We performed PCR assays for OHV-2, bovine herpesvirus 4 (BHV-4), bovine lymphotropic herpesvirus (BLHV), bovine syncytial virus (BSV, also known as bovine spumavirus), and bovine immunodeficiency virus (BIV), and conventional tests were used to detect BVDV. In addition, we performed a prospective survey to determine the prevalence of the three herpesviruses and two retroviruses in cows from dairies with a history of SA-MCF.

Using the OHV-2 PCR, the virus was found to be significantly associated with sheep-associated malignant catarrhal fever (SA-MCF) in terminal cases of disease in 34 cattle and 53 bison (see table below). OHV-2 was not detected in 38 cattle and 10 bison that succumbed to other diseases. The persistent herpesviruses, retroviruses, and pestivirus were not associated with the disease. This indicated that the OHV-2 PCR was a sensitive and specific test for MCF, and most likely the virus acts alone to induce the disease. The OHV-2 PCR results correlated well with lesions observed histopathologically, resulting in a variety of tissues (lung/liver/kidney/spleen) yielding positive PCR tests for OHV-2 sequences.

**Association of Histopathologic Diagnosis of MCF with OHV-2 PCR Amplification**

|                 | PCR/Bison |     | PCR/Cattle |     |
|-----------------|-----------|-----|------------|-----|
|                 | Pos       | Neg | Pos        | Neg |
| Histopathologic | Pos 53    | 1   | 30         | 0   |
|                 | Neg 0     | 10  | 1          | 38  |

A PCR survey for OHV-2 in DNA from individual cows' peripheral blood lymphocytes in four dairies showed that a dairy in close contact to sheep had a prevalence of OHV-2 of 21.3 percent. In three other dairies, cows did not have OHV-2, and had little contact with sheep. We are investigating whether the presence of OHV-2 in normal animals means that they will eventually develop MCF, or that they are potential carriers and, perhaps, infectious sources of the virus.

Prevalence of the other herpesviruses and retroviruses in the dairy cows was variable, ranging from 2 to 51 percent for BHV-4, 52 to 78.7 percent for BLHV, and 10 to 34 percent for BSV. These viruses have not been associated with a specific infectious disease(s), but further investigation is warranted.

PCR for OHV-2: Submit fixed or fresh tissue, or blood in an EDTA tube, Fee=\$22, (\$10 if >20 samples).

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## GET TO KNOW YOUR LAB/Meet Our Client Services Staff

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Our Diagnostic Laboratory Office is managed by Carrie Schmer. Carrie's name and face might be quite familiar to many of you since she has been working here for 14 years. Carrie comes to us from an agricultural background and is a CSU graduate with an Animal Sciences degree. In addition to her management duties of the reception, sample entry and processing areas, she also spends her time coordinating special project cases within the laboratory. Please give her a call with any problems or concerns you may have in these areas.

Nancy Ault supervises our staff in the phone reception, data entry and billing areas. She also is a long time employee, working on her seventh year, and has both large and small animal experience. She works primarily on billing necropsy and sample evaluation cases, and coverage for employee absences. You may speak frequently with her over the phone or in person when dropping off samples. Nancy also is responsible for all samples referred to other laboratories for testing, and can answer questions you may have regarding these send-outs.



Carrie Schmer, Nancy Ault, LeeAnn Mitchell, and Erica James.

LeeAnn Mitchell was welcomed as a transfer from the Department of Microbiology one year ago. LeeAnn spends her days inputting cases into our database system as well as billing and distributing results and invoices to clients. She also helps maintain our pending case files and the daily distribution of results to clinicians within the Veterinary Teaching Hospital.

Erica James is our newest employee in the office area. Like Carrie, she also is a CSU graduate in biology and was hired in early July. Erica works mornings in the sample entry area, processing and routing samples arriving through the mail as well as those dropped off by clients locally. Her

afternoons are spent as our main phone receptionist. We hope it won't be long before you have an opportunity to visit with her.

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## QUALITY CONTROL

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Dwayne Hamar

For several years, all sections of our Diagnostic Laboratory have included various quality control samples as part of their routine sample analyses. The American Association of Veterinary Laboratory Diagnosticians (AAVLD) has become increasingly concerned and more stringent about quality control as a part of their accreditation review. This is of special importance as we deal more and more with international trade issues. In fact, in the last year there were several articles in the AAVLD newsletter titled "Quality Corner." We are very pleased we initiated Standard Operating Procedures (SOPs) and quality control procedures long ago. Recently, we have compiled all of the quality controls used in each laboratory section into one document as our Quality Control Manual. This document will be maintained on our Web site for use by employees of the Diagnostic Laboratory, as well as keeping a hard copy in the main office.

Our quality control program includes testing samples from National Veterinary Service Laboratory (NVSL), National Institute for Standards and Technology (NIST) and commercial sources, while other samples are maintained within our own sections or exchanged with other laboratories. Some of these quality controls are:

Anatomic and Clinical Pathology--All of our pathologists are diplomates of the American College of Veterinary Pathologists (ACVP), and we hold weekly pathology slide and general conferences. Informal slide consultation between pathologists also is common. We include twice daily controls in clinical pathology analyses. An outside agency performs quarterly proficiency examinations for clinical pathology.

Bacteriology—We perform annually NSVL (certifying samples) and AAVLD samples for aerobic and anaerobic cultures and antibiotic susceptibility tests. We include internal and commercial quality control samples for organisms with each test or weekly. Internal controls with each set and annual NVSL tests for serology for Mycoplasma, Salmonella, Johne's disease and Leptospira are done.

Chemistry/Toxicology—Outside agencies provide monthly blood lead and quarterly proficiency examinations. We include samples from NIST, commercial and/or in-house samples with each analysis.

Drug Monitoring and Endocrine—We use controls with every analysis. We also do a quarterly proficiency test with Michigan State University.

Parasitology—We use internal and commercial samples with each test.

Virology—We perform NSVL (certifying samples) annually for serology for BLV, PRV, BT, EIA, and EVA. We include internal positive and negative samples with every virus isolation, fluorescent antibody, immunoperoxide and PCR procedure.

Some of these internal and/or external samples are used to validate new methods as well as to validate that day's results. The objective of our quality control program is to provide you, our clients, with the best possible results at a reasonable cost.

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## FROM THE WESTERN SLOPE

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Dr. Darrel Schweitzer

We are very proud that Dr. Darrel Schweitzer was chosen as the 1999 Guest of Honor of the Colorado Wool Growers Association. Darrel is a native Coloradoan and graduated from Kansas State University in 1974. He practiced for two years in Canada, then did graduate studies in pathology at Colorado State University, receiving his MS degree in 1978. He has been Director of the Grand Junction Diagnostic Laboratory since 1978. Darrel has worked closely with sheep producers in Colorado and his laboratory is known for performing a large volume of ELISA tests for *Brucella ovis* every year. The Grand Junction Laboratory also provides a variety of other diagnostic tests including pathology, bacteriology, serology, virology, and parasitology.

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## BOVINE TRICHOMONIASIS, Something New

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John Cheney

**B**ovine trichomoniasis (caused by *Trichomonas foetus*) often is endemic to beef herds which practice natural breeding. This is especially true in the Western states where cattle are allowed to co-mingle in grazing associations, and on state and federal grazing permits. Prevention and control

of bovine trichomoniasis relies on management practices. One preventive practice recommended is the use of young bulls for breeding. This recommendation has been made because young bulls, under 2 years of age, usually are resistant to infection and, if they become infected, are probably less likely to become permanent carriers. In young bulls, the epithelial crypts in the mucus membrane of the preputial cavity and on the glands penis are not as deep as they are in older bulls. For this reason, yearling bulls, especially those thought to be virgin bulls, are not tested prior to sales and use for breeding. In some Western states, which require testing of bulls for trichomoniasis before entering their state, yearling bulls are exempt from the testing.

This past spring, one Western state required that all bulls used for breeding, no matter what age, be tested for trichomoniasis prior to entry into the state. In testing yearling bulls going into this state, a yearling bull was found to be infected. Tests were then conducted on the other yearling bulls from this same sale and 21 more were found infected. In another herd in a different Western state, two yearling bulls were found infected, and there was no contact between the bulls in the two herds. How these yearling bulls, which were thought to be virgin bulls, became infected is not known.

These findings have to change our thinking and the recommendation we make regarding the use and testing of young, especially yearling, bulls for trichomoniasis. At this time, all bulls used for breeding, regardless of age, should be tested prior to using them. In most herds, testing for trichomoniasis should be a part of the breeding soundness examination.

Because the bull is more likely to be a carrier, most diagnostic effort should begin with the bull. It is very important that the sample collected from the bull be taken properly. To obtain this sample, attach a clean dry artificial insemination pipette to a 10cc syringe and insert into the prepuce to the level of the fornix. Move the pipette back and forth scraping vigorously across the mucus membrane of the prepuce and glans penis while applying negative pressure to the syringe. Before removing the pipette, relieve the negative pressure on the syringe. The pipette must contain some cloudy material which is often blood-tinged. If the sample in the pipette is clear, this is an inadequate sample, and a new sample must be taken. This sample is then flushed into a clean vial containing 2cc of lactated Ringers solution for transport to the Diagnostic Laboratory. Please submit as soon as possible within a 48-hour period and notify us in advance if a large quantity is to be submitted.

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Trichomonas culture: Submit sample as described, Fee=\$8 (\$7 if >50 samples).

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**NEW TEST FOR INSULIN LEVELS**—The endocrinology section is offering a new test for measuring insulin levels. This is done on the IMMULITE analyzer and requires about 0.5ml of serum in a red top tube. Keep the sample cold (refrigerate or icepack) prior to submission. Fee=\$30.

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**NEW TESTS FOR DIFFICULT-TO-DIAGNOSE CASES OF LYMPHOID MALIGNANCY IN DOGS**

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Anne Avery

While most cases of malignant lymphoma and leukemia in dogs are simple to diagnose, all practitioners have encountered cases in which they suspect a lymphoid malignancy, but cannot confirm the diagnosis despite numerous diagnostic tests. For example, a dog's large lymph nodes might be called hyperplastic by the pathologist, or a bone marrow sample might have increased numbers of plasma cells, but not enough to diagnose multiple myeloma. Chronic lymphocytosis may arouse suspicions of chronic lymphoid leukemia (CLL), but the lymphocytes may appear relatively normal by cytology. We recently have developed a diagnostic test that can help solve some of these diagnostic dilemmas.

One of the most important features of malignancy is all of the neoplastic cells arose from a single cell, making the process a clonal one. Therefore, to distinguish lymphocytes that are neoplastic from lymphocytes that are reacting to antigen, one needs to determine if the population is clonal or not. One of the most important features of lymphocytes is that each one is different because each lymphocyte carries a unique receptor for antigen. T-lymphocytes express the T-cell receptor and B-lymphocytes express immunoglobulin. Plasma cells are derived from B-lymphocytes and also have unique immunoglobulin genes. Other leukocytes, such as NK cells, neutrophils and macrophages, have no such unique feature.

We have developed a polymerase chain reaction (PCR) assay that allows us to determine if a given population of lymphocytes is composed primarily of a clonal cell population, or if it is composed of numerous different clones, such as would be seen during the response to an infectious disease. We can do this because the antigen receptor expressed by each lymphocyte is slightly different in size. We amplify the antigen receptor gene from DNA extracted from your sample and separate all of the amplified products on a gel by size. The presence of a single band means neoplasia. The presence of a smear (representing a large number of different antigen receptor genes) means a heterogeneous population of lymphocytes likely to be responding to antigen.

In preliminary studies with this method using a total of 72 dogs, 55 of which had lymphoid malignancies, we found that the assay was 93 percent sensitive and 93 percent specific. Therefore, we failed to detect about 7 percent of the dogs that we knew, by other criteria, to have lymphoma. We had a single false positive – a dog with no evidence of lymphoid neoplasia that had a positive result on PCR. It is possible that this dog will eventually develop leukemia or lymphoma, but he remained healthy six months after the PCR assay was done. Like any diagnostic test, this one must be interpreted with the other clinical data available.

Appropriate times to ask for this assay would include:

- When biopsy samples have been diagnosed as hyperplastic but you still suspect lymphoma,
- In cases of chronic lymphocytosis,
- Evaluation of the bone marrow of dogs that have unexplained peripheral cytopenias,
- Evaluation of the bone marrow of dogs that have hypercalcemia, and you are suspicious of myeloma,
- Evaluation of masses from dogs that have been previously treated for lymphoma and are presenting with a new tumor (the size of the PCR product offers us a molecular fingerprint of the tumor that will help us to determine if the recurring tumor is the same as the initial one).

The test can be run on the following material – a purple top tube containing blood or bone marrow, lymph node or mass aspirates, lymph node or mass biopsies; or a purple or red top containing cavity fluid (ascites, pleural effusion, CSF). For aspirates, take several (at least three) good aspirates of a lymph node, eject the contents of the syringe into a red top tube that has about 1ml of saline (LRS, NormR, 0.9 percent saline), and rinse the syringe in the saline after each aspirate. We cannot do this analysis on formalin-fixed tissue. We do not believe treatment with steroids affects our results, although we have not tested this rigorously.

As a practical matter, if you suspect lymphoma or leukemia and are submitting samples, it would be appropriate to submit enough material for the PCR analysis with your initial mailing. If the pathologists do not feel that they can make a diagnosis by cytology or histology, they can submit samples for PCR. Submit a purple top of bone marrow rather than slides; submit lymph node aspirates in saline as well as slides; put a small piece of your biopsy in saline as well as formalin. This is especially important for samples you don't want to obtain twice (ultrasound or endoscopically-guided biopsies, or difficult-to-do bone marrow biopsies).

PCR results are available twice a week on Wednesdays and Fridays. Material received up to Tuesday afternoon will be available on Wednesday, and up to Thursday afternoon available Friday afternoon. When possible, we also will do flow cytometry on the sample (only peripheral blood or cavity fluids if there is sufficient material). Using flow cytometry, we can establish the phenotype of the cells in question. We use

antibodies specific for canine CD4, CD8 (T-cell markers), CD21 (a B-cell marker), and several others. This assay can be particularly useful in cases of lymphocytosis, and in thoracic effusions composed primarily of small lymphocytes.

At present, **there is no charge** for the assay. After approximately six months, we will re-assess the need for this service, the amount of time it takes, and the cost of materials. At that time, we will institute charges for the PCR and flow cytometry tests.

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**BOVINE RESPIRATORY SYNCYTIAL VIRUS (BRSV) INFECTION IN RANGE COWS**

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**Charles Dickie/Rocky Ford**

**B**ovine respiratory syncytial virus (BRSV) was involved in a prolonged disease outbreak in a 1,271 Hereford cow herd before BRSV vaccination was available. There was a rapidly fatal, acute interstitial pneumonia in cows. Normal appearing, vigorous cows would become dyspneic and within hours were in extreme respiratory distress. The swiftness with which some cows died was dramatic. Serology for BRSV, multinucleated syncytial cells, interstitial pneumonia, and pulmonary emphysema established the diagnosis. We can also confirm this with immunohistochemistry on formalin-fixed tissue. A hypersensitivity factor in disease caused by BRSV has been discussed in the literature.

Vaccination of the entire herd prevented additional disease in the herd. BRSV now has more notoriety as a cause of disease in the younger animals, but it can be rapidly fatal for older cows.

**HELP US HELP YOU**—Please be sure to fill-out request forms completely, including your clinic name and address, owner and animal identification, and history and test request. Please be sure to label samples. We have been receiving blank or incomplete request forms and unlabelled samples!

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**IONIZED CALCIUM SAMPLE REQUIREMENTS**

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**Linda Vap**

**I**onized Calcium (i-Ca<sup>++</sup>) determination is useful for evaluation of calcium levels to confirm clinical signs suspected to be due to hypocalcemia. Collect whole blood samples for i-Ca<sup>++</sup> analysis anaerobically in a syringe rinsed with heparin. The i-Ca<sup>++</sup> will be falsely lowered if the sample contains excess heparin. Place the syringe in a ziplock-type of bag of ice water and submit within two hours of collection. If this is not possible, serum that is obtained,

handled and stored anaerobically can be accurately measured for i-Ca<sup>++</sup> concentration after three days refrigerated or at room temperature, and after seven days if frozen. Mixing serum with air results in significant decreases in the i-Ca<sup>++</sup> concentration and increases serum pH.

To obtain and store samples anaerobically, collect blood directly into a vacutainer tube without removing the top, allow to clot, spin, and remove the serum as soon as possible through the cap with a syringe and needle. Remove all air. Freeze the sample and ship with dry ice if it will take 37 days to arrive or, refrigerate and ship with an ice pack if it will be received within three days. Note that frozen samples develop air bubbles upon thawing during shipping unless freezer temperatures are maintained with dry ice. The sample should be tested immediately upon arrival.

**CSU/VTH Heparinized Whole Blood  
Reference Range for i-Ca<sup>++</sup>**

| Species | mmol/L    | mg/dl   |
|---------|-----------|---------|
| Canine  | 1.12-1.40 | 4.5-5.6 |
| Feline  | 1.20-1.32 | 4.8-5.3 |
| Equine  | 1.25-1.75 | 5.0-7.0 |
| Bovine  | 1.00-1.25 | 4.0-5.0 |

Ionized Calcium Testing: Submit as described, Fee=\$14.

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**PRODUCERS CAN TEST THEIR CATTLE FOR INTERNAL PARASITES**

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**John Cheney**

**T**his fall, beef cattle producers in the Rocky Mountain region will be weaning their calves and working the cow herds. This is the time of year when these cattle should be tested for internal parasites (worms) and treated if counts can be used to determine which cattle on the ranch or farm should be treated and which cattle will not benefit from treatment. To get an accurate picture of the internal parasite problem in the herd, submit 5-10 individual fecal samples from each group of cattle on the farm or ranch. For example, the calves, the yearlings, and the adult cows if the young cattle are found to have sufficient worm burdens. Cattle on different types of pasture should be considered as different groups and sampled as such. Collect fresh fecal samples individually but these can be picked up from the ground as soon as the animal defecates. The sample should be about one-quarter cup in size and placed in a plastic baggie that can be sealed. Keep the samples cool and please notify the laboratory prior to sending. Send to the ATTN of Dr. John Cheney or Ms. Glenda Taton-Allen by overnight or priority mail. Do not send samples to the laboratory to arrive on the weekend.

Fecal testing: Submit one-quarter cup feces kept cool, Fee=\$5 (regular cost is \$8 per sample).

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## CHRONOLOGICAL SALMONELLA ISOLATIONS

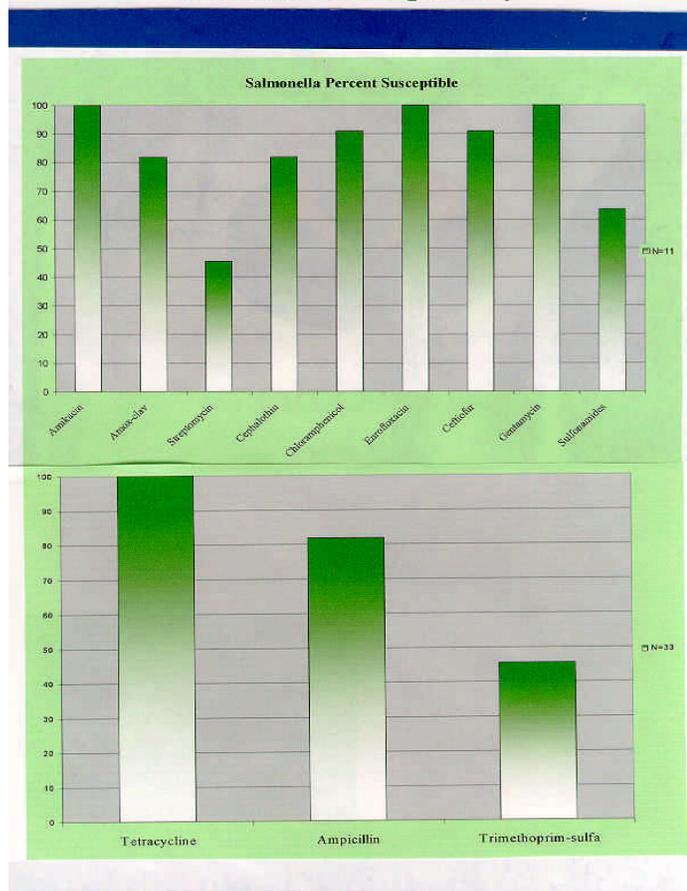
Doreene Hyatt

We have examined and tabulated our Salmonella isolates from January through August, 1999. We isolated eight different serotypes on 13 different occasions. The table below details our findings.

| MONTH | ANIMAL                        | SEROTYPE                            | SAMPLE  |
|-------|-------------------------------|-------------------------------------|---|
| Feb   | Python                        | <i>S. arizona</i>                   | Wound   |
| Feb   | Porcine ( <i>kunzendorf</i> ) | <i>S. cholerasuis</i>               | Lung/kidney/liver/nasal   |
| Feb   | Lizard                        | <i>S. arizona</i>                   | Fluid   |
| Feb   | Canine                        | <i>S. typhimurium (copenhagen)*</i> | Large intestine   |
| Mar   | Bovine (23 animals)           | <i>S. monevideo</i>                 | Feces   |
| Mar   | Bovine (2 animals)            | <i>S. typhimurium (copenhagen)</i>  | Small intestine   |
| Mar   | Green water dragon            | <i>S. flint</i>                     | Unknown   |
| Apr   | Bovine                        | <i>S. montevideo</i>                | Small intestine   |
| Jun   | Equine                        | <i>S. anatum</i>                    | 1<br>iver/liver abscess/colon/lymph node/bile<br>duct/feces/heart valve/small intestine/colon content   |
|       | Equine farm                   | <i>S. anatum</i>                    | Sponge/stall/gravel/stock drain/hose/mat/feed/bag/<br>walls/sludge/water supply/dead mice/flies/beetles |
|       | Equine farm                   | <i>S. montevideo</i>                | Mud and water   |
|       | Equine farm                   | <i>S. newport</i>                   | Grave   |
| Aug   | Elk                           | <i>S. kentucky</i>                  | Uterus  |

\*Suspected *S. typhimurium* DT104, results pending.

### Antibiotic Susceptibility



We also examined the antimicrobial susceptibility patterns of the Salmonella isolates. This information is shown in the chart to the left. The antibiotics read left-to-right, top row: Amikacin, Amox-clav, Streptomycin, Cephalothin, Chloramphenicol, Enrofloxacin, Cefixim, Gentamycin, Sulfonamides; bottom row: Tetracycline, Ampicillin, Trimethoprim-sulfa.

To submit samples for bacteriologic cultures, including Salmonella, submit fresh tissue in a sterile container and ship overnight on ice **or** submit a Port-A-Cul swab. For suspected outbreaks of Salmonella, we also have a rapid PCR test that provides results in 24 hours (see LabLines, Vol. 3, No. 2).

Salmonella culture: Submit samples as described above. Fee=\$11, plus \$9 for sensitivity. PCR test plus culture: Fee=\$41.

### BACTERIAL NAME CHANGES!

The bacteriology section reports are now using the new designation "*Arcanobacterium pyogenes*" for the organism formerly known as "*Actinomyces pyogenes*." Future watch—The genus designation for *Pasteurella haemolytica* and *P. granulomatis* have been changed to the genus *Mannheimia* (*M. haemolytica* and *M. granulomatis*).

### ERADICATION OF OPP OR CAE: Testing with AGID vs PCR

Jim Collins

We recently completed a comparison of serologic diagnosis to polymerase chain reaction (PCR) diagnosis of lentivirus- (OPP and CAE) infected small ruminants. Serologic diagnosis usually is performed with the agar-gel immunodiffusion (AGID) test and detects serum antibodies resulting from infection with one of these viruses. The PCR detects the presence of the virus genome in blood cells. It detects a common genetic region of the virus core protein of OPP and CAE viruses as identified from sequences deposited in GenBank, at the National Center for Biotechnology Information.

We sampled groups of animals from four infected sheep herds and performed both tests, using an AGID test kit (Veterinary Diagnostic Techniques, Wheat Ridge, CO) and a previously published PCR test. We expanded the PCR to be a "double PCR" (double amplifications) so as to increase sensitivity. We obtained the results shown below.

#### Number of Animals in Results Categories Comparing AGID to PCR for OPP/CAE Diagnosis

|                   |          | PCR Test Results |          |
|-------------------|----------|------------------|----------|
|                   |          | Positive         | Negative |
| AGID Test Results | Positive | 38               | 5        |
|                   | Negative | 4                | 26       |

We considered an animal to be positive if either PCR or AGID gave positive results. PCR detected additional sheep that were AGID negative. These cases consisted of four older sheep that may have had declining antibody. Serology also detected additional sheep that were PCR negative. These cases may have represented infections with viruses having diverse genetic sequences, a propensity of the lentivirus group of viruses.

Using our criteria, the sensitivity of PCR was 89 percent and the sensitivity of AGID was 91 percent. Since neither test

detected all infected animals, our recommendations would be:

- For eradication and control purposes, both tests should be used.
- AGID screening could be performed first since it is less expensive, followed by PCR testing of the negative animals.
- In all likelihood, multiple testing of animals (at least two) should be performed with either test over a period of several months.

AGID for OPP or CAE: Submit 1ml serum, Fee=\$5.

PCR for OPP or CAE: Submit blood in EDTA tube, Fee=\$22.

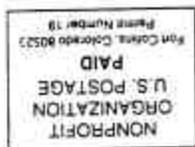
### CORYNEBACTERIUM IN HORSES

We have recognized an increase in isolations of *Corynebacterium pseudotuberculosis*, the cause of "pigeon fever" or "pigeon breast," from horse abscesses and wounds since September. We normally isolate this organism from horses one or two times a year, but we have had nine isolates in the last two months, all from the Larimer County area. See LabLines Vol. 2, No. 2 for previous article on this subject

**OOPS!**—In our last newsletter, we mistakenly printed "snakewood" instead of "snakeweed."

## WHAT'S INSIDE THIS ISSUE OF LABLINES

- Hypothyroidism in Dogs
- Chronic Wasting Disease Updates
- Calculi Analysis
- New Tests for Malignant Catarrhal Fever
- Quality Control in the Laboratory
- Bovine Trichomoniasis
- New Tests for Lymphoid Malignancy in Dogs
- BRSV in Range Cattle
- Ionized Calcium Determination
- Salmonella Isolations
- New Tests for OPP and CAE



**Colorado State University**

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