

# Colorado State University Veterinary Diagnostic Laboratories



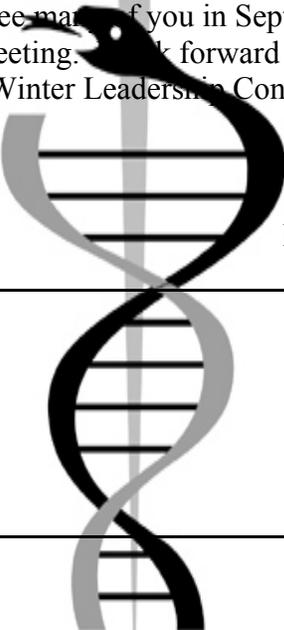
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## Letter from the Director

We hope this issue of LabLines finds you healthy and in good spirits! The last year has been challenging for us all with state-mandated budget cuts, continued drought, and increased disease challenges such as West Nile Virus. We continue to meet these challenges. Through a grant from USDA in support of the National Animal Health Laboratory Network and the American Association of Veterinary Laboratory Diagnosticians, we have completed our modular BSL-3 Laboratory unit. This allows us to do surveillance for foreign animal diseases, either accidentally or purposely introduced into the country. We have sent our technicians to Ames, Iowa and Plum Island to learn how to do this testing. Our virologist, Dr. Hana Van Campen, also has been to Plum Island and assisted in California with the Exotic Newcastle's Disease outbreak. One of our pathologists, Dr. Gary Mason, recently attended the foreign animal disease course at Plum Island.

We finished our fiscal year June 30 with a 37% overall total increase in accessions (31.5%/Fort Collins; 182%/Grand Junction; and 42%/Rocky Ford). Unfortunately, with the state budget cuts, we have been forced to raise prices to make ends meet. Our facilities remain inadequate at the Fort Collins Laboratory, and we continue working to obtain funding for a new building. We have hired a new pathologist, Dr. Karamjeet Pandher, who begins December 1, to replace Dr. Robert Norrdin who is going on transitional retirement. Please see inside for a bio on Dr. Pandher. It was wonderful to see many of you in September at the annual Colorado Veterinary Medical Association Meeting. Look forward to seeing many of you soon at the CSU Annual Conference and CVMA's Winter Leadership Conference.



*Barbara E. Powers*

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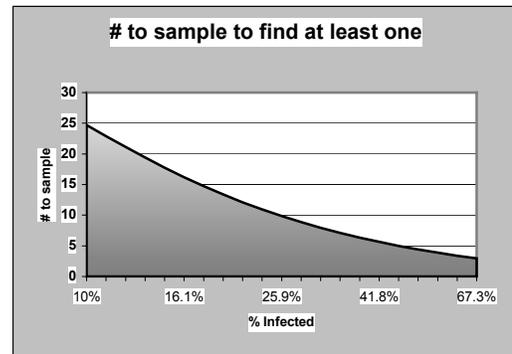
## DEVELOPING A BIOSECURITY PROGRAM, PART III

—James Kennedy/Rocky Ford

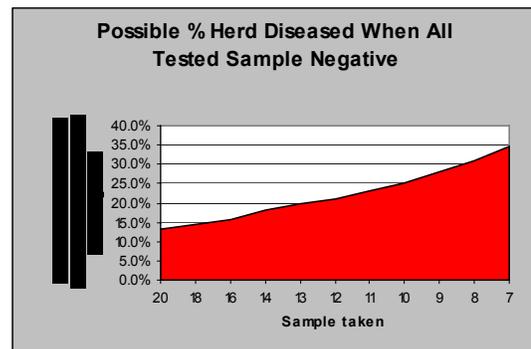
In this series, we have addressed the concepts of client education and proper disinfectant usage as parts of a biosecurity program. The next areas of discussion are diagnostic testing and minimizing the risk of introducing pathogens into the beef herd. Before discussing diagnostic testing, however, we need a reminder of what constitutes a biosecurity program. Homeland security and biosecurity, though intimately intertwined, are two separate and distinct entities. Biosecurity has been practiced for years, but our awareness of disease and realization that agriculture is a business that must operate efficiently to be successful has brought to the forefront the need for good animal husbandry that incorporates biosecurity as a key component. Whenever we minimize the risk of introducing infective agents into a herd, we are practicing biosecurity. One of the ways we can minimize introduction of infective agents is proper use of disinfectants. Another way is diagnostic testing.

We routinely draw blood samples and conduct antibody tests to detect the presence of disease-causing agents. We have infective antigens and graded responses to detect disease, such as tuberculosis, and we culture viruses, bacteria, and protozoa. The type of test selected is determined by our goal, cost, and ease of collection of necessary samples. As veterinarians, we expect tests to be fast, reliable and inexpensive; a combination not always easily obtainable. Antibody tests rely on the animal's ability to produce antibodies and to produce those antibodies at a detectable level. A level of detectable antibodies may not be produced until after the animal already has shed the virus or bacteria of concern. If we base a biosecurity program on an antibody test, we may accept animals that are infective but have not reached a detectable level of antibody production. Because of this, it would appear that a test detecting antibody levels would be inappropriate for a biosecurity program. This would be correct if we are planning on testing one animal and, based on the results of that single test, allowing that animal into our herd. On the other hand, we might use an antibody test if we did paired samples or if we tested an appropriate number of herd mates, screening the herd for the presence of a disease. This raises the question – what is an appropriate number to test? Determining sample size can be difficult. It is impacted by prevalence, herd size, and the amount of confidence of detecting a diseased animal you desire. Graph 1 shows the number of head you would need to sample at different prevalence levels to detect a single positive animal based on a perfect test in a herd of 100 animals with 95% confidence.

If we have a herd of 100, but test just 10, how many could be infected that we missed? What is the herd prevalence? Graph 2 depicts the prevalence that could exist despite negative tests on a certain number of animals. Again, we have a herd of 100 animals and want to be 95% sure that, if we detect zero positive animals, the prevalence of disease in that herd is less than the value of the Y axis. As an example, you randomly



select 10 animals from a herd of 100. You test them for a certain disease and they all test negative. We conclude that if the disease is present, it is below the 25% prevalence level. That is a frightening concept when developing a biosecurity program, but a meaningful reminder when designing a testing program. We could improve the results by selecting animals by clinical signs, age, or some other parameter, but we still have left the door open for a large number of infectious agents. Another point to keep in mind is that the graphs shown are generated assuming a perfect test. If the test is an imperfect one, the size to sample becomes an even more complex problem.



It is important to test often and test enough. If you are about to undertake a herd testing program, contact us and discuss what number you might need to test, what test results can be expected, and how to interpret those results.

## A CASE OF CANINE RABIES IN COLORADO

—Kristy Pabilonia

On July 13, a family adopted and transported a male Border collie puppy from a ranch near Amarillo, Texas, to their home in Fort Morgan, Colorado. The puppy had survived an attack by a skunk on July 1. All other members of its litter died in the attack. On July 28, the family took the puppy to their veterinarian with complaints of lameness and fever. During the next three days, the clinical signs progressed to paralysis and dyspnea. The puppy died on July 31.

The veterinarian suspected rabies and submitted the head to us for testing. The brain was removed and sections taken for histopathology and fluorescent antibody testing. Histopathology revealed severe, non-suppurative encephalitis. The cerebellum, medulla, and hippocampus were used to make impression slides and were stained for rabies virus by direct immunofluorescence. This fluorescent antibody test was positive for rabies. Additional brain tissues were submitted to the Colorado Department of Public Health for fluorescent antibody and mouse inoculation testing for confirmation. Brain tissues also were sent to the Centers for Disease Control and Prevention for monoclonal antibody testing to determine the strain of rabies virus involved. The skunk is thought to be the source of rabies for this case.

Rabies is one of the oldest and most lethal of all known infectious diseases. All warm-blooded animals are susceptible to rabies and almost all cases result in death. Six rabies virus variants are prevalent in terrestrial animals in North America. The distribution includes most of the eastern and central United States and parts of California, Texas, Arizona, New Mexico, Alaska, Canada, and Mexico. The terrestrial reservoir host species include skunks, raccoons, coyotes, and red, gray and arctic foxes.

Two clinical forms of rabies, the furious form and the dumb/paralytic form, are recognized. The furious form is thought of as the classical presentation of rabies and is easily recognized by the public. The furious form is characterized by a variety of clinical signs including aggression, decreased fear of humans, hypersalivation, hyperesthesia, and hydrophobia. The furious form often, but not always, precedes the dumb/paralytic form which is characterized by ataxia, seizures, wandering, progressive paralysis, and respiratory failure.

Colorado has not had a case of terrestrial (non-bat strain) rabies since an outbreak of rabies in skunks from 1988-89. The last case of rabies in a terrestrial animal was a bat strain in a bobcat last year. The last reported case of canine rabies in Colorado was in 1974. Since January 2003, we have received more than 100 submissions for rabies testing and have diagnosed seven cases. One case was the dog described in this article and the other six cases were bats. This case emphasizes the need for testing of animals that die of neurologic disease, as well as the importance of a vaccination program to protect against rabies.

Rabies testing—Submit brain (fresh) or entire head. Fee=\$60.

\*\*\*\*\***REMINDER**\*\*\*\*\*

Please be complete when filling out ALL submission forms. For West Nile virus submissions we need owner, animal location, city, zip, and county. This information is required by the Colorado Department of Public Health.

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## FELINE IMMUNODEFICIENCY VIRUS

—Andrea Torres and Hana Van Campen

**Virus**—Feline immunodeficiency virus (FIV) is in the family Retroviridae and belongs to the Lentivirus gene. Typical of retroviruses, FIV RNA is reverse transcribed into DNA which then integrates into the host cell chromosome. The virus then persists for the life of that particular cell. FIV can infect all species of cats, however, it causes no discernible disease in non-domestic felids. Other animal lentiviruses include caprine arthritis-encephalitis virus, maedi-visna/ovine progressive pneumonia virus, equine infectious anemia virus, bovine immunodeficiency virus, simian immunodeficiency virus, and also human immunodeficiency virus.

**Genetics**—There are five recognized FIV clades or subtypes—A, B, C, D, and E. Each clade contains many strains. The most commonly isolated clades in the United States are A and B. Clade A is more frequently isolated in the western United States and Clade B in the eastern United States. FIV, being an RNA virus, has high error frequencies in RNA replication and, thus, vast genomic sequence diversity. Because of this, there is significant intra- and inter-clade sequence variation. All clades are capable of causing disease in domestic cats.

**Transmission**—The primary route of FIV transmission is through biting. Free-roaming male cats are at the greatest risk of becoming infected. Although vertical transmission is less common, FIV can be transmitted from an infected queen to her offspring *in utero*, during the peripartum period, and via milk. Although prevalence varies by location, 2% to 3% of healthy domestic cats throughout the United States are FIV positive. Up to 15% of sick cats in the United States are FIV positive.

**Disease**—FIV infection in domestic cats, similar to HIV-1 infection in people, is characterized by a terminal immunodeficiency syndrome. During the acute phase of infection, cats have an easily detectable antibody response and an early progressive decline in CD4+ T-lymphocytes. This is followed by a prolonged asymptomatic phase with eventual immunological dysfunction. Terminally, these cats have wasting, recurrent fever, opportunistic infections, and various secondary diseases. Additionally, some infected cats never develop FIV-related disease.

**Diagnostic Assays**—The pathogenesis of FIV dictates the types of diagnostic assays that are currently available.

**Virus Isolation**—All infected cats have virus somewhere in their body. Isolating this virus, the definitive diagnosis, is possible at certain research laboratories but it is time intensive, cumbersome, and expensive.

**Antibody Detection**—Antibody levels are high in the serum of infected cats and the presence of these antibodies correlates with isolation of the virus. Detecting these antibodies is the

routine method of diagnosis because it is quick, easy, and inexpensive. ELISA or Western Blot (WB) are used to detect antibodies against FIV, irrespective of the clade with which the cat is infected. Most cats produce antibodies within 60 days of becoming infected. In addition to vertical transmission resulting in infection and thus antibody production, kittens also can acquire maternal-derived antibodies from infected queens despite not being infected themselves. Kittens in both scenarios will test antibody positive.

**Antigen Detection**—In contrast to antibodies, there is limited circulating viral antigen expression with FIV infection. Consequently, there are not any diagnostic assays to detect FIV antigen directly in serum or circulating blood cells.

**Viral Nucleic Acid Detection**—Once FIV DNA integrates into a lymphocyte's chromosome, this proviral form will persist for the life of that cell. Potentially, this DNA can be detected using Polymerase Chain Reaction (PCR) technology. However, the sequence of the target DNA to be amplified must be known prior to using a PCR assay. Because of the diversity of FIV genomic sequences, it is difficult to design a PCR assay that can detect all of the five known clades and the numerous strains with which one cat may be infected. In addition, the presence of circulating blood cells with integrated FIV DNA is not consistent. A negative result could be a false negative due to the inability of the PCR assay to detect the particular infecting FIV strain or because of low levels of circulating blood cells with integrated FIV DNA. FIV research laboratories also can detect viral RNA using reverse-transcriptase PCR.

**Vaccination**—In July 2002, Fort Dodge Animal Health released a whole, inactivated FIV vaccine which contains strains from clades A and D. According to peer-reviewed published reports, 67% of the vaccinates were protected and 74% of the unvaccinated control cats became persistently infected. Therefore, the preventable fraction for the vaccine is 55%. Results regarding protection for clades other than A and D have not yet been published. Vaccinated cats will develop antibodies to FIV. Consequently, vaccinated cats will test positive via currently available ELISA and WB assays. Therefore, ELISA and WB cannot distinguish if an FIV antibody positive cat is infected, vaccinated, or infected and vaccinated. Cats vaccinated with the inactivated vaccine will not test positive by virus isolation, PCR, or antigen detection.

#### ***Diagnostic Assays Offered by the Diagnostic Laboratory***

**ELISA**—The SNAP® feline combo test (IDEXX Laboratories, Inc.), based on ELISA technology, is used to detect antibodies to FIV. A positive test indicates the cat has antibodies to FIV. This test cannot distinguish antibodies as a result of natural infection, vaccination, or infection and vaccination. Cats infected with any of the five clades will test FIV antibody positive. Kittens with maternal-derived antibodies from infected queens or vaccinated queens may test antibody positive. A positive ELISA sample should be

confirmed with the more specific WB assay. A negative test indicates the cat does not have antibodies to FIV. It is possible for an infected cat to have delayed seroconversion. Re-testing these antibody-negative, at-risk cats a minimum of 60 days later is recommended.

**PCR**—The Diagnostic Laboratory offers a PCR to detect FIV DNA (referred to as provirus) integrated into the host cell chromosome. In experimental settings, this assay detects DNA from isolates belonging to clades A, B, and C. A positive test indicates the cat is viremic and permanently infected with FIV. Cats are most likely to be viremic during the acute phase following infection and in the terminal stage of immunodeficient disease. This PCR assay does not indicate a prognosis for the development of disease. A negative test indicates that FIV DNA is not detected in circulating blood cells. A negative result can occur if the cat is not infected with FIV, the cat is infected with FIV but it is not present in circulating blood cells, or the cat is infected with FIV but the PCR assay cannot detect the infecting FIV strain. If an infected cat does not have any FIV DNA in its circulating blood cells, it may have FIV in some covert location like bone marrow stem cells. Vaccinated cats that are not infected will test PCR negative.

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**ELISA for FIV**—Submit serum (red-top tube), refrigerated and sent by overnight mail service on an icepack. The tests are run every day and results are reported the same day. The ELISA test also detects FeLV antigen. Fee=\$30/sample.  
**PCR for FIV**—Submit whole blood in an EDTA (purple-top) tube, refrigerated and sent by overnight mail service or an icepack. The tests are run on Wednesday and the results are reported on Friday afternoon. Fee=\$30/sample.

*See Also*—[American Association of Feline Practitioners Information Brief](#): In response to inquires regarding Fel-O-Vax® FIV, September 2002.  
[http://www.aafponline.org//fiv\\_info\\_brief.htm](http://www.aafponline.org//fiv_info_brief.htm) and [2001 Report of the American Association of Feline Practitioners and Academy of Feline Medicine Advisory Panel on Feline Retrovirus Testing and Management](#).

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#### **BVD CONTROL/ERADICATION**

—Dr. Jim Kennedy/Rocky Ford

**B**ovine virus diarrhea (BVD) is a devastating disease of all cattle. The disease reduces productivity and increases mortality of beef cattle. The clinical signs of mucosal erosions and diarrhea have obvious impacts on infected animals. Probably more devastating than those that exhibit clinical illnesses, are those animals that do not live up to their potential because of lower weight gains, increased disease susceptibility, and diminished reproductive performance. Vaccinations to prevent BVD exist and are an integral part of any control or eradication program but, due to the highly changeable nature of the virus, these are not a panacea for controlling or eradicating the disease. Knowing the nature of

the disease and the virus raises the question—can it be controlled or eradicated? To undertake a BVD control/eradication program is no small task. Several organizations with vested cattle interests, including the Academy of Veterinary Consultants and the American Association of Bovine Practitioners, have proposed programs to address BVD control/eradication. Key to the success of these programs is testing, a challenge for the veterinary practitioner and for the Diagnostic Laboratory.

The table below lists tests that might be done to identify animals infected with BVD virus, along with when those tests might be appropriate. It also is important when selecting tests to consider the availability, cost, and the timeliness of the test. Virus isolation certainly identifies the presence of the virus time needed and the cost to return results may not fit into a producer's management scheme. Another issue of concern is the existence of persistently infected (PI) animals. It is thought that PI animals may be a key viral source for infection. PI calves are a result of infection of the dam, usually during the first four months of gestation. When born, these calves may show no signs of harboring the virus, yet will shed the virus and possibly infect siblings or mature animals within the herd. Regardless of the test selected, suspect PI animals should be isolated and tested again to be certain they are truly persistently infected. This avoids confusing PI animals with acute infections where the viremia is transient and will no longer be present.

Testing strategies for herds should be modified to account for different degrees of BVD risk. High-risk herds require more

aggressive testing and management protocols than low risk herds. A key component of controlling BVD is the detection of PI animals. PI animals may be present in a herd and escape detection by even the best herdsman if a diagnostic testing program is not initiated. As indicated in the chart above, the testing for PI calves may be accomplished by one of two methods—IHC or tissue AC-ELISA. Samples for both tests are identical, requiring a minimal of 1cm<sup>2</sup> tissue sample usually from the margin of the ear. In the case of the IHC, the sample is identified and placed in formalin. If using the AC-ELISA, the sample is placed in a phosphate-buffered saline (PBS) solution. PBS filled tubes may be obtained from the Diagnostic Laboratory or purchased through Syracuse Bioanalytical. Samples, regardless of method, should be shipped to the Laboratory so that they arrive within 24 hours of sampling time. Which test to use is best decided by what is most readily available. Although the IHC test is regarded as the "Gold Standard," recent work at Wyoming Veterinary Diagnostic Laboratory indicated that at minimum, the AC-ELISA has excellent correlation with IHC (.999), is less prone to human error in reading stained slides, and, as the table indicates, is faster and cheaper (personal communication, Wyoming State Veterinary Diagnostic Laboratory). The number of animals to test is relative to how aggressively the problem needs to be attacked. In cases where the risk of BVD is high, the situation may require nearly a whole herd test. If the risk is low, a screening of animals may be appropriate.

As we look at issues confronting the cattle industry, it is obvious that BVD is an emerging issue that, with appropriate diagnostic testing, can be controlled if not eliminated.

TEST	WHEN TO USE	COST	TIME FOR RESULTS
Immunohistochemistry* (IHC)	Cows without calves Replacement stock Calves of any age When PI calves suspected	\$20	Three to 7 days
Virus Isolation	Cows without calves Replacement stock	\$25	One to four weeks
PCR	Cows without calves Replacement stock	\$30	3 days
Antigen capture ELISA* (AC-ELISA)	Calves of any age Replacement stock Cows without calves When PI calves suspected	<10--\$7 10-50--\$5 >50--\$4	Within 24-hours of receipt
Serum neutralization	Only in special cases (contact lab)	\$5	3 days

\*Should be confirmed by second test in 3 to 4 weeks.

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## OVINE Q FEVER ABORTION

—Dan Gould

In July 2003, an aborted ovine fetus was presented to the Diagnostic Laboratory. This was one of four abortions that had occurred in a group of 12 ewes. The fetus presented for evaluation was a 38cm crown-rump length male with little wool development. Moderate autolysis was present and the abdominal cavity contained abundant red, thin fluid. The placenta was free of gross lesions.

Aerobic culture of lung, liver, and stomach contents failed to reveal bacterial growth, including *Campylobacter*. Fluorescent antibody testing of liver, spleen, and placenta failed to demonstrate chlamydial antigen. Histological evaluation of a variety of internal organs failed to reveal microscopic lesions. However, the chorioallantois had significant histologic lesions. A small amount of necrotic debris was present on the surface. The subepithelial connective tissue was lightly infiltrated with lymphocytes and macrophages. Scattered on the surface in the epithelial layer were enlarged trophoblast-derived cells with rounded borders and filled with basophilic microorganisms 0.5-1 micron in diameter. This placental lesion pattern is characteristic of infection with *Coxiella burnetii*, the Q fever rickettsia-like agent. Immunohistochemical staining of the placenta with anti-*C. burnetii* antibody revealed abundant specific staining.

Q fever is a zoonotic disease that most commonly produces a mild, flu-like illness in humans. However, serious heart or respiratory complications can occur. Aborted lambs or goats and their placentas contain large numbers of the organisms and constitute an important source of infection. Care should be taken when there is contact with ewes during parturition and when handling aborted lambs. Aerosolization of the agent represents an infectious hazard. A spore-like form of the organism is resistant to degradation in the environment. This case report highlights the need for a thorough diagnostic workup for abortion cases.

Abortion screen—Submit fetus or appropriate fresh and formalin-fixed tissues. Fee: Equine/camelids/small animals=\$90; all other species=\$65.

### \*\*\*NEW UPS SERVICE\*\*\*

The Diagnostic Laboratory is pleased to announce an arrangement with UPS as another delivery service option for sending your samples to us. They offer an overnight priority service that will get samples to us before 10AM. Please call our office for more information on UPS rates and how to use this service. We continue to also work with the Fed Ex service as a provider of sample delivery.

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## FEES FOR CLINICAL PATHOLOGY TESTS

—Linda Vap and Mary Anna Thrall

We occasionally receive comments or questions regarding fees for services provided by the Clinical Pathology Laboratory, usually for Complete Blood Counts (CBCs) and Diagnostic Panels (DPs). We wanted to take this opportunity to explain how our fees are established.

Fees are directly related to costs, as we do not profit from submissions. Costs arise from several general sources – staff, instrumentation, supplies, reagents, quality control, service, and overhead. The technical staff in Clinical Pathology is comprised of the most qualified people possible. The coordinator has degrees in veterinary medicine and medical technology, has completed a residency in clinical pathology, and has over 25 years of laboratory experience. Clinical Pathology employs five ASCP certified medical technologists and one certified veterinary technician with a total of 68 years experience at the Veterinary Teaching Hospital and over 120 years of total laboratory experience. This level of quality should be and is appropriately compensated within the limits of the Colorado state classified system. Our faculty is comprised of four ACVP board certified clinical pathologists, and one part-time ACVP board eligible clinical pathologist. In addition, numerous residents (clinical pathologists in training) help serve the Clinical Pathology Laboratory.

### Hematology (CBCs)

Our automated instrument is a state-of-the-art Bayer Advia 120 Hematology Analyzer with specific veterinary software. The Advia provides cell analysis by flow cytometry, using the most sensitive and accurate methods available. In addition, the Advia provides information about reticulocytes not available by any other hematology system. This function currently is being evaluated on in-patient samples. All blood films are examined by medical technologists to ensure that morphologic abnormalities are identified. We perform differential nucleated cell counts manually as this continues to be more accurate than counts provided by flow cytometry. If any abnormality is present on the blood film or in the data, the blood film and data are reviewed by a veterinary clinical pathologist at no extra charge to the veterinarian. In addition, we perform a plasma protein estimate by refractometry on all CBC samples. Any abnormality in this estimate should trigger total protein, albumin, and globulin determination on the biochemical profile. We provide reticulocyte counts on anemic patients so that bone marrow regeneration can be assessed. We perform fibrinogen determinations on every large animal CBC request to better identify inflammation.

### Clinical Biochemistry

We use the Hitachi 971 Automated Discrete Chemistry Analyzer, an instrument used by many human laboratories.

This instrument enables us to add tests that meet specific veterinary needs not readily available by other instruments such as SDH, fructosamine, and antithrombin.

We may determine biochemical profile results by actual measurement of a substance in serum, or by performing a calculation based on other measurements. Examples of calculated results include anion gap, calculated osmolality, globulin, A/G ratio, TIBC, and percent iron saturation. When comparing prices of various laboratories, one should compare numbers of measured tests offered. For example, our small animal panel is comprised of 23 measured tests – glucose, BUN, creatinine, calcium, phosphorus, magnesium, protein, albumin, cholesterol, total bilirubin, amylase, CK, ALP, ALT, AST, GGT, sodium, potassium, chloride, bicarbonate, iron, and UIBC. Many laboratories include calculated tests in their list of tests provided. The large animal panel includes 18 measured tests, including SDH. SDH is a relatively expensive test but provides valuable information specifically related to liver cell integrity in large animals.

Tests provided by other laboratories for large animals sometimes include ALP and ALT which are inexpensive to run but add no diagnostic value. Our complete large animal panel includes glucose, BUN, creatinine, phosphorus, calcium, magnesium, protein, albumin, total bilirubin, CK, AST, GGT, SDH, sodium, potassium, chloride, and bicarbonate. Calculated tests include globulin, A/G ratio, anion gap, and calculated osmolality.

### Cytology

Our fees for fluid analyses and examination, and interpretation of cytologic specimens are comparable to those of other diagnostic laboratories. ACVP Board Certified Clinical Pathologists make or review all interpretations. Cytology is a technique that is relatively inexpensive and non-invasive, and often provides a diagnosis.

The Clinical Pathology Laboratory is a proud provider of quality service. In addition to excellent personnel, we ensure the quality of our results by taking extensive quality control measures. Internal calibration and quality control runs are performed multiple times within a day and external quality control samples are evaluated quarterly. Veterinary Clinical Pathologists and the Laboratory Coordinator review results on a regular basis.

Prompt service also is high on our list of priorities. Routine turnaround time is typically within hours, depending on the request. Our laboratory receives samples from local outside clinics until 9PM weekdays, 7PM weekends, and 3PM on holidays. Stat and ASAP requests are processed accordingly and none of these services are charged additionally despite considerable personnel-related expenses.

### A Word About Sample Quality

The quality of results is absolutely dependent on the quality of the specimen submitted. In order to maximize the quality of results, please consider the following.

#### *Hematology*

- Despite using a preservative anticoagulant, cells will swell, degrade, or lyse in a short period of time. A blood film made shortly after collection preserves the morphology in its original state. No additional steps are needed except to protect the film from freezing and condensation.
- Whole blood samples should be shipped overnight with cold packs. Avoid placing the tubes directly on the cold pack.

#### *Biochemistry*

- Clotted or heparinized samples should be spun, and serum or plasma separated within 30 minutes of collection. If using a serum separator tube, ensure the separating material has completely separated the cells from the serum.
- Since some enzymes are altered by long-term storage and/or freezing, samples should be shipped overnight with but not directly on cold packs.

#### *Cytology*

Common problems we encounter that render cytology specimens non-diagnostic are:

- Formalin fume contamination of sample. When a cytology specimen comes into contact with formalin fumes, it will not stain appropriately. DO NOT SEND cytology specimens in the same container as formalin-fixed tissues.
- Specimens that are hemodilute or clotted. Using a small (22 gauge or less) needle may help prevent blood contamination. Once samples have been aspirated, one has approximately 30 seconds in which to make the preparation before the tissue clots, rendering it non-diagnostic.
- Preparations that are too thick or in which the cells are all broken. One should spread out the cells gently, particularly when dealing with fragile tissues such as lymph node aspirates.
- Samples that have been exposed to moisture. This may occur when slides are placed in the refrigerator with tubes of blood. Slides should be kept at room temperature to avoid condensation.
- Unstained samples older than five days. Slides should be stained within two to three days of collection and preparation.

## UPDATE ON PCR TESTING FOR FHV AND CHLAMYDIA

We have developed a combined feline herpesvirus-1 (FHV) and Chlamydia duplex polymerase chain reaction (PCR) test. Testing for both agents is done at the same time, thus reducing the cost. The charge will be \$35 for the duplex tested compared to \$55 if the tests are performed separately.

## UNUSUAL WEST NILE VIRUS RESULTS IN THE 2003 SEASON

—Brendan Podell

This season, we performed an IgM ELISA for West Nile virus on 674 horses and detected antibodies in 350 (51%). This IgM ELISA test is available at all three laboratories in Fort Collins, Rocky Ford, and Grand Junction. In addition, at Fort Collins, we have performed West Nile virus polymerase chain reaction (PCR) on approximately 100 samples. Positive results encompassed numerous horses and a variety of birds, including blue jays, magpies, crows, grackles, falcons, and a pelican. However, the most notable positive cases included those not previously reported in alpaca, coyote, and mule deer species. Positive results also were found in unusually affected species like raccoons and squirrels. A sheep also was PCR and IHC positive. In addition, a serum sample from a calf with neurologic signs was sent to Cornell and was found to be positive.

The IgM ELISA test for West Nile virus is available for equine serum samples. West Nile virus PCR can be performed for any species on frozen tissues, preferably brain, and also on sterile swabs placed in 0.5ml of viral transport media or sterile saline.

West Nile Diagnostics--Submit 1ml serum for IgM ELISA, horses only. Frozen tissues or swabs for PCR, any species. Fee: PCR=\$30; IgM ELISA=\$7 (>2=\$5).

## BLUE-GREEN ALGAE POISONING

—Tony Knight/Clinical Sciences

Despite the fact that summer has ended, temperatures have not dropped low enough in most areas to remove the threat of a variety of cyanobacteria (blue-green algae) from growing on the stagnant, brackish water of ponds and reservoirs. The sudden proliferation (called “blooms”) of the algae in water has been reported worldwide and is associated with a variety of disease symptoms in humans,

cattle, horses, sheep, birds, dogs, and fish that drink or live in the water contaminated with these organisms and their toxins.

Blue-green algae species, which occur as either single cell organisms or long filamentous chains of cells, will, under optimal conditions, proliferate to produce a visible green “scum” on the water. This occurs particularly where prevailing winds have blown and concentrated the organisms into one area. As the mass of algae die, they produce a variety of potent, water-soluble toxins. If consumed by animals and birds, these toxins can cause nervous signs, liver failure, and death. The blue-green algal toxins generally are either hepatotoxic or neurotoxic. Occasionally, humans exposed to algal lipopolysaccharides have developed gastroenteritis, skin and eye irritation, and hay fever-like symptoms.

The most common of the blue-green algae affecting animals, the toxins produced, and the target organ affected are listed in the table below.

<i>Cyanobacteria</i> sp.	Toxin	Target Organ
<i>Microcystis</i> sp.	Microcystin	Hepatic
<i>Anabaena</i> sp.	Microcystin	Hepatic
<i>Planktothrix</i> sp.	Microcystin	Hepatic
<i>Cylindrospermopsis</i>	Cylindrospermin	Hepatic
<i>Nodularia spumigena</i>	Nodularin	Hepatic
<i>Oscillatoria</i> sp.	Microcystin	Hepatic
<i>Anabaena</i> sp.	Anatoxin	Nervous

Microcystin and nodularin are the two most common blue-green algae toxins associated with liver necrosis in animals. These complex peptides cause acute intrahepatic hemorrhage and hepatic necrosis. So rapid and severe is the hepatic necrosis that only a few rows of periportal hepatocytes can be observed histologically. In experimental studies, hepatocytes have been found in pulmonary vessels, indicating the peracute nature of microcystin poisoning.

Blue-green algae poisoning should be considered when sudden death occurs in livestock during the warm months of the year when algal blooms are likely to occur. Dogs that develop acute liver necrosis and neurologic signs are good candidates for microcystin and nodularin poisoning, especially if they have been swimming or drinking from stagnant ponds. Death in cattle from acute hepatic necrosis warrants consideration of microcystins, nodularins, industrial solvents such as carbontetrachloride, and the potent hepatotoxin carboxyatractyloside found in the cotyledons of cockleburs (*Arctium* spp.) when they emerge as seedlings.

According to the World Health Organization (WHO) standards, water containing more than 1 microgram microcystin/liter should be considered toxic. Recent studies

in Australia have shown that milk from dairy cows fed *Microcystis aeruginosa* had no detectable levels of microcystin in milk, leading the researchers to conclude that milk was not a likely source of these algal toxins.

Samples to submit for the detection of blue-green algae should include at least 50ml of the suspect water containing the algal bloom. This should be refrigerated, but not frozen. Another 50ml of the water should be frozen for algal cyclic peptide determination at a laboratory capable of high-performance liquid chromatography (HPLC), mass spectroscopy (MS), or thin-layer chromatography (TLC), and an ELISA for microcystin. Identification of the algae themselves will necessitate sending the water sample to an experienced microbiologist familiar with the blue-green algae. Liver samples should be both fixed and frozen for histopathology and microcystin immunohistochemistry.

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#### FROM THE WESTERN SLOPE

--Darrel Schweitzer

Digital cameras have become quite commonplace in our culture and many of you probably have one available for personal use. Even if you don't, here are some ideas that may help justify the purchase of one for clinical use. With thanks to a colleague, Dr. Duane Moore, here is one idea which is of great value to me as a pathologist -- use the digital camera to photograph skin tumors, which then can be transmitted to your pathologist via e-mail. This is especially helpful if there is an area, such as a questionable margin, which you would like the pathologist to pay particular attention. Most cameras come with photo editing software that you can use to label the photo with pertinent data, arrows, etc. This will allow the pathologist to orient the tumor as it was in vivo, to precisely identify any area of interest to you, and to provide him or her with a view of the tumor's clinical appearance. These factors can aid in diagnosis and will definitely allow more informed gross sectioning of the tumor. On a technical note, you may need to decrease the resolution or size of the resultant file for faster transmission. If done properly, this does not compromise photo quality to a great extent. However, to be useful, you do need a camera with enough resolution to get a photo of high quality. Very cheap cameras may leave you wanting.

Digital pictures also are useful in documenting abuse cases. It is a great advantage to be able to immediately view the pictures to be sure they show exactly what you want. According to Dr. Annette Rauch (2003 AVMA Convention Lecture), such photos probably can be used as evidence provided the original file on the "film" card is available. You may need to invest in extra cards that can be kept with case records until a case is resolved. I suggest getting small, for example, 8MB cards for this use, and reserve larger cards for routine, repeated usage.

Digital photos also may be useful in following the progress of wound healing, tumor growth, or other conditions which change over time. Photos of distinctive features on an animal may be useful to identification. Transmitting photos of plants to an expert can possibly save time in deciding the plants' involvement in suspected poisonings.

Digital photos can be useful as records of clinic equipment and, of course, you can use them to develop Web pages. Use your imagination and I'm sure you will think of much more useful information that can be recorded using a digital camera.

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#### COLORADO CATTLEMAN'S ANIMAL HEALTH GOALS 2003-2003

—John Cheney

The Colorado Cattleman's Association (CCA) has 18 standing committees, one of which is the Animal Health & Welfare Committee. For several years, this committee was headed by Dr. Don Klinkerman from LaJunta and Dr. Marv Hamann from Pueblo. The committee currently is headed by Dr. Blaine Evans from Walden.

Each year, the committee sets certain goals for the coming year. Currently, the Animal Health & Welfare Committee has set three goals, each of which will affect veterinarians in Colorado. These goals are as follows:

1. Continue to push for coordination between federal and state regulations on animal movement intrastate and nationwide, especially regarding the federal government's recognition of stricter state regulations.
2. Prepare and present to the Resolutions Committee at the Mid-Winter Conference Meeting
  - A resolution urging that BSE standards be applied equally to all countries.
  - A resolution agreeable to the State Veterinarian supporting testing for bison and free-roaming elk in Wyoming and Utah.
3. Continue efforts to obtain adequate funding for the CSU Diagnostic Laboratory, including support for a new building, especially in light of the demand for food animal disease diagnostics and emergency preparedness

The committee welcomes any input from state veterinarians regarding these goals or other items handled by the Animal Health & Welfare Committee. Contact Dr. Evans (970-723-4927) or Dr. Cheney (970-491-1281) with your ideas and other livestock concerns.

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## GET TO KNOW YOUR LABORATORY/Necropsy Area

**D**ennis Madden is the Necropsy Laboratory Coordinator. He started working at CSU in 1980. Over that time, he has conducted over 40,000 necropsies. His expertise is in gross pathology and necropsy technique. Dennis really enjoys teaching veterinary students, training pathology residents, and serving the clients of the Diagnostic Laboratory. Feel free to call Dennis any time you have questions concerning a necropsy. Dennis was awarded the College of Veterinary Medicine and Biomedical Sciences Outstanding Employee of the Year Award in 1996. For those CSU graduates that remember Dennis, he is still playing soccer and handball.



**Dennis Madden, Lee DeBuse, and Larry Ludden**

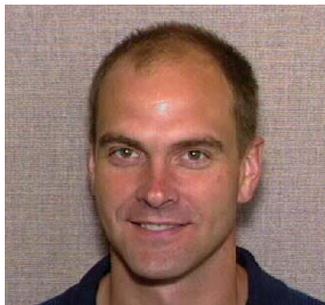
Lee DeBuse has been with the Diagnostic Laboratory for 13 years. As a Laboratory Technician, she is responsible for trimming biopsies, coordinating necropsy and sample evaluation cases for pathologists, histomailers, and coordinating entry of chronic wasting disease samples.

Larry Ludden started working at CSU in November 2000 working as courier serving the combined departments of Pathology and the Diagnostic Laboratory, as well as the Dean's office. In the summer of 2001, he joined the staff of the Diagnostic Laboratory as a Laboratory Technician in the pathology/biopsy section. His duties include processing histopath mailers, preparing tissues for the histology laboratory, processing frozen section slides, necropsy photography, preparing decalcified bone samples and fielding questions from clinics concerning histopath samples and mailing supplies. Larry attended the University of Nebraska for five years studying mathematics and accounting.

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## GET TO KNOW YOUR LABORATORY/Two Pathologists

**D**r. EJ Ehrhart joined us in August 2002. Experience includes 6 years as an Assistant Professor at the Veterinary Diagnostic Laboratory at the University of IL. He completed his Veterinary Medicine degree at University of MO/Columbia in 1987, worked in private practice for three years before entering the combined anatomic residency/PhD program at CSU (completed 1996). Dr. Ehrhart is



appointed as an Associate Professor at CSU with a joint appointment in the Diagnostic Laboratory and the Department of Microbiology, Immunology, and Pathology. His diagnostic special interests are in oncology, radiation pathology, and ophthalmology. He directs research at the Animal Cancer Center in the Jorgensen Molecular Pathology Laboratory. Research is centered on comparative oncology with emphasis on cancer protein alterations and how they can predict tumor behavior, tumor response, and act as potential targets for therapeutic intervention.

Dr. Karamjeet Pandher recently joined our laboratory as Assistant Professor with a 50% appointment with the Diagnostic Laboratory and a 50% appointment in the Department of Microbiology, Immunology, and Pathology. He was previously with Pfizer Research Laboratories in



Groton, CT and the Diagnostic Laboratory at Kansas State University in Manhattan. Dr. Pandher completed his BVSc (DVM) in India and his PhD in bovine respiratory disease at Oklahoma State University. He became ACVP board-certified in 2001. We welcome him!

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### \*\*\*\*\*NOTICE\*\*\*\*\*

**Regrettably, our clinical consultation for dermatopathology service is no longer available. Dr. Bettenay joined her husband in Germany. Of course, we continue to offer the histopathology service. If there are any queries relating to the clinical management of these cases, the CSU dermatology service or a local dermatologist should be consulted.**

### CHRONIC WASTING DISEASE TESTING FOR CAPTIVE ELK

Chronic Wasting Disease (CWD) testing for captive (or farmed) elk is now **free of charge for Colorado elk**. The testing is now paid by USDA to CSUVDL. This system requires that immunohistochemistry be performed. The obex is required, but data indicate that it is very important to submit the medial retropharyngeal lymph nodes as well. Send samples in formalin to any of our three laboratories. We will ensure that the proper forms are filled out and signed by Dr. Cunningham, which is required for USDA payment. Results are available in 5 to 7 days following sample receipt. This system applies **only** to captive elk (not hunter-kill elk).

### HISTO MAILER SERVICE

We recently have streamlined the histomailer process. In an attempt to minimize delays in shipping time, mailers do not have a return address. The return address label will only include the Diagnostic Laboratory address. As a result, the information sent to us with each mailer and its sample becomes much more important. Recently, we received a few samples in mailers without any information accompanying them. In this situation, a sample can be diagnosed but the diagnosis cannot be released. For each mailer, please include a completed submission form and some identifying information, including the patient's name, clinic or hospital name, and the practitioner's name on the sample jar. Your efforts will help assure a more timely diagnosis.

### DID YOU KNOW???

Our website, [www.dlab.colostate.edu](http://www.dlab.colostate.edu) has some very useful information. Give Carrie a call at 970-297-4418 if you would like to view your results online. She can set up password-protected access for you

### CHRONIC WASTING DISEASE TESTING FOR HUNTERS

The CWD testing season, through the Colorado Division of Wildlife and CVMA's Hunter Assistance Program is in full operation. We have over 13,000 tests in and completed from this year's hunting season. Remember we are testing the medial retropharyngeal lymph node with the rapid ELISA test, confirming ELISA suspects with immunohistochemistry. We can test the obex if the lymph node is not available. The new data processing and barcode system is providing even faster results than last year!

### UPDATE ON PORCINE DIAGNOSTIC TESTS

As of November 2003, the following porcine diagnostic tests will be offered at the Rocky Ford Laboratory and no longer will be available at the Fort Collins laboratory –

- PRRS ELISA Serology
- Pseudorabies (PRV) LAT Serology
- Swine Influenza HI Serology

Serum samples should be sent directly to the Rocky Ford Laboratory. Samples sent to the Fort Collins laboratory will be forwarded to Rocky Ford. Dr. Jim Kennedy at the Rocky Ford Laboratory is available for consultation on test interpretation and diagnosis of swine diseases.

The SN tests for PRV, FA for TGE on small intestines, and diarrhea screens still will be performed at the Fort Collins Laboratory.

## WHAT'S IN THIS ISSUE OF LABLINES

- Biosecurity, Part 3
- Feline Immunodeficiency Virus
- BVD Control & Eradication
- Ovine Q Fever Abortion
- Clinical Pathology Testing
- West Nile Update
- Blue-Green Algae Poisoning
- From the Western Slope
- Rabies in a Dog
- Colorado Cattlemen's Animal Health Goals

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