Colorado State University Veterinary Diagnostic Laboratories

LabLines

Volume 9, Number 2 **Fall 2004**

Letter from the Director

Welcome to another issue of LabLines! We hope you enjoy the informative articles within. I'd like to start with a brief overview of what has been happening at the Laboratory. As we ended our fiscal year on June 30, we noted an overall increase in accessions at our laboratories in Fort Collins, Rocky Ford and Grand Junction. We currently are working on our annual report which details all of our diagnostic, teaching and research activities. This report will be available in May 2005. Since the beginning of the school year this fall, we have acquired new students, but also have seen quite a change in our office staff (see inside for details). In January, we look forward to our annual meeting with our External Advisory Committee.

Major highlights of disease diagnostics in the last year are detailed in this edition of LabLines. While our emphasis has been on working with the Colorado Department of Agriculture and the United States Department of Agriculture developing disease surveillance programs and protecting our agricultural base, we continue to provide excellence in diagnostics for all animal species. We also continue to be active in educating the next generation of veterinary laboratory diagnosticians to help meet the critical national shortage in this area. Despite the serious budget cuts we have experienced in our state funding, we have acquired federal funding that covers these operating shortfalls. Unfortunately, our facilities upgrade plan remains on hold indefinitely.

In September, I ended my term as President of the Colorado Veterinary Medical Association at the CVMA Annual Convention in Steamboat Springs. It was a most enjoyable experience and I will continue to be active with the CVMA. I enjoyed visiting with you in September and hope to see many of you at CSU's Annual Conference in January. In October, I started my term as Vice-President of the American Association of Veterinary Laboratory Diagnosticians and look forward to working in the organization at the National level.

Barbara E. Powers

Barbara Powers, DVM/PhD/DACVP

Colorado State University
Diagnostic Laboratories
300 West Drake
Fort Collins, CO 80523
Phone 970/297-1281
Fax 970/297-0320
email: dlab@colostate.edu
http: //www.cvmbs.colostate.edu/dlab

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

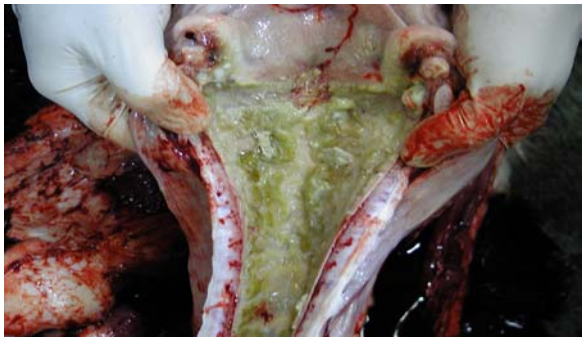
INCREASED IBR CASES: FALL 2003-WINTER 2004

—Hana Van Campen and Karamjeet Pandhar

We saw an increased number of cases of infectious bovine rhinotracheitis (IBR) submitted between October 2003 and February 2004. Bovine herpesvirus-1 (BHV-1) is the etiologic agent of IBR, as well as keratoconjunctivitis (pinkeye), abortions, infectious vulvovaginitis, and balanoposthitis.

In two separate cases of abortions in dairy cows, fetuses of 210 to 240 days of gestational age were positive for IBR by FA staining and had compatible histopathologic lesions. A late-term fetus from a first-calf beef heifer also was found to be IBR positive by FA staining in a group where earlier abortions had been attributed to high nitrate content of feed.

IBR was demonstrated by FA staining in two cases of pneumonia in dairy calves and one case in an adult cow. *Pasteurella multocida*, *Mannheimia hemolytica*, and BRSV also were found in the lungs of two affected animals. IBR also was demonstrated in a herd outbreak of pneumonia in vaccinated beef cattle. Both calves and adult cows were affected with bronchopneumonia. Intranuclear inclusions also were observed in the liver of a 6-month-old calf from this herd.



Larynx and trachea with IBR lesions

In this same period, five cases of respiratory disease in feedlot calves had evidence of IBR infection. Typically, calves had fibrinous bronchopneumonia and necrotic tracheitis. In addition to positive FA staining in lung samples, IBR viruses were isolated from three of the cases. Concurrent infections with BVD, BRSV, *Mycoplasma bovis*, *Pasteurella*, *Mannheimia*, and *Arcanobacterium pyogenes* were common. Affected calves were in the feedlots from 10 days to two months. All were vaccinated with 4-way viral vaccines and many had received prophylactic treatment with antibiotics. The IBR isolates have been sent to Dr. Robert Fulton, Oklahoma State University, to determine if there are significant genetic differences between the field viruses and the vaccine strains of IBR.

BHV-1 infections often have characteristic gross and histopathologic lesions. Diagnostic tests that give supporting evidence for BHV-1 include fluorescent antibody (FA) staining to detect viral proteins, polymerase chain reaction (PCR) to detect the virus's DNA, and virus isolation to detect infectious virus. Antibodies to IBR/BHV-1 are measured by serum neutralization (SN). Diagnosis of acute respiratory infections can be obtained by comparing SN titers between acute and convalescent serum samples. We offer all of these diagnostic techniques. For further advice on sample selection and cost, please call us (970-297-1281).

NATIONAL ANIMAL HEALTH LABORATORY NETWORK UPDATE

—Barb Powers

The United States Department of Agriculture (USDA) and the American Association of Veterinary Laboratory Diagnosticians (AAVLD) joined in 2002 to establish the National Animal Health Laboratory Network (NAHLN). This is a system of linked state and federal laboratories that provide surveillance testing and emergency laboratory response testing for foreign animal diseases or reportable domestic diseases. We are one of five core NAHLN laboratories, which now includes more than 30 laboratories across the country. Active disease surveillance programs in this system include avian influenza, exotic Newcastle's disease and transmissible spongiform encephalopathies (see related articles in this issue of LabLines). We have passed proficiency tests for these diseases as well as for classical swine fever,

vesicular stomatitis, and foot and mouth disease. We soon will begin "negative cohort" studies for these latter diseases. The testing for all these diseases is done in our recently constructed, high-security BSL3 modular laboratory, operational since August 2003.

Jay Kammerzell, our computer and business manager, is a co-chair of the information technology portion of the NAHLN. High security data transfer and reporting of results is a key component of the NAHLN. For more information about the NAHLN, visit the website at www.nahln.us.

*****REMINDER*****

We continue to receive submissions that do not include all requested information or sometimes no information at all!. Complete information enables us to better serve you and identify your samples. We urge all of you to submit complete submission forms with your samples.

LEPTOSPIROSIS PCR NOW AVAILABLE

—Doreene Hyatt, Nick Haley and Cindy Hirota

Due to recent concerns about the emergence of leptospirosis in the state, we are offering a PCR test specific for *Leptospira interrogans*. The test is not able to differentiate specific serovars, but is useful in the diagnosis of leptospirosis. Serology for leptospiral serovars is available and costs $12.50 for a 5-serovar assay. Because leptospiral organisms may be shed intermittently in the urine, a negative test result does not rule out infection with *Leptospira*. Similarly, the test should not be used to determine when shedding of the organism has ceased or when antimicrobial therapy should be halted. The test can be run on urine, blood, ocular fluid or tissue. Ideally, samples should be sent chilled. Sample volumes should be at least 2ml of urine, 0.5ml of blood, 0.5ml of vitreal/aqueous humor, or 1-2cm^3 of tissue. Commonly tested tissue samples include kidney, liver and ocular tissues. The test is $25. Tests are run on Tuesdays and Thursdays, with results available on Wednesdays and Fridays.

Additionally, seven tests for *L. brataslava* were conducted in 2004. Three were positive with the highest titer being 1:800. In 2003, four samples were tested and three were positive with the highest titer being 1:400.

We are performing the Lepto-5 Microagglutination titers on Wednesdays with results available at the end of that day. Samples need to be in the laboratory before noon the day of the testing. In addition, the Brataslava serovar is available at an additional change of $6.00, but must be requested at the time of sample submission.

Our laboratory has seen an increase in testing equine serum for *Leptospira interrogans* to aid in the diagnosis of equine recurrent uveitis, also known as moon blindness. There are many studies correlating equine uveitis with positive titers to the *L. interrogans*, especially the Pomona serovar. For more information, see the Dwyer AE, Crockett article in the Journal of the American Veterinary Medical Association, 1995.

Table 1. Number of serum samples tested for titers to *Leptospira interrogans* between January 1 and September 13 in 2003 and 2004 by animal species. Included is the percent change in testing requests between 2004 and 2003.

Species	2004	2003	% Change
Bovine	164	252	-35
Camelid	0	3	-100
Canine	102	77	+32
Caprine	2	1	+100
Equine	31	22	+41
Ovine	2	3	-33
Zoological	5	5	0

Table 2. Leptospirosis serology results for each of the five serotypes for serological samples submitted between January 1 and September 13 in 2003 and 2004 for ALL animal species. The total number of samples tested (N) and the number of positive results (P) as defined as a titer greater than or equal to 1:100, as well as the highest titer reported during the year (High) is given below.

Year	N	*L. canicola*		*L. grippo*		*L hardjo*		*L. ictero*		*L. pomona*	
		P	High	P	High	P	High	P	High	P	High
2003	363	79	3200	119	6400	79	1600	115	1600	93	3200
2004	307	37	800	45	102,400	29	800	84	3200	61	102,400

AVIAN INFLUENZA/EXOTIC NEWCASTLE DISEASE SURVEILLANCE PROGRAM

—Kristy Pabilonia

We have joined forces with the Colorado State Veterinarian's Office, the National Veterinary Services Laboratory, and the United States Department of Agriculture to create and implement an avian disease surveillance program. The purpose of this program is to monitor the occurrence of economically important infectious avian diseases, primarily in poultry. The two diseases of most concern are highly pathogenic avian influenza and Exotic Newcastle disease.

The program incorporates a number of disease surveillance and education components. We are sampling birds from all over Colorado, including chickens, turkeys, ducks, geese, game and pet birds. Samples are collected at fairs, shows and exhibitions, bird swaps, bird enthusiast meetings, and commercial poultry production facilities. Birds brought to us or the Veterinary Teaching Hospital, either as patients or to the necropsy service, also are sampled. Additionally, veterinarians travel to sites where sick or dying birds are reported. There, the veterinarians can investigate possible causes of illness and death, and collect samples for further testing. All samples are tested for avian influenza and Exotic Newcastle disease viruses by real-time PCR. Further testing of the samples for other infectious diseases may be done where appropriate.

In addition to diagnostic testing, the program works to educate veterinarians, commercial poultry producers, bird enthusiasts, backyard flock owners, and the general public on avian diseases and biosecurity practices.

Dr. Kristy Pabilonia is the veterinarian who oversees this program. She has traveled throughout Colorado collecting samples for surveillance testing, investigating cases of sick birds, and lecturing to bird enthusiasts. She has visited farms in a multitude of cities and towns including LaJunta, Pueblo, Calhan, Colorado Springs, Evergreen and Denver. She also has attended numerous fairs and meetings throughout Colorado. To date, more than 260 samples have been collected from more than 130 birds. These birds come from more than 50 sites in Colorado.

This program already has experienced numerous successes. Colorado has collected some of the highest numbers of samples out of the states participating in this program. Colorado also is one of the only states to create a direct link between the disease surveillance program and backyard flock owners, in an effort to educate bird owners and protect our state from major disease outbreaks. Future directions for this program include the establishment of regulations for interstate movement of birds, and the implementation of an active Colorado Poultry Improvement Plan that meets national guidelines.

Dr. Kristy Pabilonia is very enthusiastic about her work in this program and enjoys working with poultry/bird groups and backyard flock owners. If you would like her to come present at a meeting, attend a fair, or visit your farm, please contact her at 970-297-1281. She also is happy to answer questions over the phone or via Email. Her e-mail address is kpabilon@colostate.edu.

Please report sick/dying birds to Dr. Kristy Pabilonia at 970-297-1281. Services and some diagnostic testing are provided free of charge (paid for by USDA).

TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHY DIAGNOSTICS

—Barb Powers

The transmissible spongiform encephalopathies (TSEs) include chronic wasting disease (CWD) of deer and elk, scrapie of sheep and goats, and bovine spongiform encephalopathy (BSE) of cattle. Since we have two of these three TSEs in Colorado (CWD and Scrapie) we have for years emphasized diagnostic excellence in this area. Our programs for CWD include working with the Colorado Division of Wildlife and the Hunter Assistance Program through the Colorado Veterinary Medical Association to provide CWD testing of hunter-harvested deer and elk. We currently are in our third year of large-scale testing using the BioRad ELISA (which we validated in 2002) and project slightly more than 12,000 tests this year. We also provide CWD testing for the farmed elk industry through the Colorado Department of Agriculture and USDA which pays for the cost of the test. At this time, only immunohistochemistry (IHC) testing is available through this program. Also, through the USDA, we perform IHC tests for scrapie in Colorado and many other states.

In June, through the USDA's BSE program, we were designated one of the "high throughput" laboratories to perform BioRad ELISA testing for BSE. The goal of this program is for the entire system to test as many as possible of the estimated 440,000 downer or non-ambulatory cattle that occur in a 12-to-18 month period. An estimated 268,000 tests will provide the USDA with a 99% confidence level of us being able to detect a BSE case if it occurs at a rate of 1 in 10 million cattle. Currently, the entire system has performed more than 150,000 tests with no confirmed positive cases. Our laboratory has completed more than 33,000 tests since June 1, when the program began.

Recently at the October annual meeting of the American Association of Veterinary Laboratory Diagnosticians, our work in this area was recognized as we won the "Best Manuscript of the Year" Award for our publication describing the field validation of the BioRad ELISA for CWD; a joint publication with the Colorado Division of Wildlife.

HYPERTROPHIC CARDIOMYOPATHY IN AN OBESE RABBIT

—Cristie Kamiya (PVM 2005) and Dan Gould

A 3-year-old, castrated male, domestic rabbit was presented for veterinary care with a health problem characterized by a six-day duration of lethargy, partial anorexia and diarrhea. There were no known previous medical problems. The rabbit was an indoor pet and lived with one other rabbit. Both rabbits had full, supervised access to the house. Their diet consisted of orchard grass hay, grains, kale, cilantro, romaine lettuce, and few pellets. The rabbit failed to improve despite syringe feeding and fluid administration. During a physical examination, cardiac arrest occurred and the rabbit could not be resuscitated.

At necropsy, the rabbit was observed to be obese. The main macroscopic finding was cardiomegaly. The right ventricular wall was thinner than normal and the left ventricular freewall and septum were thicker than normal. The septum bulged into the right ventricular lumen. The abdominal cavity contained clear ascitic fluid with occasional strands of fibrin. A large volume of the abdominal cavity

was occupied by adipose tissue. Multifocal erosions and ulcerations were present in the cardiac and pyloric regions of the stomach mucosa. The liver had regional areas of congestion and accentuation of the lobular architecture. Fibrin strands were present on the serosa of the liver. The thoracic cavity was filled with a clear pleural fluid. The lungs were atelectatic and congested.

The main microscopic changes were in the heart and consisted of large regions of myocardial fibrosis, with more localized areas of myofiber degeneration and necrosis accompanied by heterophil infiltration. The liver had areas of hepatocyte vacuolar degeneration. The final diagnoses were – hypertrophic cardiomyopathy, pulmonary atelectasis, hydrothorax and peritoneum, and gastric ulceration.

Comment – Although domestic rabbits have been used in research as a model for human cardiac disease, naturally occurring rabbit cardiac disease is not well understood. It is only recently that cardiac disease is being recognized in pet rabbits, due to advanced diagnostic capabilities such as echocardiogram. Rabbit hearts differ from other small mammals in that the tricuspid valve only has two cusps, and the aorta is not associated with chemoreceptors, but with baroreceptors. The pulmonary artery and its branches are heavily muscular, and the myocardium has limited collateral circulation, which predisposes to ischemia and infarction. Most affected rabbits are treated for cardiomyopathy with treatment regimens extrapolated from successful dog and cat cardiomyopathy treatment. The obese state of this rabbit may have been a contributing factor to the development of hypertrophic cardiomyopathy. A condition described in the human medial literature is known as "Pickwickian Syndrome." This is a complex of abnormal clinical signs in patients who are morbidly obese, and that result in mechanical interference with respiratory and high blood CO2 pressure (PCO2) due to excess fat in the body cavities.

A LESSON ON INTERPRETING DIAGNOSTICS (AND TRUSTING YOUR CLINICAL IMPRESSION)

—Nicholas Haley/Microbiology Resident

In August 2004, a young, neutered male, domestic shorthaired feline presented to us with a history of recent bat contact, followed several days later by fever, vague neurologic signs, and swollen submandibular lymph nodes. Initial testing for rabies and *Yersinia pestis* (the causative agent of plague) conducted by the Centers for Disease Control (CDC) was negative.

Gross necropsy revealed markedly enlarged submandibular lymph nodes, while no gross lesions were evident in the brain. Histopathology of the lymph nodes showed evidence of suppurative inflammation with small colonies of bacteria, again, with no evidence of rabies noted in brain tissue. Despite negative tests conducted by the CDC, the primary differential remained *Y. pestis*, and a routine aerobic culture of lymph node and splenic tissues was performed. After culture and biochemical analysis, an isolate was obtained that could not be definitively identified, and further DNA testing was performed to rule out *Y. pestis*. Using 16S rRNA PCR, the organism was narrowed down to a *Yersinia* species, and pure cultures were sent to the CDC for further testing. Again, initial results were negative for *Y. pestis*, though further testing involving mouse inoculation and subsequent testing on mouse tissue was performed. Only after the mouse inoculation was the agent unequivocally determined to be *Yersinia pestis*. Contact with the bat is not believed to have been the route of infection with *Y. pestis* though, due to the patient's history of neurologic disease, it was considered a relevant aspect of the case history.

History--*Yersinia pestis* is an organism that has a very important place in history and in the future because of its potential use as an agent of bioterrorism. There have been three major worldwide plague pandemics and several smaller epidemics that have been attributed to *Yersinia* since the dawn of the Roman Empire. The second pandemic, occurring in the 14th and 15th centuries, is thought to have promoted the value of the working class and the fall of serfdom in Europe. The most recent pandemic is thought to have started in the Orient in the late 1800s, at which time its introduction into the United States is believed to have taken place. According to the Colorado Department of Public Health and Environment, there have been about 50 cases of plague in various wild and domestic animals in Colorado this year, up from roughly 25 in 2003, and there is some concern this trend will continue in the coming years.

Natural Hosts and Transmission--In the United States, plague commonly is carried by wild rodents, including rats and ground squirrels. In Colorado, the agent most commonly causes disease in prairie dogs, where transmission to domestic cats via flea bite or ingestion of infected animals is the most commonly seen veterinary entity. Dogs and other species appear to be relatively resistant, thus infection in these species is extremely rare. Interestingly, reports of infected lynx kittens are becoming more common, and the organism is considered a strong threat to lynx reintroduction in Colorado. Humans may be infected after being bitten by an infected flea, improper handling of infected tissues, or contact with aerosolized bacteria from infected humans, pets, or culture plates.

Clinical Signs in Veterinary Species--Common clinical signs in cats include enlarged submandibular lymph nodes, fever, lethargy and pneumonia. In rodents, such as prairie dogs, clinical signs range from enlarged lymph nodes, epistaxis, and pneumonia, to death.

Diagnosis—A diagnosis of plaque may be tentatively made after examining fluid or tissue smears with a Gram stain. Typically, the organism appears as a bipolar staining, gram negative, spore-forming rod. The definitive test method for *Yersinia pestis*, a fluorescent antibody (FA) test looking for the *Y. pestis*-specific FA antigen, is performed by laboratories that have met specific government standards for biosecurity. The organism only expresses the antigen when cultured at >33°C. There is a risk for false negatives if cultures are improperly handled. (Proper tissue/culture handling and preparation was carried out by both laboratories in the aforementioned case. It remains unclear why the tissues and pure culture both were initially negative for *Y. pestis* by FA testing.)

Prevention and Treatment—Currently, there is no vaccine available for preventing infection with *Y. pestis* in the United States. Production of an injectible vaccine was discontinued in the United States in 1999 while, just recently, a Canadian firm was awarded an $8 million NIH grant to develop an intranasal vaccine amid fears of a potential bioterrorist attack.

The primary means of prevention involves control of rodent and insect vector populations. Avoiding contact between susceptible species, such as humans and felids, and rodent populations is another important means of prevention. Flea control in areas of known *Y. pestis* prevalence is recommended.

Human plague cases are most commonly treated with doxycycline, though ciprofloxacin also may be used. In veterinary cases, aminoglycosides, fluorquinolones or tetracyclines are recommended.

How to Handle Suspect Cases—If a case of suspected plague presents to your practice, proper personal protection, such as gloves, gown and face mask, should be worn. Patients should be treated immediately with anti-flea medication and isolated from other individuals. Since plague is a reportable disease, it is important to contact the Colorado State Veterinarian's Office (303-239-4166) upon confirmation of disease. If plague is a differential diagnosis for a sample submitted to us for testing, please make this clear on the submission form. Suspect samples submitted to us are forwarded to the CDC for FA testing. Proper samples for testing include lymph nodes or carefully collected lymph node aspirates, splenic tissue, blood or sputum.

Cleaning areas where suspected or confirmed cases of plague have been, such as cages or exam tables, should involve general cleaning followed by cleaning with a 10% household bleach solution with about 30 minutes of contact time.

More information on plague transmission, prevention and control may be found on the following websites:

- www.ext.colostate.edu/pubs/insect/05600.html
- www.cdphe.state.co.us/dc/zoonosis/plague/plaguehom.html
- www.bt.cdc.gov/agent/plague/index.asp

SUSPECTED COMPANION ANIMAL TOXICITY

—Dwayne Hamar, Cathy Bedwell, and Dan Gould

Following the sudden, unexpected death of a companion animal, owners frequently suspect that the animal was poisoned, especially if there had been complaints or conflict about the pet. In many cases, the animal is found dead without observation of any clinical signs. To investigate an unexpected, unobserved death in a companion animal as a toxicology case is difficult and expensive. There are numerous classes of chemicals that can result in death and each would have to be analyzed separately. A more practical and productive approach begins with systematically gathering appropriate history and necropsy findings.

The first step is to develop a complete history. Carefully note any clinical signs observed prior to the animal's death. It may be important to talk to more than one member of the household to determine if there is conflicting information. A very important element of the history is to determine whether or not the animal's environment is well defined. Is the animal confined to a cage or kennel, or is it allowed to roam freely? Note any changes in a confined animal's environment, for example, the introduction of a new (possibly poisonous) plant. Are there any suspicious substances in the animal's environment?

The next step is to conduct a complete necropsy including the central nervous system. Keep an open mind. Avoid allowing the owner's assumptions of a poisoning to bias your investigation. Examine all organ systems for any gross abnormality. In many cases, the gross necropsy will provide the diagnosis (for example, gastric volvulus dilatation or exsanguinations due to a ruptured hemangiosarcoma). Carefully examine the contents of the stomach for extraneous, suspicious materials. Collect samples of all major organs, including the brain, and preserve in formalin for histological examination. Collect fresh (unfixed) samples of liver, kidney, stomach contents, urine (if available), fat, and one-half of the brain. Place all tissues in separate, labeled containers and freeze (fat should be wrapped in aluminum foil or placed in a glass container). Remember – it is better to sample extensively! Tissues always can be discarded if not needed, but cannot be recovered if the need arises.

Send the formalin-fixed tissues to us for histological examination. Histologic changes may indicate the cause of death, or give an idea of what to investigate further. Retain the frozen samples for subsequent toxicological analysis if additional history and/or pathology findings incriminate particular candidates. For example, histological observation of calcium oxalate crystals in the kidney would indicate testing for ethylene glycol and save the owner the cost of many screens.
Some laboratories offer test panels of related toxicants (eg., organophosphate, carbamate, anticoagulant, etc. "screens"). However, it should be made clear that these panels will not test for all possible intoxicants and performing all available panels quickly becomes prohibitively expensive.

FOUR CASES OF CANINE DISTEMPER PNEUMONIA IN NEONATAL PUPS

—Karamjeet Pandher

Canine distemper, caused by a *Morbillivirus* (canine distemper virus), is a disease of dogs, foxes, wolves, coyotes, ferrets, raccoons, and exotic cats (domestic cats are resistant to experimental parental infection).

The disease is transmitted by aerolosization of viral particles, mostly from respiratory exudates, and is highly contagious. The virus is shed from most epithelial cells during infection including respiratory, intestinal, and urinary tract epithelial cells.

Disease course and manifestation largely is dependent on viral strain and host immune status. Following respiratory infection, the virus multiplies in macrophages and is distributed to regional lymphoid organs. By six days post-infection, there is widespread systemic infection with concomitant pyrexia and lymphopenia. Subsequently, the virus spreads to various epithelial and nervous tissues. With adequate immunity, the virus is cleared by neutralizing antibody and cell-mediated mechanisms without clinical disease. Dogs with

inadequate-to-poor immunity develop variable levels of systemic disease, often with resulting acute or chronic neurologic complications.

The disease is most prevalent at 3-to-6 months of age (when passive, maternally-derived immunity fades), and exhibits protein manifestations like pneumonia, acute and chronic encephalitis, diarrhea, conjunctivitis, nasal and digital hyperkeratosis, and pustular dermatitis.

CDV has been demonstrated to transcend the placental barrier causing abortions and weak puppies, and development of neurologic disease in infected newborns. Neonatal infections are reported to be associated with thymic atrophy, necrotizing encephalitis and, experimentally, with necrotizing cardiomyopathy.

Lanes 1,2,4 represent canine lungs PCR positive for canine distemper virus; lane 3 is PCR negative; lane 5 is positive control; and lane 6 is negative control (water).

We recently received four necropsy submissions from three different litters from a region in Colorado. The pups were between 5-and-12 days old and presented with respiratory disease (sneezing, labored breathing, increased lung sounds), with or without emesis and diarrhea affecting some or most of the littermates. The gross appearance of the lungs was similar in all submissions and was characterized by increased firmness, incomplete collapse upon opening the thoracic cavity, and a mottled red appearance. All other organs examined were grossly unremarkable.

Histologically, the prominent and consistent lesion was an interstitial pneumonia. The alveolar septa were expanded by an infiltrate of mononuclear cells (morphologically compatible with macrophages). The alveolar sacs were filled with edema, occasional fibrin aggregates, foamy macrophages and degenerate cells, and occasional multinucleate cells. There was multifocal hemorrhage in the alveoli. All other organs including the brain and digestive tract were histologically normal. The characteristic intracytoplasmic eosinophilic inclusion bodies of canine distemper virus were not observed in any of the four submissions (most likely the result of the transient nature of these inclusions). Three of the four puppies were PCR positive for CDV (the fourth PCR negative puppy had a littermate that tested positive). All four specimens were positive by immunohistochemistry of the lung performed at OADDL (Oklahoma Animal Disease Diagnostic Laboratory). The pups were negative for Canine Parvovirus-1 by PCR; variable secondary bacteria were isolated from the lungs. The histologic lesions also were incompatible with canine herpesvirus infection.

Previous reports have associated CDV infection in neonatal animals with neurologic disease and generalized immune cell depletion whereas respiratory disease is associated with post-natal infection. However, given the findings in these four pups, canine distemper could be a differential for neonatal canine respiratory disease. No maternal clinical signs were noted in any of these cases. The infection could have been acquired *in utero* (bitches might have had asymptomatic infection) or immediately post-partum. Submission of the entire body (chilled over ice, but not frozen) is desirable. Alternatively, if necropsy is performed at the clinic, all major organs including lungs, liver, brain, spleen, thymus, stomach, kidneys, and urinary bladder should be submitted to us in formalin along with fresh and chilled lungs, spleen, and thymus for analysis. Routine but diligent disinfection procedures usually are adequate in the neutralizing clinic contamination.

BIOSECURITY V

—James Kennedy

We have covered a number of important issues in this series of articles on biosecurity. The initial article stressed the importance of client education, the next dealt with disinfectants and

ways to prevent disease spread, the third with diagnostic tests and sampling, and the fourth interpreted diagnostic tests on a herd basis. Now, we'll examine the role vaccinations play in biosecurity.

Veterinarians routinely sell and administer vaccines, but a vaccination program alone does not make for a biosecurity program. Vaccines are tools that can aid in the prevention of disease but do not prevent disease in all animals. Vaccine efficacy is best determined by computing the preventable fraction, where the preventable fraction is the percent of controls dying, less the percent of vaccinates dying, divided by the percent of controls dying.

$$\frac{\text{(\% controls dying - \% vaccinates dying)}}{\text{\% controls (dying)}} = \text{Preventable Fraction (PF)}$$

You would expect a good vaccine to have a PF of more than 80%, leaving as many as 20% of the animals unprotected. Vaccine companies may talk about their vaccine increasing the antibody titer to a specific antigen, but if that antigen is not presented to those antibodies, the vaccine accomplishes nothing in preventing the disease. An example is BVD with type 1 and type 2 viruses. Vaccinating for one type does not provide the same protection against the other. This points out that although a herd is vaccinated, the risk of the animals having a particular disease still exists, and drives home the concept that vaccination does not equal biosecurity. Vaccines also can negatively impact a biosecurity program by confusing diagnostic test interpretation. We might consider foot and mouth disease. Vaccines to prevent some strains exist and can be used, but vaccinated animals will test positive and not be suitable for export. A more familiar disease is brucellosis. Strain 19 vaccines were notorious for creating vaccination reactions. Reactor animals required special handling and could result in quarantines of healthy herd mates. Although it is unrealistic to not vaccinate for diseases, the better course of action is testing and managing a herd to prevent disease. These components should be the focus of any biosecurity program.

Vaccinations fail for many reasons, including improper handling and administration, immune incompetence, and poor antigenicity. A vaccination program without good management and proper diagnostic testing is destined for failure. Vaccinations are tools of a biosecurity program, but not the key element of the program.

GET TO KNOW YOUR LABORATORY

—Hellos and Goodbyes

A recent visit or phone call to us may have surprised a few of you with some new faces or voices. Early in September, we welcomed two new employees to the office. Joanie Olsen has joined our staff as the main receptionist. Joanie, along with being a long-time CSU employee, has years of animal experience and is a welcome addition with our constantly busy phone lines. Lisa Wolfe, also another long-time CSU employee, is assisting in the sample entry area. Lisa is a recent certified veterinary technician graduate and is happy to be able to put her education and skills to use in our laboratory. Please join us in making them feel welcome!

Hellos meant some goodbyes had to be said. Amber Reeve and Hallie Willmore both left the laboratory this fall to pursue their education—Amber in the field of human radiology and Hallie to attend her first year of veterinary school. We wish them good luck!

Residency Training News

Cases presented to us are used, in part, for training the next generation of veterinary diagnosticians, meeting a critical need in this area.

Five new trainees were appointed in the combined Residency/PhD program on July 1. New anatomic pathology residents are Greg Wilkerson, a 2001 graduate of Oklahoma State University with three years emergency medicine experience, and Stuart VandeVenter, a 2004 graduate of Iowa State University. Philip McKee and Matthew Williams are the new clinical pathology residents. Dr. McKee is a 2004 graduate of Oklahoma State

University and Dr. Williams is a 2001 graduate of Tuskegee University with three years experience in mixed practice. Nicholas Haley, a 2004 graduate of Cornell University, is the new microbiology resident.

Greg Wilkerson

Stuart VandeVenter

Nick Haley

Five current or former CSU trainees successfully completed board certification examinations administered by the American College of Veterinary Pathologists in September. Debra Kamstock and Oliver Turner were certified in anatomic pathology; and Karen Zaks, Linda Vap and Jeremy Johnson were certified in clinical pathology.

CLINICAL TRIALS OFFERED THROUGH THE ANIMAL CANCER CENTER

—Susan Plaza

Melanoma Vaccine Study—Canine patients with oral melanoma are invited to enroll in a clinical study to investigate the efficacy of vaccine therapy designed to inhibit angiogenesis in the tumor environment. The disease must be surgical respectable and have not metastasized to the lungs. There may be regional metastasis to the lymph node. The patient will receive eight vaccines during a period of 21 weeks. All treatment visits are covered by the study, as well as partial compensation for the definitive surgery at the fifth week.

Hemangiosarcoma Low Dose Chemotherapy Study—Canine patients that have been diagnosed with hemangiosarcoma and have undergone a splenectomy are invited to enroll in a clinical trial. This study will examine the effect on survival time of patients receiving continuous low doses of oral chemotherapy versus traditionally administered chemotherapy at two-week intervals. This is a randomized clinical trial with one group receiving Adriamycin every two weeks and the other group receiving oral etoposide, cyclophosphamide and piroxicam. The study will cover the costs of all treatment visits, however; the owner is responsible for the costs of the initial staging visit.

For further information on any clinical trials, please consult our website at www.csuanimalcancer-center.org or contact the clinical trial coordinator, Dr. Susan Plaza at 970-297-4001.

CSU VETERINARY TEACHING HOSPITAL LAUNCHES PET HOSPICE PROGRAM

Practitioners understand how serious illness in a pet not only affects the patient, but family and loved ones as well. Because of this, a dedicated group of veterinary students, with guidance from the Argus Institute for Families and Veterinary Medicine and James L. Voss Veterinary Teaching Hospital clinicians, has developed a first of its kind Pet Hospice service, serving clients in the Fort Collins and Loveland areas. The mission of the Pet Hospice Program is to provide compassionate end-of-life care for pets and emotional support and education for their families. Pet Hospice volunteers visit homes of terminally ill patients on a schedule dictated by the referring veterinarian, and provide pain control and physical comfort. They also assist with any prescribed hydration and nutrition therapies. Volunteers serve as teachers, assisting the family in caring for the pet's needs in the home setting. The volunteer Case Manager updates the referring veterinarian after each visit so that he/she understands the exact physiological status of the patient. At all times, the patient remains under the direct care and supervision of the referring veterinarian. Pet Hospice allows terminally ill pets to spend their final days in the comfort of their own home, with the families who love them. Pet Hospice volunteers are available to assist with at-home euthanasia of Hospice patients as well.

Veterinarians need only complete a short training session in order to understand the procedures of

CSU's Pet Hospice and to familiarize themselves with the type of patients that may be referred to the Pet Hospice. For more information about CSU's Pet Hospice and how you can offer this groundbreaking service to your clients, call Debra Stirling or Christie Long at 970-219-7335. Presently, we are limiting our services to within a thirty minute drive from Fort Collins.

WHAT'S IN THIS ISSUE--

Colorado State University

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