Colorado State University Veterinary Diagnostic Laboratories

LabLines

Volume 11, Number 1 **Spring/Summer 2006**

Letter from the Director

Welcome to the Spring/Summer edition of LabLines! In this issue, there are a number of updates and diagnostic information. An important inclusion on the first page is a listing of our External Advisory committee. The members of this committee all volunteer their time and provide the laboratory with extremely valuable advice to help us produce timely and quality diagnostic services. For those of you on the Western Slope, we are excited to announce that the new director of the Grand Junction Laboratory is Dr. John Andrews, a highly-regarded pathologist. Very soon, you will seen an increased quality of service as well as an expansion of services. Throughout the laboratory system, we have had a number of new staff additions and shifts. We have added staff to the busy Virology Section and expanded the Avian Disease Section. There is a new group of Anatomic Pathology residents starting July 1 and these are: David Gardner and Monali Bera. We are very sad to see Dr. Norrdin retire July 1, but we are sure he will still visit us and we wish him well! A search for a new pathologist to fill the void is underway. Our new parasitologist, Dr. Lara Ballweber, will begin August 1. We will tell you more about her in our fall newsletter.

In addition to the interesting articles, please also see the note about the availability of our 2005 Annual Report. It was good to see many of you at the Winter and Spring Colorado Veterinary Medical Association (CVMA) Leadership Conferences, and hope to see more of you in September in Keystone as CVMA celebrates its 100th Annual Convention.

And now for the most exciting news, I am most pleased to announce that the Colorado State legislature has approved funding for our new building, the Diagnostic Medicine Center, with Phase 1 funds of $3.5 million to be available July 1, 2006!!! Our sincere thanks and gratitude to all who have supported this long-term effort to obtain funding for this much needed new facility.

Barbara E. Powers

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Dr. Mike Gotchey/Equine	1878 Lincoln Avenue	Steamboat Springs, CO
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Mr. Ed Hansen/Beef Cattle	4554 CR 74E	Livermore, CO 80636
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Our External Advisory Committee members volunteer their time to meet with us annually and assess our progress, as well as provide input to our future directions. We are grateful for their time and advice, and hope they feel that they are an integral part of the laboratory.

POSSIBLE 3-METHYLINDOLE PNEUMOTOXICITY IN A LIVESTOCK EXHIBITION STEER

—Dan Gould

A 19-month-old steer in excellent body condition was brought to a livestock show for exhibition. Shortly after arrival, the steer developed labored respiration. During the next 48 hours, this condition progressed to severe dyspnea with open-mouth breathing. Fever also was present. The condition was non-responsive to antibiotic therapy and the steer died. The gross necropsy revealed abnormalities only in the lungs. The lungs failed to collapse and the parenchyma was meaty and mottled dark red and pink. Interlobular septae were widened and lobules were independently movable. Airways contained pink froth. A prominent observation made during the necropsy was that the entire carcass was permeated with a peculiar, very strong, foul, fecal-type odor.

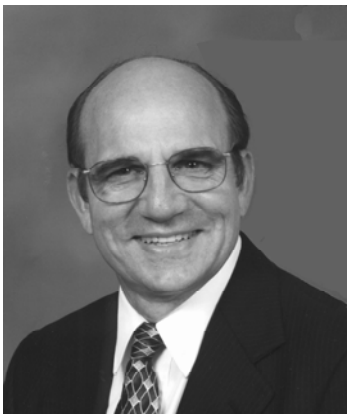
Histopathology of the lung tissue revealed severe, diffuse, subacute interstitial pneumonia characterized by fibrin and polymorphonuclear leukocytes in alveolar spaces and type II pneumocyte proliferation and syncytial cells. Acute and subacute interstitial pneumonia in cattle can be caused by pneumotoxicity or bovine respiratory syncytial virus (BRSV) infection. Fluorescent antibody and virus isolation testing of lung tissue for BRSV and other viruses was negative. The prototype toxicant for bovine pneumotoxicity is 3-methylindole (3-MI, skatole), which is generated by ruminal microbial metabolism of tryptophan. Typically, excessive tryptophan intake occurs when cattle are subjected to a dietary change to lush, rapidly growing pasture. The occurrence of such a situation would have been unlikely given the dietary regimen of this animal. A form of acute interstitial pneumonia is observed in feedlots. This form is not related to a dietary change.

Follow-up studies on lung tissue were conducted in a research laboratory specializing in 3-MI pneumotoxicity (Dr. Garold S. Yost/University of Utah/801-581-7956; www.pharmacy.utah.edu/ pharmtox/faculty/yost). Laboratory personnel frequently work with purified 3-MI and are familiar with the characteristic odor. They noted that a strong odor of 3-MI emanated from the show steer lung sample, however, they were unsuccessful in chemically identifying metabolites of 3-MI in the lung.

In order to compare the strong odor emanating from the steer carcass during necropsy with the odor of purified 3-MI, the veterinarians who conducted the necropsy were supplied with purified 3-MI dissolved in methanol. The odor of the purified 3-MI was identical to the distinctive odor of the carcass at necropsy.

Overall, on the basis of the lung histopathology and the odor of the carcass, it is likely that this animal died of 3-MI pneumotoxicity. This suggests that the steer might have consumed material rich in tryptophan that was converted in the rumen to 3-MI and absorbed systemically. It is noteworthy that some commercially available food supplements used for excitable horses contain L-tryptophan as a "calming" ingredient. Evidence for administration of such a supplement to this steer could not be demonstrated. However, misuse of an L-tryptophan-containing equine product in cattle could result in 3-MI pneumotoxicity. Use of such a supplement for cattle should be strongly discouraged.

WESTERN SLOPE ANIMAL DIAGNOSTIC LABORATORY WELCOMES NEW DIRECTOR



We are pleased to welcome John J. Andrews, DVM/PhD/DACVP as the new Director and Veterinary Pathologist at the CSU Western Slope Animal Diagnostic Laboratory (WSADL) located in Grand Junction.

"The success of a branch veterinary diagnostic laboratory depends heavily on the service attitude of the personnel of the local laboratory staff in Grand Junction, as well as the quality of faculty and staff of the main laboratory in Fort Collins," said Dr. Andrews. "I found both to be excellent during the interview process and believe that collectively, we will be able to provide an excellent service to veterinarians, animal owners and others, particularly on the Western Slope of Colorado. We should be able to assist and augment diagnostic services to the rest of the state and the region as well."

Dr. Andrews said his team at the Western Slope Animal Diagnostic Laboratory will strive to provide a high quality diagnostic laboratory service, using their facilities and the main Diagnostic Laboratory in Fort Collins to provide the very best scientific investigations possible to identify and solve animal disease problems on the Western Slope.

"Specifically, we will immediately be able to provide necropsy and histopathology services for all species," said Dr. Andrews. "We will be developing and adding capabilities for infectious disease antigen detection through immunohistochemistry and fluorescent antibody procedures in the near future. The general microbiology services already present will continue to improve as well as the specialty services of *Brucella ovis* serology and *Tritrichomonas* fetus cultures that the WSADL has been providing for many years."

Dr. Andrews noted that renovation and updating of the facilities and equipment in the necropsy, histopathology and microbiology areas will enable the laboratory to improve services markedly. Rapid reporting methods such as faxed reports will continue and WSADL will be working with the main laboratory information technology personnel to provide 24/7 on-line access to laboratory results. Molecular detection of specific infectious agents will be added as they are needed by the western regions of Colorado. Other services such as clinical chemistry and hematology will be considered and may be offeredWas the need is identified.

"We will be meeting with local veterinarians and other potential users of the WSADL and exploring other possible means to assist them in their disease investigations and other diagnostic challenges," said Dr. Andrews.

ANNUAL REPORT--Our 2005 Annual Report is now available. Find it on our Web site at www.dlab.colostate.edu under "Annual Report." If you would like a hard copy, contact Mary at mlindbur@colostate.edu.

USER SURVEY--In our last newsletter, we sent out a user survey. So far, we have received 55 responses. If you still have not filled it out, please do so and we will have results in our next newsletter.

INFLUENZA A VIRUS IN DOGS

—Hana Van Campen

In March 2004, influenza A virus was isolated by Dr. Ed Dubovi, Cornell University, from a sample of lung obtained from a racing greyhound. The influenza A virus was associated with an outbreak of severe respiratory disease which occurred earlier in the year in Florida. Dogs exhibited coughing, fever (≥30°C) and, in some cases, hemorrhage and bronchopneumonia. The influenza A virus was most similar to recent isolates of equine influenza A viruses of the H3N8 subtype. Retrospective study traced the influenza A virus infection in dogs to 2000. Evidence also has been found for the spread of the infection in pet dogs and in dogs in shelters.

Clinical Presentation—The majority of dogs infected with influenza A virus infections are reported as being ill. Two disease entities have been recognized—a "kennel cough"-like disease and a severe, hemorrhagic pneumonia in racing greyhounds.

Differential Diagnoses—Currently in Colorado, canine distemper virus and *Bordetella bronchisepticum* infections should be considered. Other less common agents of respiratory disease in dogs include canine adenovirus 1 and 2, and parainfluenza virus-2.

Diagnostic Tests—We offer the following diagnostic tests for canine influenza:

Virology: To demonstrate the presence of influenza A virus in the acute phase (first 5-to-7 days after infection).

- Influenza A PCR (Test #788) detects viral RNA in the acute phase of infection. The PCR test is a very sensitive and specific test; however, viral RNA is easily degraded if improperly handled.

    1. Sample—Nasal or pharyngeal swab, transtracheal wash, lung
    2. Submission instructions—Send overnight on ice pack
    3. Turnaround time—One week
    4. Cost--$30.00

- Influenza A ELISA (Test #730) detects the influenza A viral antigens in the acute phase of infection. The ELISA is less sensitive than PCR; however, viral proteins are more stable than RNA.

    1. Sample—Nasal or pharyngeal swab, transtracheal wash, lung
    2. Submission instructions—Send overnight on icepack
    3. Turnaround time—Run same day as received
    4. Cost--$20.00

- Virus isolation (companion animal) (Test #713) detects infectious influenza A virus which is present in greatest amounts in the first 3-to-5 days after infection. Influenza A viruses are easily inactivated by soap, disinfectants, heat and UV light; therefore, careful handling is required to preserve viral infectivity.

    1. Sample—Pharyngeal swab, transtracheal wash, lung
    2. Submission instructions—Send overnight on icepack
    3. Turnaround time—1-to-3 weeks
    4. Cost--$40.00

Serology: To demonstrate the presence of antibodies to equine influenza A2 virus of the H3N8 subtype in the serum of dogs. Any positive test indicates post-exposure to and infection with influenza A virus of the H3 subtype assuming that the dogs have not been previously vaccinated with a product for horses.

- Submit for influenza A hemagglutination inhibition (equine influenza A2 HI) (Test #841).

    1. Sample—Acute and convalescent (obtained two weeks later) serum samples in red-topped tubes
    2. Submission instructions—Send overnight
    3. Turnaround time—HI tests are set up on Wednesdays and Fridays, and are reported out the next day
    4. Cost--$7.50

To-date, there have been 4 canine flu positive dogs by PCR and one isolate grew. There have been numerous antibody positive dogs which increase in number weekly. Nationally, Colorado has the highest number of seropositive samples submitted from dogs. Dr. Dubovi reports that Colorado is the nation's canine influenza hotspot at this time (5-16-06).

AVIAN INFLUENZA SURVEILLANCE IN COLORADO

—Kristy Pablionia

The Colorado Avian Disease Surveillance Program is now in its third year of avian influenza surveillance and monitoring. The program, which started in April 2004, is a cooperative effort among the Colorado State University Veterinary Diagnostic Laboratory, Colorado Department of Agriculture, Colorado Department of Public Health and Environment and Colorado Division of Wildlife. Many other agencies are involved in the program, including the Colorado Veterinary Medical Foundation, Colorado Livestock Association, and regional offices of the United States Department of Agriculture and United States Fish and Wildlife Service. To date, more than 1,400 birds have been tested for avian influenza virus and highly pathogenic avian influenza virus has not been detected.

The Colorado Avian Disease Surveillance Program is continually expanding in response to the growing threat of avian influenza virus introduction into North America. Beginning this summer, Colorado will take part in a national wild bird surveillance program for avian influenza virus. Colorado also will receive cooperative funding from the USDA for increase avian influenza surveillance and monitoring in backyard flocks, commercial poultry, game bird operations and live bird markets.

All avian influenza virus testing will be conducted at our laboratory, a core member laboratory of the National Animal Health Laboratory Network (NAHLN). We have high-throughput capabilities for avian influenza testing and laboratory technicians are proficiency-tested annually. If needed, more than 800 samples can be tested for avian influenza virus every day.

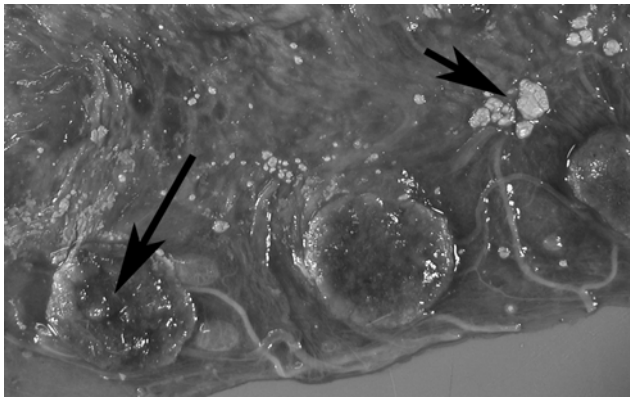
Please report sick and/or dying birds to the Colorado Health Emergency Line for the Public (CoHELP) at 1-877-462-2911. Reports will be referred to the Colorado Avian Disease Surveillance Program.

QUEST FOR Q-FEVER COMPETENCE

--Jonathan Arzt

"Q-fever" refers to any of several distinct disease syndromes caused by the zoonotic, obligate intracellular bacterium, *Coxiella burnetii*. Historically classified as a rickettsian, sequence analysis has recently led to reclassification of *C. burnetii* within the order *Legionellales*. The agent and the disease exist worldwide with the exception of Antarctica. The disease was first recognized in the 1930s when it received its "Q" moniker to signify the query or uncertainty of its etiology. Three quarters of a century after the first description of the syndrome in humans, many aspects of this disease remain a query.

C. burnetii is capable of infecting humans and numerous animal species including mammals, birds and arthropods. The most publicized route of transmission is inhalation of aerosolized organisms. An infamous cluster of human cases of coxiellosis termed "poker players' pneumonia" occurred after an ill-fated game was held while sharing a room with an infected parturient cat. In ruminant herds, the organism is likely maintained through a combination of aerosolization and ingestion of bacilli shed in placentas, lochia, milk and feces. Rare incidents of venereal transmission among humans are documented, and this route likely is relevant (but not described) among animals. Arthropod-mediated transmission is supported by the isolation of *C. burnetii* from numerous species of ticks. The role of wild animals in maintaining the organism is not well-established but is likely, given the broad range of species susceptible to infection.

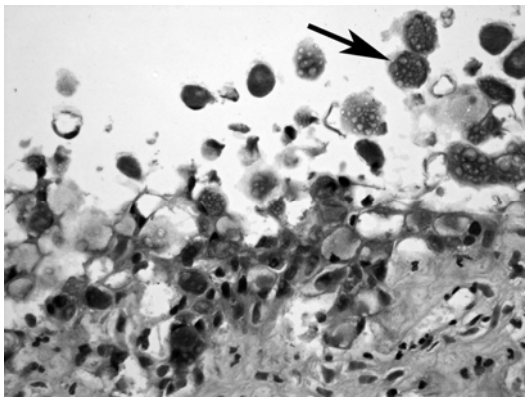


Placenta from caprine Q-fever abortion case showing cotyledonary necrosis (long arrow) and intercotyledonary plaques (short arrow).

It is generally accepted that across species, more than half of *C. burnetii* infections are asymptomatic. In animals, the most important syndrome associated with the disease is late-term abortions, stillbirths, and "poor-doer" live births in small ruminants and to a lesser extent cows. Affected dams typically manifest no other signs of disease, but may have an associated metritis. Aborted fetuses often have no lesions, but may have granuloma-

tous hepatitis or pneumonia. The most consistent (though non-specific) lesion associated with these abortions is a necrosuppurative placentitis which may affect cotyledons and intercotyledonary regions. Vasculitis is variably present. Within affected regions, trophoblasts often contain abundant bacilli which are readily identifiable with routine H&E staining. While this trophoblast change is quite similar to the microscopic appearance of other causes of ruminant abortion (*Chlamydophila, Campylobacter, Brucella*), the foamy vacuolation of the trophoblasts is said to be characteristic of coxiellosis.

In humans, Q-fever typically manifests as an acute, febrile, flu-like syndrome which may be associated with atypical pneumonia or hepatitis. Symptoms are non-specific and may persist for up to three weeks if untreated. Rare components of the acute human syndrome include meningoencephalitis, abortion and pericarditis. A small proportion of human cases progress to a chronic phase which may include endocarditis, repeated abortions or chronic fatigue syndrome.



400X microscopic view of superficial surface of placenta from caprine Q-fever abortion case showing vacuolated trophoblasts distended with copious *C. burnetti* bacilli (arrow).

Currently at the Veterinary Diagnostic Laboratory, a multifaceted project is underway aimed at elucidating the pathophysiology of Q-fever and determining its prevalence in Colorado. The development of molecular diagnostic techniques is central to this project and these tests will be available to our clients shortly. We are evaluating utility and cost-effectiveness of polymerase chain reaction (PCR), immunohistochemistry (IHC), and serology. The specific goals of the project are:

- Targeted Surveillance for *C. burnetii* as a cause of abortion among livestock species in Colorado using Veterinary Diagnostic Laboratory-generated case material. Abortions for which no etiology is determined by routine abortion-screen protocols will be tested for *C. burnetii.*
- Molecular Pathogenesis Study. Working with a goat herd with known enzootic coxiellosis, we are collecting numerous samples for PCR- and IHC-based analysis with the hope of better describing host-agent relationships in fulminant disease and chronic asymptomatic shedders. This portion of the project also will include an evaluation of a clinical treatment trial aimed at determining efficacy of herd-level eradication of *C. burnetii* under environmental conditions present in Colorado.
- Validation of above-mentioned diagnostic tests so that accurate diagnostics can be offered to our clients.

Much of the attention focused on Q-fever recently is due to the potential use of *C. burnetii* as an agent of bioterrorism. The cause for this concern is the high infectivity, aerosol route of exposure, and availability of the agent. Realistically, the large percentage of asymptomatic infections probably makes *C. burnetii* an unlikely candidate for malicious use. The designation of Q-fever as an "emerging" disease also is questionable, as increased awareness and diagnostic acuity may be the cause of the current surge of confirmed cases. However, recently increasing goat prices have led to more importation of goats into Colorado, which may play a real role in the prevalence of disease.

Regardless, several facts make Q-fever worthy of further study and concern, particularly among individuals with small ruminant or bovine exposure. Infected ruminants are known to shed *C. burnetii* in feces and milk for extended periods of time while asymptomatic. The most consistent risk factor for human disease worldwide is exposure to small ruminant parturition. And, lastly, there is still far too much query associated with this disease; basic aspects such as prevalence, pathogenesis and treatment protocols are still not established.

Veterinary Diagnostic Laboratory clients concerned about potential Q-fever cases are encouraged to contact us for up-to-date test availability and sample collection strategy. Coxiellosis should be considered a differential diagnosis in all small ruminant abortions and fever of unknown origin cases in any species. Similar to management of other infectious diseases, new animals should be quarantined, tested and, if appropriate, treated prior to introduction to established herds.

Q-fever diagnostics—No charge at this time. Not available for on-line diagnostics at this time.

BVD IN ALPACAS: TESTING AND CONTROL PRACTICES FOR ALPACA HERDS

—Rob Callan and Hana Van Campen

In 2005, alpaca crias that were persistently infected with bovine viral diarrhea virus (BVDV) were found in Ontario, Canada, by Dr. Susy Carman, University of Ontario/Guelph, and in New York and New Jersey by Dr. Ed Dubovi, Cornell University. One of these animals traced back to a farm in Colorado. Since the release of this information, alpaca breeders and veterinarians have been very concerned about identifying exposed herds and persistently infected animals. To help veterinarians make these diagnoses, the following tests are recommended.

Screening for Exposed Herds—The purpose is to identify animals and herds that have been exposed to BVDV.

- Collect serum samples (red-topped tubes)
- Submit for BVDV Serum Neutralization Test
  --Detects antibodies to BVDV type 1
  --Any positive tests indicate exposure to BVDV
  --The cost is $5.00 per sample
  --Currently, testing for BVDV type antibodies is sufficient for a diagnosis of exposure to BVD
- Select animals with a greater likelihood of exposure to BVDV
  --Females that have gone to another farm for breeding
  --Animals that attend shows
  --Animals exposed to visiting animals (eg., ex posed to breeding animals and their crias from other farms)
  --Recommend screening at least 10 percent of your animals or a minimum of 15 animals

Identifying PI Animals—The purpose is to identify animals with viremia (circulating BVDV) which may be persistently infected (PI) and a major source of infection for other animals.

- Collect whole blood (EDTA) samples (purple-topped tubes)
- Submit for BVD PCR test
  --Detects RNA from the BVD virus
  --Test costs $30.00 per sample
- Positive test indicates that BVDV is circulating in blood
- To establish whether the animal is persistently infected, a second blood sample should be obtained three to four weeks later and tested for the presence of virus by PCR

Tests or samples that are NOT recommended for alpacas--

- Skin samples; holes or notches in ears or skin are not generally acceptable to owners
- AC-ELISA tests do not reliably detect BVDV in alpacas
- Serum samples for BVD PCR (whole blood is needed)

Biosecurity and Biocontainment—Prevent the introduction of BVDV into a herd.

- Do not allow animals into the herd without testing for BVDV PI status
- Isolate new animals from the herd for at least three weeks
  --The isolation area should be at least 10 meters from pregnant females
- Utilize breeding farms with an active BVDV control program that only accepts tested dams and crias from herds with an active BVDV control program
- Utilize shippers that are willing to ship only animals with confirmed BVDV PI negative status
- Restrict as best as possible, contact with other animals of undetermined BVDV status at shows and other events
  --Direct contact is the primary means of transmission
  --BVDV can spread by aerosol at distances of at least 10 feet
  --BVDV can be spread by items in contact with infected animals such as feeders, waterers, equipment, clothing, shoes, etc.
  --BVDV is susceptible to many disinfectants including regular detergents (soap and water), bleach, povidone iodine, chlorhexidine, etc).

SCREENING FOR BVD PERSISTENTLY INFECTED CATTLE USING PCR

—James Kennedy/Rocky Ford Laboratory

The Rocky Ford Diagnostic Laboratory has implemented a screening test to detect BVD-persistently infected (PI) animals. The process utilizes ear notches soaked in phosphate-buffered saline. The process allows as many as 100 animals to be pooled and tested for persistent BVD infections. Pooling samples provides an

economical alternative to testing individuals, yet due to the high sensitivity of PCR, provides a high degree of accuracy in detecting PI cattle. When the virus is detected in a pool, antigen capture ELISA is then utilized on the original samples to detect which individual(s) are the suspected PIs.

Submission of Samples for Pooled BVD PCR Testing—Please use forms provided by the Diagnostic Laboratory making sure to complete the top of the form so results may be mailed to you. Additional forms may be downloaded from our Web site at www.dlab.colostate.edu/BVDControlProgram/bvdcontrolprog_main.cfm under the Forms link on the right side of the Web page. Tubes should receive consecutive numbers beginning at 1 and ending at the last tube included in the submission to be shipped. Individual IDs can be placed on the submission form for further reference if needed. From a clean portion of the ear, collect a one square cm notch and place it in a sterile red top tube. If necessary to disinfect the ear notcher, be sure to thoroughly rinse any disinfectant from the tool before collecting the next sample. Samples may be frozen for up to one week before submitting. When boxing samples for shipment, pack tubes carefully with plenty of ice-packs. UPS provides the best service for delivery to the laboratory in our area, however, other shippers such as Fed Ex, DLH and the US Postal Service also may be used. The shipping address is: Colorado State University, Veterinary Diagnostic Laboratory, Rocky Ford Branch, 27847 Rd 21, Rocky Ford, CO 81067.

Interpreting Results of Pooled PCR Testing for BVD Persistent Infections—There are three possible outcomes for PCR results. The first is "BVD not detected" which is self-explanatory, however, the two other possibilities are less obvious. A result stating "BVD detected," means the virus was identified in the pool but may be the result of a persistently infected animal or on rare occasions, an acutely infected animal. Less than 2 percent of pools have detected the virus but failed to contain a positive antigen-capture ELISA; this could be the result of acute infections or vaccinations with a MLV product. The third possible outcome is "PCR inhibition." This occurs when some foreign substance contaminates the sample such as excessive manure or disinfectants (less than 4 percent of the pools show inhibition). Whenever the virus is detected or when pools show inhibition, individual samples are automatically evaluated using AC-ELISA on the individual notches submitted. With the low prevalence of PI animals, the cost of pooled PCR at $50 per pool, even with the potential for identifying acute infections and inhibition, is a more cost-effective process than testing individual samples. The estimated sensitivity (chance of false negative) of this test is 100% and the estimated specification (chance of false positive) is 97%.

If you have any questions, please feel free to contact the Rocky Ford Laboratory at 719-254-6382 or you may contact me directly at James.Kennedy@colostate.edu.

UNSURE WHAT SAMPLE IS NEEDED, HOW TO SEND IT OR WHAT THE COST IS? Check our User's Guide on-line at www.dlab.colostate.edu or request a hard copy. As always, please call our client services section and/or laboratory sections at 970-297-1281 if you have a specific question.

HIGH-THROUGHPUT SYSTEM DEMONSTRATION FOR FOOT AND MOUTH DISEASE VIRUS DETECTION

—Christina Gerhard

On July 26-28, we will be collaborating with Lawrence Livermore National Laboratory (LLNL), the Department of Homeland Security (DHS), the US Department of Agriculture (USDA) and Animal and Plant Health Inspection Service (APHIS) to demonstrate a high-throughput system for the detection of foot and mouth disease (FMD) virus.

Although the last outbreak of foot and mouth disease virus in the United States occurred in 1929, it is still enzootic in many parts of the world.[1] Outbreaks create severe economic impacts by halting trade in the cattle industry, creating animal welfare problems, and hindering tourism.[1] There are currently no active surveillance systems for FMD[2] in the United States and suspect cases currently are tested only at Plum Island. In Colorado, outbreaks of vesicular stomatitis, when affecting cattle, are indistinguishable from FMD. Pre-clinical detection by a method with quick turnaround time, reduced cost, increased efficiency and the ability to differentiate between foot and mouth disease and other clinically similar diseases is key.

LLNL has developed a multiplexed FMD virus rule-out assay that has the ability to simultaneously detect the presence of viral DNA or RNA for seven different viruses, including those that closely mimic foot and mouth

disease, such as bovine herpesvirus, bovine popular stomatitis virus, bovine viral diarrhea virus, bluetongue virus, swine vesicular disease, and vesicular exanthema of swine. This assay also distinguishes between multiple serotypes of each of the viruses listed above including 2 FMDV serotypes (FMDV-1 and FMDV-2). The assay, based on luminex bead technology, hybridizes PCR product from samples to a bead suspension that is labeled with a fluorescent probe. Fluorescence produced by these beads is measured and, based on the individual sample's ratio of red to near-infrared, identifies the presence or absence of the target nucleic acid sequences from the viruses listed above.

After an interlaboratory comparison using the same technology, during which 14 laboratories that are part of the National Animal Health Laboratory Network (NAHLN) processed and reported results from 200 samples, we were selected as one of two laboratories to participate in this surge-capacity demonstration. The goal of this demonstration, as stated by the USDA/APHIS, is to "demonstrate to state and federal authorities and laboratory personnel an integrated high-throughput laboratory system capable of processing the surge in sample submissions that would result from a foot and mouth disease outbreak."

Over the course of three days, technicians from LLNL will demonstrate an automated robotics system that will allow the complete processing—from sample receipt by the laboratory through data analysis and results reporting—of a minimum of 1000 samples per day with two technicians. During this time, our technicians will be cross-trained on the equipment and have the opportunity to run many of these samples.

In conjunction with the laboratory demonstration, personnel from LLNL, DHS, USDA, APHIS, NAHLN, and other organizations will be on hand Thursday, July 27, to conduct an overview and review the pilot execution and data collected. Previously invited guests will have the opportunity to tour the laboratory, observe the equipment in operation, and attend scientific presentations related to the demonstration.

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[1]Sutmoller P, Barteling SS, Olascoaga RC and Sumption KJ. Control and eradication of foot and mouth disease. Virus Research. 91(2003)101-144.

[2]Hullinger P. Multiplexed Diagnostic Technologies for Agricultural Security. NAHLN Briefing. Lawrence Livermore National Laboratories. December 2005.

LEPTOSPIROSIS SEROLOGY UPDATE

—Doreene Hyatt

As an update of information given in the 2005 LabLines, the table below gives the number of serum samples tested for titers to *Leptospirosis interrogans* between January 1 and June 1, 2006, and the entire year for 2003 through 2005 by animal species.

Species	2006	2005	2004	2003
Bovine	103	338	231	361
Camelid	4	0	2	3
Canine	120	426	181	106
Caprine	17	12	2	2
Equine	13	38	34	23
Ovine	0	8	5	3
Zoological	1	3	5	7
Porcine	0	6	0	0
Feline	2	1	2	0

Lepto serology results for each of the five serotypes for serological samples submitted between January 1 and June 1, 2006, and all of 2004 and 2005 for all animal species are shown in the following table. 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.

Year	N	*L. canicola*		*L. grippo.*		*L. hardjo*		*L. ictero.*		*L. pomona*	
		P	High	P	High	P	High	P	High	P	High
2004	467	49	800	76	819,200[a]	33	800	102	3200	87	102,400[b]
2005	858	152	1600	135	51,200	117	1600	165	3200	123	12,800
2006	264	51	800	34	6,400	21	1600	26	800	38	6,400

Additionally, 18 tests for *L. brataslava* were conducted in 2004 and five were positive with the highest titer being 1:3200. In 2005, 225 samples were tested and 10 were positive with the highest titer being 1:3200. From January 1 to June 1, 2006, a total of six tests were done with no positives.

CONFIRMING LEAD TOXICITY IN CATTLE

—Dwayne Hamar/Cathy Bedwell and Dan Gould

Lead is a common toxicant in both large and small animals. Ingestion is the primary route of exposure to lead for small and large animals alike. Potential sources of lead include lead-based paints, ashes from wood painted with lead-based paints, ammunition, old plumping materials and automobile batteries. Small animals displaying neurological signs are commonly tested antemortem; therefore, whole blood is usually the tissue of choice for analysis. Large animals with a history of neurological signs are commonly tested postmortem; therefore, tissues are submitted for lead quantitation and brain submitted for histopathologic examination.

Although liver tissue is usually considered sufficient for the demonstration of elevated tissue lead concentration, kidney tissue is likely to have higher lead concentrations than liver and can better serve as the basis for the diagnosis of lead toxicity. In cattle, we have confirmed, on average, two to three cases of lead intoxication per year. In many cases, we cannot confirm lead intoxication because we have received only fresh liver for quantitation. Of the last 16 cases submitted, in which both liver and kidney were analyzed for lead, five of the liver samples contained less than 10ppm, whereas the corresponding kidneys contained greater than 27ppm. In general, a liver concentration greater than 10ppm wet weight should be considered diagnostic for lead. Of these 16 cases, the kidney cortex lead concentrations averaged 6.7 times that of the liver concentrations, which serves to emphasize the importance of submitting fresh/frozen kidney for confirmation of lead intoxication.

For cattle, other toxicoses need to be differentiated from lead toxicity. These include sulfur related-polioencephalomalacia and water deprivation/sodium toxicosis (salt toxicity). For cattle with neurological signs, always submit fresh/frozen kidney and liver for lead analysis, fresh/frozen brain for sodium and infectious disease analysis, and formalin-fixed brain for histopathological examination.

Lead toxicity: Submit liver and kidney fresh or frozen. Fee=$10.

Tired of waiting for that fax? Would on-line access to your results be useful? E-mail Carrie at cschmer@colostate.edu. She will create your account so you can see your results as soon as they are complete and at your convenience, day or night.

MYCOBACTERIUM AVIUM SUBSPECIES *PARATUBERCULOSIS* (AKA AGENT OF JOHNE'S DISEASE) CULTURE

—Doreene Hyatt

The culture and isolation of *Mycobacterium avium* subspecies *paratuberculosis* (*Mycobacterium paratuberculosis*) is a time-consuming, laborious and expensive process. A part of quality control for culturing this organism is by use of proficiency tests sent to diagnostic laboratories from the National Veterinary Services Laboratory (NVSL) in Ames, Iowa. These tests contain between 20 and 26 samples with known amounts of *Mycobacterium paratuberculosis* in them as well as some samples that are negative. For the last five years, we have passed this proficiency testing and continue to participate in the tests.

In the past few years, new techniques using liquid culture systems (originally for culturing human blood-borne pathogens) have been developed for culturing *Mycobacterium paratuberculosis* in a much shorter period of time. We received one of these liquid culture systems and started using it in October 2005. We are using the current proficiency test on the liquid culture system (as well as on the old solid culture system) and should have results within the next month.

Since October 2005, we have cultured 55 samples using both the older method (NVSL centrifugation method using solid media culture) and the liquid culture system. Of the 15 samples that have been completed and reported, there was 100 percent agreement between the two culture systems for positive samples (4/15) and negative samples. The liquid culture system has been

reported to decrease the culture time to as little as 28 days. Assuming that the correlation between results from the two systems is maintained, this will significantly shorten the amount of time for reporting *Mycobacterium paratuberculosis* results. The liquid culture method should be available as an option for *Mycobacterium paratuberculosis* testing in the near future at a minimally higher charge than the current solid media culture system.

Johne's culture: Submit 1ml of fresh feces. Fee=$20.

CORYNEBACTERIUM PSEUDOTUBERCULOSIS IN COLORADO

—Doreene Hyatt

Corynebacterium pseudotuberculosis infections cause a disease in equids commonly referred to as "pigeon fever," "pigeon breast," or "dryland distemper" and are the most common cause of ulcerative lymphangitis in equids. As mentioned in our 2000, 2001 and 2002 Lab-Lines, before 1999, we annually reported approximately one isolation of *C. pseudotuberculosis* from horses statewide. That changed in 2002 when we had a high of 89 isolations in the calendar year. Whether that increase was because of an increased prevalence or because of an increased awareness of the disease is unknown, but the number of isolations has steadily decreased since 2002. Again, whether the decrease is because of an actual decrease in disease or a decrease in the number of cultures performed is unknown. Updated statistics of the number of isolations of *C. pseudotuberculosis* from horses in the past six years are given in the table below.

Isolations of *C. pseudotuberculosis* from samples taken from horses in the years 1999 to current.

Year (Jan-Dec)	# Positive Samples
1999	15
2000	7
2001	12
2002	89
2003	53
2004	33
2005	31
2006 (Jan 1-June 1)	2

IT'S YOUR RESPONSIBILITY!

You are responsible for safely packaging and shipping your diagnostic specimens. The Federal Regulation can be found at this link: http://a257.g.akamaitech.net/7/257/2422/05dec20031700/edocket.access.gpo.gov/cfr_2003/octqtr/49cfr173.199.htm. Current US Postal Service regulations may be found at www.usps.gov. Other carriers may have additional requirements. Be sure you know and are able to apply the requirements, or fines and penalties may be imposed.

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- New Director for the Western Slope
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