

# LAB LINES

Diagnostic news and trends from the Colorado State University Veterinary Diagnostic Laboratories  
Volume 13, Number 2  
Fall/Winter 2008

## Mycobacterium avium paratuberculosis

# Johne's Progress...and Challenges

— John Maulsby, DVM, CSU VDL Case Coordinator

New information released by USDA-NAHMS in September shows dairy veterinarians nationally are making progress in educating producers about the impacts of *Mycobacterium avium paratuberculosis* (MAP), or Johne's disease. However, challenges remain both nationally and here in Colorado.

■ The good news is that although the similar survey from 1996 revealed almost 10 percent of producers hadn't heard of Johne's disease, only 1.5 percent claimed the same lack of knowledge today. Just over 94 percent of dairy producers now say they have a fair or basic knowledge of the disease, compared to just under 60 percent then.

■ Only about one third of all surveyed dairies say they test individual animals. Testing in order to help identify animals that are shedding and thus present a risk to noninfected animals on the operation forms the basis for a control program. Medium-sized dairies were most likely to test.

■ Similarly, just over three in 10 operations reported they participate in a Johne's disease control program. About 16 percent said they used a program developed specifically for their operation; about one-quarter said they were involved in a state-sponsored program.

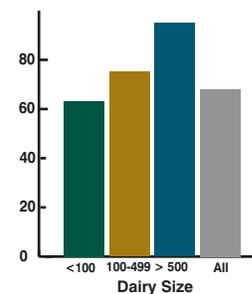
■ In 2007, according to USDA, MAP was isolated from at least one environmental sample on 68.1 percent of operations, and prevalence increased as herd size increased. About one-fourth of operations reported six culture-positive environmental samples, and dairies with only one to five culture-

positive samples were less common. These results suggest that at least 25 percent of U.S. dairies may have a relatively high percentage of infected cows in their herds.

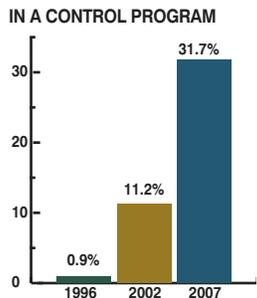
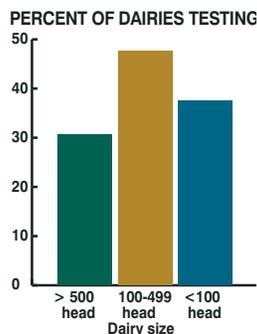
A cross-sectional observational study of 15 Colorado dairies reported in 2004 found 11 had serological positives. In-herd prevalence ranged from 0 percent to 7.82 percent, with a mean of 2.6 percent. Large herd size and buying replacements increased risk of seropositivity.



AT LEAST ONE POSITIVE\*



\* Based on environmental sampling.



## FOOD-CHAIN PRESSURE GROWING?

In August, the American Academy of Microbiology released its 41-page summary of a research colloquium assessing the potential involvement of MAP in human Crohn's disease. MAP, which can survive conventional milk pasteurization, poses "a plausible but uncertain risk to human health," according to the report.



Though it stopped short of officially invoking the Precautionary Principle to mandate agricultural control of the pathogen, the colloquium participants did call for the possible connection between Crohn's and MAP to be explored "exhaustively," and that minimal steps, like removing clinically diseased animals from the food chain, be instituted immediately. To read the entire report, go to [www.dlab.colostate.edu/webdocs/general/2008Fallwinter.pdf](http://www.dlab.colostate.edu/webdocs/general/2008Fallwinter.pdf), and click on this story.

## SUBMISSION GUIDELINES FOR SUCCESS

### Johne's Disease Testing

- Pooled fecal culture  
Submission tips  
Fee
- Tissue culture  
Submission tips  
Fee
- DNA probe  
Submission tips  
Fee
- Histological exam  
Submission tips  
Fee
- ELISA  
Submission tips  
Fee

- Questions about test selection or applying results in a herd-management plan?

Contact CSU VDL's new case coordinator John Maulsby at [John.Maulsby@colostate.edu](mailto:John.Maulsby@colostate.edu)

CSU VDL Educational Outreach

# Targeted Treatment: A Fresh Approach to Strongyle Control

About 40 years ago, a novel approach to equine parasite control was introduced. Called the interval dose system, it advised owners to treat horses every six to eight weeks to prevent maturation of *Strongylus vulgaris* and prevent pasture contamination. As part of the movement towards strategic parasite control, this approach addressed not only treating parasites within the animal, but also preventing future infections and disease. Widely accepted, this approach is credited towards the dramatic reduction in equine colics associated with *S. vulgaris*. By the 1980s, *S. vulgaris* was becoming relatively uncommon, and small strongyles (cyathostomes) had begun to account for the majority of strongyle egg output in grazing horses.<sup>1,2</sup>



EQUINE

aggressive treatment became routine. Unfortunately, the efficacy of the program in controlling *S. vulgaris*, combined with the availability of safe and inexpensive anthelmintics, led many owners to adopt a zero tolerance towards the presence of any strongyle egg. Over the years, the focus shifted from control to total eradication. The goal became to keep fecal egg counts (FEC) at or as near zero as possible, and rote deworming by the calendar became so ingrained in many management schemes that a lot of owners absolutely refused to consider skipping a scheduled treatment—needed or not.

## AGGRESSIVE TREATMENT BECAME ROUTINE

At the time the interval dose system was introduced, cyathostomes were considered to be of minor consequence, especially when compared to the highly pathogenic *S. vulgaris*. However, as *S. vulgaris*-induced colics decreased, the pathogenic effects of cyathostomes became evident. Today, they are considered to be the primary parasitic pathogen of horses.

Unfortunately, the suppressive use of anthelmintics has contributed significantly to the increased prevalence of anthelmintic-resistant cyathostomes around the world. Resistance to benzimidazoles is widespread, and resistance to pyrantel salts (tetrahydropyrimidines) is becoming more common.<sup>2,3</sup> Although cyathostome populations resistant to the macrocyclic lactones have yet to be identified, resistant *Parascaris equorum* populations have emerged.<sup>4</sup> U.S. studies suggest 40 percent of farms studied harbored anthelmintic resistant-cyathostome populations.<sup>2</sup>

Unfortun-

—Lora Ballweber, Associate Professor CSUVDL, Parasitology Section Head

## TIME TO ADJUST OUR THINKING

What does this mean in the practical sense? First and foremost, we must realize that one size does not fit all. Programs must be developed for each individual farm and monitored to evaluate effectiveness. We must break the decades-old habit of mechanically deworming by the calendar and realize that the goal is not eradication, but control.

As counterintuitive as the concept may seem, in order to select against resistance, we have to keep the susceptible parasites in the population to dilute out the resistant ones. Adopting that approach to resistance management will require major adjustments to our thinking. In order to achieve our goal, it means not every horse is dewormed every time

## TACTICAL DEWORMING

It's called tactical or selective deworming. To identify those horses that need treatment vs. those that do not, monitoring with routine FEC, reported in eggs per gram, is necessary. Before any program is put into place, it is imperative to test all anthelmintics used on a farm to determine whether they still work.

At present, the only way to do that is perform a Fecal Egg Count Reduction Test. This is a relatively easy procedure to perform using the steps outlined in the sidebar at left. Then, determine the average percent reduction for all horses treated with the same compound based on the effectiveness targets at left.

It is best if all horses are examined initially. This gives the combined benefit of determining whether any individual horse may be harboring resistant strongyles as well as provides a starting point for the identification of those horses that consistently shed higher numbers of eggs. However, if the entire herd cannot be tested, then eight to 12 horses should be examined. Fewer animals can be used, but confidence in the results diminishes as numbers decrease.

## TARGETING TREATMENTS

Once we know which anthelmintics are working, how do we determine who to treat? Again, FECs are the answer. There is no absolute cutoff in FEC that triggers whether to treat or not to treat. As with all diagnostic tools, FEC must be interpreted in light of the animal's

## SUBMISSION GUIDELINES FOR SUCCESS

### Quantitative fecal float

Submit X grams of fresh feces

Fee:

## FECAL EGG COUNT REDUCTION TEST

1. Collect a fresh fecal sample from each animal at the time of treatment and then again 10 to 14 days after treatment.
2. Perform a quantitative flotation procedure on both samples.
3. Calculate the the individual percentage reduction according to this formula:

$$\% = \left[ \frac{\text{pre-treatment FEC} - \text{post-treatment FEC}}{\text{pretreatment FEC}} \right] \times 100$$

## RESISTANCE TARGETS

For benzimidazoles and tetrahydropyrimidines:

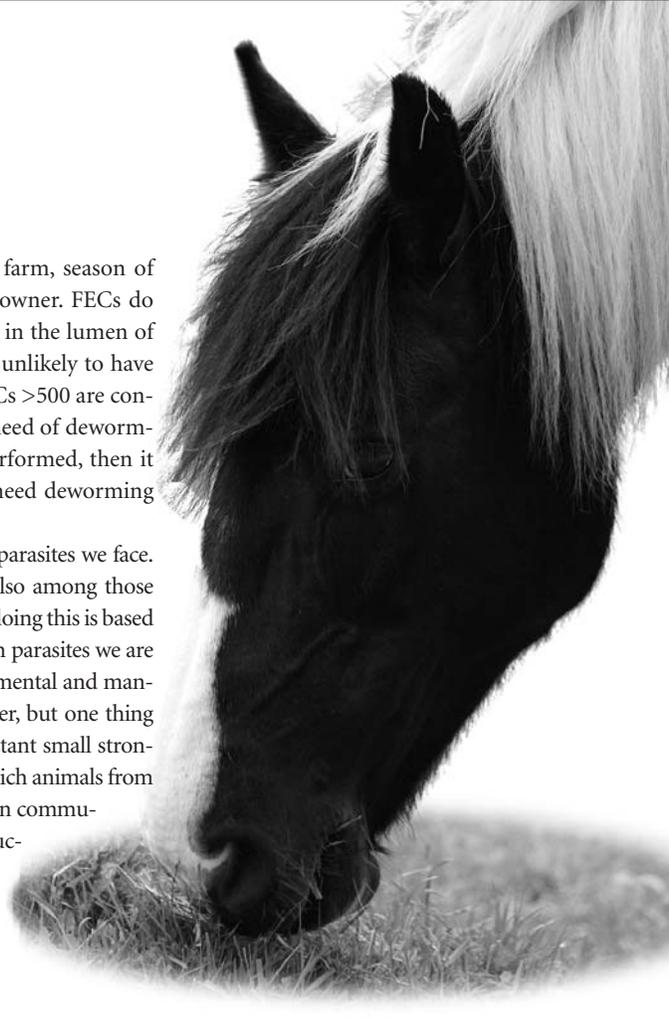
- >90% reduction = still effective
- 80-90% reduction = suspect resistance
- <80% reduction = resistance is present; compound is ineffective

For the macrocyclic lactones:

- Any reduction <98% = cause for concern.

overall health, management practices of the farm, season of the year, stocking rate, and tolerance of the owner. FECs do not directly correlate to numbers of parasites in the lumen of the gut; however, horses with FECs <200 are unlikely to have ill effects from the infection.<sup>2</sup> Horses with FECs >500 are considered to have high counts and are likely in need of deworming. If farm-wide individual FECs are not performed, then it is generally agreed that most horses do not need deworming until the mean herd FEC is 100 to 300.<sup>1</sup>

Of course, small strongyles are not the only parasites we face. *Parascaris equorum*, tapeworms and bots are also among those parasites we work to control. How we go about doing this is based on a number of considerations, including which parasites we are trying to control at the time, as well as environmental and management considerations. There is no easy answer, but one thing is clear: The transmission and dispersal of resistant small strongyles is virtually assured given an industry in which animals from widely separate areas are mixed together, often in communal grazing situations. If we are going to be successful in our battle to minimize the impact of these parasites, we must be willing to re-examine what we are doing and recommend changes where appropriate.



## CSUVDL In Press

# Gut Inflammation to Predict DI Johne's

**D**isseminated infection (DI) of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in cattle may impair cow health, potentiate spread of disease, and is a potential food-safety risk. The objectives of this study were to determine the association between severity of histologic enteric lesions and the occurrence of DI, clinical signs, and positive fecal culture and serum ELISA results.

Bacteriologic fecal culture and serum ELISA were performed on 40 dairy cows from MAP-infected herds. Cows were classified as having DI if MAP was isolated from any of 11 extra-intestinal tissues collected post-mortem. A grade of 0 to 3, corresponding to the severity of histologically evident granulomatous inflammation was determined for sections of ileum, jejunum, mesenteric lymph node, and ileocolic lymph node. An overall intestinal inflammation (OII) grade of 0 to 3 was assigned to each cow. The proportion of cows with DI increased with tissue-specific lesion grade and OII

—Dennis MM,<sup>1</sup> Antognoli MC, Garry FB, Hirst HL, Lombard JE, Gould DH,<sup>2</sup> Salman MD; College of Veterinary Medicine and Biomedical Sciences, Colorado State University

<sup>1</sup> CSUVDL Intern; <sup>2</sup> Professor Emeritus CSUVDL

grade. All cows with grade 3 inflammation in any single tissue had DI, however, some cows with DI had grade 1 inflammation or no lesions.

In general, there was a positive association between OII grade and clinical signs, gross enteric lesions, and positive ELISA and fecal culture results. However, 12 percent of OII grade 0 cows had clinical signs (explained by other conditions recognized with necropsy), and the proportion of positive ELISA results was lower for OII grade 3 cows relative to grade 2 cows.

Although MAP dissemination may occur early in the disease process, histopathology of intestinal tissue may be used to detect a substantial proportion of DI cows.

## SOURCES

- 1 Kaplan RM. Anthelmintic resistance in nematodes of horses. *Vet Res.* 2002 Sep-Oct;33(5):491-507.
- 2 Kaplan RM, Klei TR, Lyons ET, et al. Prevalence of anthelmintic resistant cyathostomes on horse farms. *J Am Vet Med Assoc.* 2004 Sep 15;225(6):903-10.
- 3 Brazik EL, Luquire JT, Little D. Pyrantel pamoate resistance in horses receiving daily administration of pyrantel tartrate. *J Am Vet Med Assoc.* 2006 Jan 1;228(1):101-3.
- 4 Slocombe JOD, de Gannes RVG, Lake MC. Macrocytic lactone-resistant *Parascaris equorum* on stud farms in Canada and effectiveness of fenbendazole and pyrantel pamoate. *Vet Parasitol.* 2007 Apr 30;145(3-4):371-6.

## CONCLUSIONS

**In general, a positive association existed between overall intestinal inflammation and clinical signs, gross enteric lesions, and positive ELISA and fecal culture results.**

**Histopathology of intestinal tissues may be used to detect a substantial proportion of Johne's disseminated infection cows.**

Dennis MM, Antognoli MC, Garry FB, et al. Association of severity of enteric granulomatous inflammation with disseminated *Mycobacterium avium* subspecies *paratuberculosis* infection and antemortem test results for paratuberculosis in dairy cows. *Vet Microbiol.* 2008 Sep 18;131(1-2):154-63.

# MRSA in Small Animals

The Colorado State University Veterinary Diagnostic Laboratories have been screening *Staphylococcus aureus* for resistance to methicillin for the past few years. Historically, methicillin resistant *Staphylococcus aureus* (MRSA) are resistant to all beta-lactam antimicrobials, including the cephalosporins and carbapenems. Isolates found in the hospital environment generally are multiply resistant to other antimicrobials.

Because of the zoonotic potential of these organisms and the increasing reports of MRSA transmission from owners to animals, this cumulative profile was generated of the *Staph. aureus* isolated from canine and

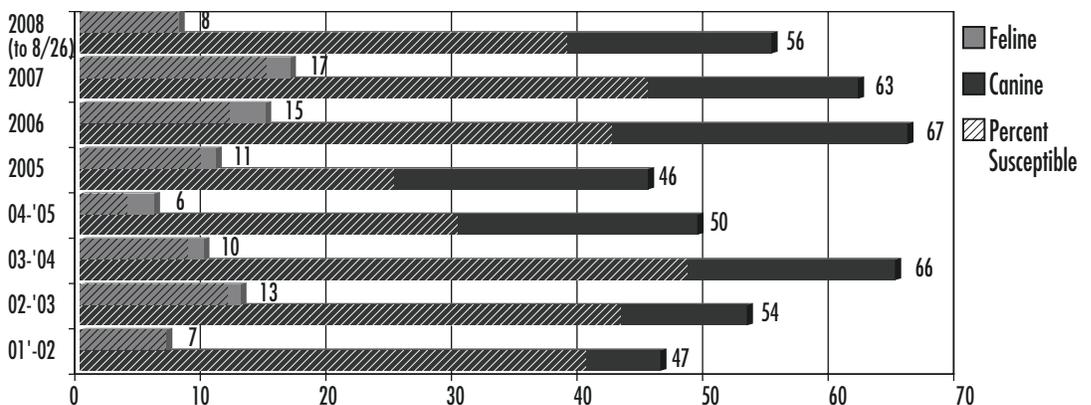
– Doreen Hyatt, Associate Professor CSUVDL, Bacteriology Section Head

feline samples. Also included is the percent of those isolates susceptible to oxacillin, the screening drug for methicillin resistance.

## SUBMISSION GUIDELINES FOR SUCCESS

- Submit swab from lesion or tissue
- Aerobic culture: \$16
- Antibiotic susceptibility test: \$12.00

Staphylococcus aureus isolated from animal samples at the CSU VDL over multiple years.



## WHAT SHOULD I DO?

If you suspect MRSA in small-animal samples, remember:

- Although not all animals colonized with MRSA will have disease, according to Weese (2005): “Among clinically infected animals, postoperative and wound infections are most commonly reported, with lesser numbers of intravenous catheter site infections, urinary tract infections, pneumonia, and skin infections.”
- Collect a sample from the lesion with a swab and submit for aerobic culture and sensitivity.
- Make sure to note your MRSA suspicion in the form’s history area or on the “other” line.

If you check your sensitivity results on the Web, make sure to check the oxacillin results; it is the screening drug for methicillin resistance.

If results show a *Staph. aureus* resistant to oxacillin, the sample contains a methicillin resistant *Staphylococcus aureus* (MRSA).

CSU VDL is developing an MRSA test number to use if you are only interested in whether or not the sample has MRSA. Watch future issues of *LabLines* for details.

## FOR MORE INFORMATION

- 1 Boost MV, O’donoghue Mm, Siu Kh. Characterization of Methicillin-resistant *Staphylococcus Aureus* Isolates From Dogs And Their Owners. *Clin Microbiol Infect.* 2007 Jul;13(7):731-3.
- 2 Kania Et Al., 2004 S.a. Kania, N.I. Williamson, L.a. Frank, R.p. Wilkes, R.d. Jones And D.a. Bemis, Methicillin Resistance Of *Staphylococci* Isolated From The Skin Of Dogs With Pyoderma, *Am Jnl Vet Res.* 65 (2004), Pp. 1265-1268.
- 3 Lee, 2003 J.H. Lee, Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans, *Applied and Environmental Microbiology* 69 (2003), pp. 6489–6494.
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animals: a review. *Vet J.* 2008 Jan;175(1):27-36. Epub 2007 Jan 9.

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- 7 Waller A. The creation of a new monster: MRSA and MRSI-important emerging veterinary and zoonotic diseases. *Vet J.* 2005 May;169(3):315-6.
- 8 Walther B, Wieler LH, Friedrich AW, Hanssen AM, Kohn B, Brunnberg L, Lübke-Becker A. Methicillin-resistant

*Staphylococcus aureus* (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations. *Vet Microbiol.* 2008 Feb 5;127(1-2):171-8.

- 9 Weese JS, Faires M, Rousseau J, Bersenas AM, Mathews KA. Cluster of methicillin-resistant *Staphylococcus aureus* colonization in a small animal intensive care unit. *J Am Vet Med Assoc.* 2007 Nov 1;231(9):1361-4.
- 10 Weese JS, Rousseau J, Traub-Dargatz JL, Willey BM, McGeer AJ, Low DE. Community-associated methicillin-resistant *Staphylococcus aureus* in horses and humans who work with horses. *J Am Vet Med Assoc.* 2005 Feb 15;226(4):580-3.
- 11 Weese JS. Methicillin-resistant *Staphylococcus aureus*: an emerging pathogen in small animals. *J Am Anim Hosp Assoc.* 2005 May-Jun;41(3):150-7.

Diagnostic sample quality assurance

# New Regulations for Shipping Diagnostic Specimens

Over the past few years, regulations for the transportation of Class 6.2 Dangerous Goods have changed. All packages containing diagnostic animal specimens that are transported by air should be labeled appropriately. While many diagnostic samples

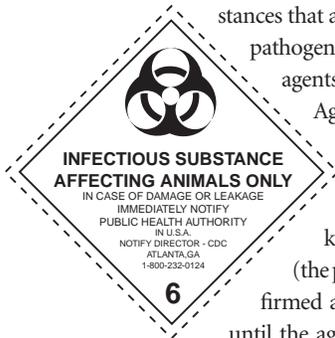
## LAB SERVICES

are considered part of this class, some of the samples sent from veterinary hospitals to the CSU VDL can be labeled as exempt. There are specific requirements for labeling, including size, wording and location of the marking.

All packages containing diagnostic animal specimens should be marked with one of the following designations:

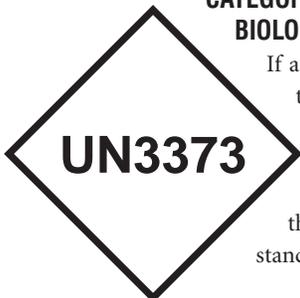
### CATEGORY A: INFECTIOUS SUBSTANCES

Category A designation can be further divided into those Affecting Animals Only (UN 2900) or Infectious Substances Affecting Humans (UN 2814). These are substances that are expected to contain highly pathogenic infectious agents, such as agents from the USDA/CDC Select Agent lists. Most diagnostic samples do not need to be shipped under this designation, as they are not known to contain these agents (the presence of the agent is unconfirmed at this time - it is not known until the agent is detected by diagnostic testing at the CSU Veterinary Diagnostic Laboratory). If the sample is highly suspected to contain a Category A agent, it should be shipped under this designation. Further training should be completed by shippers to fully understand the regulations for shipping these substances.



agents from the USDA/CDC Select Agent lists. Most diagnostic samples do not need to be shipped under this designation, as they are not known to contain these agents (the presence of the agent is unconfirmed at this time - it is not known until the agent is detected by diagnostic testing at the CSU Veterinary Diagnostic Laboratory). If the sample is highly suspected to contain a Category A agent, it should be shipped under this designation. Further training should be completed by shippers to fully understand the regulations for shipping these substances.

### CATEGORY B: BIOLOGICAL SUBSTANCE



**BIOLOGICAL SUBSTANCE  
CATEGORY B**

If a specimen is expected to contain a pathogen, the package should be labeled with a Category B UN3373 diamond-on-point mark, with the wording "Biological Substance, Category B." Minimum dimensions for this marking are 50 millimeters by 50 millimeters.

— *Kristy Pablonia, CSUVDL Assistant Professor and Avian Diagnostics Section Head, and Christina Gerhard, CSUVDL Microbiologist*

Examples of specimens that should be sent with this mark include, but are not limited to:

- Fresh tissues or swab samples sent for PCR or virus isolation.
- Fresh tissues or swab samples sent for bacterial culture.
- Fecal samples expected to contain a pathogen.
- Culture plates of organisms sent for further testing.

### EXEMPT ANIMAL SPECIMEN

When there is minimal likelihood that the sample contains a pathogen, the package may be marked as "Exempt Animal Specimen".

## EXEMPT ANIMAL SPECIMEN

Examples of specimens that may be sent with this mark include, but are not limited to:

- Serum sent for antibody testing.
- Tissues sent in 10 percent formalin. (Use of higher than 10 percent formalin requires further marking using a UN 3334 designation.)
- Samples to be tested for therapeutic drug monitoring or toxins.
- Environmental samples not expected to contain a pathogen.
- Dried blood spots placed on absorbent filter paper.

All markings should be placed on the outside of the box in a prominent location. UN3373 - Biological Substance, Category B label stickers and other package labels can be purchased online from [www.saftpak.com](http://www.saftpak.com), [www.casingcorp.com](http://www.casingcorp.com) or [www.uline.com](http://www.uline.com).

Please Note: It is the shipper's responsibility to insure that the package complies with all current regulations. Regulations change frequently, and shippers may be fined for violations of the transport regulations. Average fines exceed \$1,200 for basic infractions.



### NEED MORE?

For training materials, including manuals, CDs and pamphlets, we recommend:

- [www.saftpak.com](http://www.saftpak.com)
- [www.iata.org](http://www.iata.org).

**Any staff responsible for shipping dangerous goods should be trained by an approved training program. Training documentation should be kept on file with the employer.**

## CONCLUSIONS

**Results of histologic examination of hepatic biopsy samples obtained at the time of surgery cannot be used to predict long-term outcome in dogs undergoing surgical correction of a congenital portosystemic shunt .**

Parker JS, Monnet E, Powers BE, Twedt DC. Histologic examination of hepatic biopsy samples as a prognostic indicator in dogs undergoing surgical correction of congenital portosystemic shunts: 64 cases (1997-2005). *J Am Vet Med Assoc.* 2008 May 15;232(10):1511-4.

## SUBMISSION GUIDELINES FOR SUCCESS

- Submit either serum (royal blue-topped tube) or plasma (green-topped tube) for zinc analysis \$10 per sample
- Remove the serum or plasma from the red cells as soon as is feasible
- Avoid hemolysis if at all possible.

## CSUVDL In Press

## Can Histology Predict Longterm PSS Outcome?

This study attempted to determine whether results of histologic examination of hepatic biopsy samples could be used as an indicator of survival time in dogs that underwent surgical correction of a congenital portosystemic shunt (PSS).

It involved 64 dogs that underwent exploratory laparotomy for an extrahepatic (n=39) or intrahepatic



(n= 25) congenital PSS.

All H&E-stained histologic slides of hepatic biopsy samples obtained at the time of surgery were reviewed. The severity of histologic abnormalities—arteriolar hyperplasia, biliary hyperplasia, fibrosis, cell swelling, lipodosis, lymphoplasmacytic cholangiohepatitis, suppurative cholangiohepatitis, lipid granulomas, and dilated sinusoids—was graded. A Cox proportional hazards regression model was used to determine whether each histologic feature was associated with survival time.

The median follow-up time was 35.7 months, and

—Parker JS, Monnet E, Powers BE,<sup>1</sup> Twedt DC  
1 CSUVDL Director

median survival time was 50.6 months. Thirty-eight dogs were alive at the time of final follow-up; 15 had died of causes associated with the PSS, including four that died immediately after surgery; three had died of unrelated causes; and eight were lost to follow-up. None of the histologic features examined were significantly associated with survival time, suggesting hepatic biopsy samples obtained at the time of surgery cannot be used to predict long-term outcome in dogs undergoing surgical PSS correction.



## Chemistry and Toxicology

## Zinc Toxicity in Dogs

We recently received two canine serum samples (pre-treatment and post-treatment) for zinc analysis. An 11 month old Pomeranian was presented to the referring veterinarian with a history of vomiting and inappetence for three days. Radiographs revealed coins in the stomach. Hematuria had developed. The dog had a pre-treatment PCV of 16 percent, BUN of 78 mg/dL and creatinine of 1.2 mg/dL. During anesthesia, a quarter unit of packed red blood cells with a PCV of about 70 percent were administered. Two pennies were removed from the stomach endoscopically. Post-transfusion, the PCV was 26 percent. The dog was treated with IV fluids, famotidine and calcium-EDTA. Three days post-surgery, the PCV had stabilized, BUN decreased to 18 mg/dL and creatinine decreased to 0.8 mg/dL. One week post, vomiting and diarrhea ceased and the dog exhibited improved energy and appetite.

The pre-treatment serum sample contained 25.0 ppm zinc; post-treatment, 2.36 ppm. Normal canine concentration ranges from 0.70 to 2.00 ppm. These results demonstrate how quickly serum zinc concentration decreases once the source is removed and chelation begins.

—Dwayne Hamar, CSUVDL Chemistry/Toxicology Section Head, and Cathy Bedwell, CSUVDL Chemist

**Zinc is a nutritionally essential trace element; however ingestion of diets containing a high concentration (> 2,000 ppm) of**



**zinc or non-dietary items may lead to zinc toxicosis. Commonly ingested sources include galvanized metal screws, bird cages, nuts and bolts; batteries; and pennies minted since 1983. Stomach acid readily dissolves the zinc from these items, causing anorexia and vomiting, progressing to shock and seizures in more severe cases. Supportive care and removal of the zinc source are indicated.**

CSUVDL In Press

## A Rare Variant of Exocrine Canine Pancreatic Carcinoma

Exocrine pancreatic carcinoma is a particularly malignant neoplasm of the dog. This study evaluated clinical and pathologic findings of an unusual variant of exocrine pancreatic neoplasia termed “hyalinizing pancreatic adenocarcinoma” in six dogs. On microscopic examination, neoplasms were composed of tubules and acini of epithelial cells, with bright eosinophilic granular apical cytoplasm. Tubular lumina and tumor stroma contained abundant hyaline material that resembled amyloid. The hyaline material was not congophilic, and tumor cells and hyaline material were immunohistochemically negative for amyloid A, immunoglobulin light chains (kappa and lambda), amylin (islet amyloid polypeptide), laminin, and  $\alpha$ 1-antitrypsin.

Two patients survived longer than 15 months after diagnosis; one of these dogs was untreated and had

—Dennis MM,<sup>1</sup> O'Brien TD, Wayne T, Kiupel M, Williams M and Powers BE<sup>2</sup>  
1 CSUVDL Resident; 2 CSUVDL Director

grossly evident metastasis at the time of diagnosis. The deaths of the other four dogs occurred as a result of poor recovery after partial pancreatectomy in association with other concurrent life-threatening conditions. Two dogs were diagnosed with panniculitis, a condition rarely associated with pancreatic disease. Further evaluation is needed to determine the composition and biologic significance of intratumor hyaline material. Studies that associate exocrine pancreatic carcinoma grade and histologic subtype with outcomes are warranted such that appropriate therapy may be elected.

### CONCLUSIONS

Dogs with this rare neoplasm may have a longer postdiagnosis survival period than for other exocrine pancreatic carcinomas, especially when other conditions with poor prognoses are lacking. Further research is needed to delineate associations between histology, grades of pancreatic cancer and outcomes.

Dennis MM, O'Brien TD, Wayne T, Kiupel M, Williams M, Powers BE. Hyalinizing pancreatic adenocarcinoma in six dogs. *Vet Pathol.* 2008 Jul;45(4):475-83.

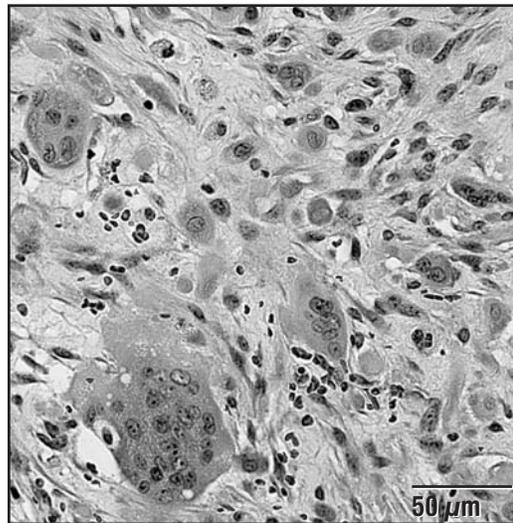
CSUVDL In Press

## Equine Giant Cell Tumors

We document 21 cases of equine giant cell tumors of soft parts clinically examined within Colorado from 2000 to 2007. The majority of cases occurred in males aged ten years or older. Nine (43 percent) arose within the hindlimbs. Key histologic features included numerous multinucleated giant cells and hemosiderin-laden macrophages admixed with a spindle-cell proliferation. The majority demonstrated liposarcomatous change, variable areas of necrosis and hemorrhage, and an intermediate number of mitotic figures. Immunohistochemical results demonstrated two distinct cell populations—vimentin-expressing neoplastic mesenchymal cells and CD18 (histiocytic marker) expressing multinucleated giant cells.

These results suggest a mesenchymal origin with possible recruitment of the secondary histiocytic population. Surgical excision was attempted in the majority and considered clinically complete. The tumors recurred in one horse and one mule. In 18, surgical excision, regardless of margin integrity, appeared successful with no documented recurrence. Unfortunately, 10 of 21 horses were lost to follow-up within three months. Of the 11 remaining available for follow-up, there has been no evidence of metastasis. A larger case series with more controlled follow-up is necessary to evaluate malignant potential and the importance of complete excision.

—Bush JM<sup>1</sup> and Powers BE<sup>2</sup>  
1 CSUVDL Resident; 2 CSUVDL Director



Histologic exam results showed characteristic sheets of swirling neoplastic spindle cells with marked liposarcomatous change, interspersed with numerous multinucleated giant cells, often containing 40 or more nuclei, plus hemosiderin-laden macrophages.

### CONCLUSIONS

Twenty one cases of equine giant-cell tumors of soft parts studied from 2000 to 2007 suggest a mesenchymal origin of this neoplasm with possible recruitment of the secondary histiocytic population. In 18 horses, surgical excision, regardless of margin integrity, appeared successful. Of 11 horses available for follow-up no evidence of metastasis has emerged.

Bush JM, Powers BE. Equine giant cell tumor of soft parts: a series of 21 cases (2000-2007). *J Vet Diagn Invest.* 2008 Jul;20(4):513-6.



### FORMER STATE VET IS NEW CASE COORDINATOR

John Maulsby joins CSU VDL as Case Coordinator, to help communicate with practitioners after lab results are obtained.

A native Coloradan, Dr. Maulsby spent 13 years in mixed practice following graduation from CSU in 1975, practicing in Boulder, Gunnison and Greeley. During this time he gained a great respect for the livestock producers of Colorado and their invaluable contribution. In 1988, he went to work for the Colorado State Veterinarian's Office as a field veterinarian, involved in animal welfare cases as well as disease control. As an assistant state veterinarian for six years, he oversaw the Bureau of Animal Protection and the Pet Animal Care Program. In August 2006, he became the State Veterinarian for two years until he retired in August 2008.

As a former practitioner, Dr. Maulsby recognizes the importance of accurate, timely results reporting to the practitioner.

"The commitment to obtain accurate results and the outstanding work ethic Dr. Powers has instilled at the CSU Diagnostic Lab is a valuable asset to Colorado's Livestock industry," he says. "I look forward to helping Colorado veterinarians and livestock producers remain profitable, stay in business and produce the safest food supply possible."

## Get to Know the Laboratory

# New Members Join the Lab Team

**Laurie Baeten** graduated from University of Wisconsin-Madison School of Veterinary Medicine in '93. After a few years in private practice, she took a position with the USGS National Wildlife Health Center, working on various wildlife disease projects. She moved to Colorado in 2001 and worked for the CSU Laboratory Animal Resources for a year before taking a position with the Colorado Division of Wildlife. After five years of wildlife disease surveillance and mortality investigation, she has come to CSU VDL for the microbiology residency.



**Brendan Podell**, a first-year anatomic pathology resident, started the program in June 2008. He received a DVM degree from CSU, during which he worked as a molecular biologist in the VDL. Brendan is interested in molecular and surgical pathology and plans to pursue a doctorate degree in infectious disease or cancer research. During his spare time, Brendan likes to bike, hike and ski.

**Laura Brandt** graduated from East Tennessee State in 2002 with a bachelor's degree in English and Spanish, and then earned a doctorate of veterinary medicine from Tennessee. Laura then did a rotating small animal internship at a private practice outside Chicago and decided to stay on for another year as a staff doctor in the Emergency Department. Now a first year resident in clinical pathology at CSU, Laura says she is happier than she has been in years. It's a good fit, and she loves the program and living in Colorado. She enjoys riding her bike to work, reading, hiking, travel, spending time with friends and studying with her two cats, Weasel and Tres Bien.

**Elizabeth Magden** is the newest laboratory animal resident. She graduated from veterinary school at CSU in May 2008 after receiving her undergraduate degree from Iowa's Grinnell College. She grew up in various small towns around Iowa and lived briefly in Montana. Prior to veterinary school, Liz worked at the Primate Foundation of Arizona in Mesa in a facility that housed 75 chimpanzees. Her current interests and hobbies include family bike rides with her husband—towing their 6-month-old son—hikes, and a coveted movie night as time allows. She says she enjoys her new residency position, and to stop by and say "hi" if you are in the Painter Building!

New Associates (clockwise, from left): Laurie Baeten, Brendan Podell, Laura Brandt, Liz Magden, Gopi Palanisamy, Erik Thamm and Deanna Dailey

**Gopinath Palanisamy** graduated from India's Tamilnadu Veterinary and Animal Sciences University in 2002. Within a few days after graduation, he moved to Champaign, Ill., to obtain a master's degree in reproductive biology from Illinois. Gopi then changed track and has been pursuing a doctorate in tuberculosis immunopathology for the past four years. He started the residency program at CSU in anatomic pathology this summer. He says he loves the things Colorado has to offer. He enjoys skiing, playing basketball, free-running, tubing and bowling, among other activities, when he finds free time.

**Erik Thamm**, new laboratory assistant in the Necropsy Section, grew up in Nashville and graduated from Tennessee with a bachelor's degree in wildlife and fisheries sciences. He moved to Colorado in spring 2003 and worked for the state Division of Wildlife for five seasons, where he became familiar with CSU necropsy. Eric is married to Melissa and has a 3-year-old son named Kolby. He says he enjoys hiking, biking, running, and basically anything else outside—as well as a good beer.

**Deanna Dailey** received her DVM from CSU in 2006. She practiced in a small animal hospital in Las Vegas for two years before returning to CSU to start her anatomic pathology residency. Her research interests are epidemiology and wildlife disease. She is originally from Baltimore, but now calls Colorado home, along with husband Chad and dog Josie. Deanna is an avid rock climber and also enjoys camping, running, yoga and relaxing in the backyard with her family.

## Population Medicine

# Pooled BVD and Trich Testing Strategies for Beef Cattle

Pooled sample strategies to screen populations have been practiced for years—in human medicine to diagnose syphilis and HIV, and in veterinary medicine to detect Bovine Viral Diarrhea Virus, *Trichostrongylus axei*, Porcine Reproductive and Respiratory Syndrome virus, *Mycobacterium paratuberculosis* subspecies *avium* and *Salmonella enteritidis* contamination.

The high sensitivity and specificity of polymerase chain reaction (PCR) make it an appealing tool to detect the presence of any etiological agent capable of providing nucleic acid for the PCR reaction, including protozoa, viruses, and bacteria. Sample pooling takes advantage of the high sensitivity and specificity of expensive diagnostic tests while minimizing diagnostic costs, especially when the etiological agent is still viable within the population via a carrier or persistently infected animal.

## Guardians of Public Health

## Passive Wildlife Surveillance?

Wild animals can play an important role in the epidemiology of infectious disease, with significant public health, economic and ecological consequences. As it is often challenging to conduct unbiased surveillance in free-ranging mammal populations, passive, opportunistic case identification has been widely used for detection of disease events in wild animals.

A study was performed to evaluate the role of different agencies and organizations in the Rocky Mountain Region to identify significant wildlife health events or aggregate information from multiple sources. Overall, wildlife rehabilitators were in contact with the greatest number of animals; however, the data from these groups, in its current state, are insufficient for surveillance purposes. Wild animal data from all survey groups aggregated at the level of state wildlife organizations; these agencies are, therefore, central in this type of surveillance activity and require sufficient resources to ensure that appropriate testing is conducted.

Duncan C,<sup>1</sup> Backus L, Lynn T, Powers B,<sup>2</sup> Salman M. *Passive, opportunistic wildlife disease surveillance in the Rocky Mountain Region, USA.* Transbound Emerg Dis. 2008 Sep;55(7):308-14. 1 CSUVDL Resident; 2 CSUVDL Director

—Jim Kennedy, DVM, Director, CSUVDL Rocky Ford

Rocky Ford has implemented two applications of a pooled testing strategy—one for BVDV and one for *T. foetus*. Results for BVDV testing identified 205 positive pools out of a total of 4,039 pools tested between July 2004 and July 2008. The 4,039 pools were composed of 265,388 animals; individual tests of the positive pools by antigen capture ELISA identified 513 positive individuals, yielding an apparent prevalence of 0.19 percent.

Pooled testing for *T. foetus* has also shown considerable promise when placed in pools of five or less. A total of 2,370 pools have been tested, with 103 pools identified between April 2007 and July 2008. The 2,370 pools represent 8,567 bulls. From 103 positive pools, 143 individual PCR positives were identified.



**A study led by CSUVDL Resident Colleen Duncan tested tissue from 2,934 harvested mule deer, 2,516 elk, 141 white-tailed deer, and six moose during the 2005-2006 hunting season. The results showed only a single adult male mule deer had BVDV antigen in the skin. Staining distribution was consistent with that seen in PI cattle. Although natural PI BVDV infection exists in Colorado cervids, its prevalence is very low.**

Duncan C,<sup>1</sup> Van Campen H,<sup>2</sup> Soto S,<sup>3</sup> LeVan IK, Baeten LA, Miller MW. *Persistent Bovine viral diarrhoea virus infection in wild cervids of Colorado.* J Vet Diagn Invest. 2008 Sep;20(5):650-3. 1 CSUVDL Resident; 2 CSUVDL Virology Section Head; 3 CSUVDL Resident

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## Colorado Bacteriology Update

## Lepto Testing Update

As an update of information given for the past few years, the table at right gives the number of serum samples tested for titers to *Leptospira interrogans* between Jan. 1 and June 30, 2008, and the entire years for 2003 through 2007 by animal species.

Lepto serology results are listed for each of the five serotypes for serological samples submitted between Jan. 1 and June 30, 2008, and all of 2004 through 2007 for all animal species. The table lists 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. Also included are the highest titer reported during the year (High).

In addition, 97 tests for *L. bratavla* were conducted in 2007, and 16 were positive with the highest titer being 1:3200. From Jan. 1 to June 30, 2008, a total of 44 tests were performed, with five positives with a high of 1:800.

—By Doreene R. Hyatt, Associate Professor, CSUVDL Bacteriology Section Head

## SUBMISSION GUIDELINES FOR SUCCESS

- ❑ *Leptospira* serology  
Submit X mL of serum  
\$XX per sample
- ❑ *Leptospira* PCR  
Submit fresh tissue or urine  
\$XX per sample

	2008*	2007	2006	2005	2004	2003
<b>Bovine</b>	143	202	103	338	231	361
<b>Camelid</b>	0	0	4	0	2	3
<b>Canine</b>	140	338	120	426	181	106
<b>Caprine</b>	13	14	17	12	2	2
<b>Equine</b>	17	33	13	38	34	23
<b>Ovine</b>	1	0	0	8	5	3
<b>Zoological</b>	71	1	1	3	5	7
<b>Porcine</b>	0	0	0	6	0	0
<b>Feline</b>	14	1	2	1	2	0

\* YTD as of July

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	33	800	102	3200	87	102,400
2005	858	152	1600	135	51,200	117	1600	165	3200	123	12,800
2006	264	51	800	34	6400	21	1600	26	800	38	6400
2007	597	90	1600	105	6400	42	3200	89	3200	98	6400
2008*	400	56	3200	73	6400	48	3200	77	6400	64	6400

\* YTD as of July

## Environmental Testing

## Water Quality Issues for Beef Cattle

Drought, heat and poor forage are once again raising concerns about beef cattle water intakes and quality. Tests from some areas indicate high levels of Total Dissolved Solids and sulfates, which can impact production. In most areas of Colorado, it is advisable to have water sources tested for TDS, sulfates and nitrates. A management program should also be implemented, including:

- Minimizing use of poor quality water, especially for confined cattle.
- Limiting use of marginal quality surface water to spring or early summer, before seasonal hot temperatures cause evaporation which concentrates sulfates.
- Developing alternative water sources where water quality is continually poor and unsuitable.
- Employing early weaning to decrease calf exposure to poor quality water and reduce cow water demands.
- Avoiding high-sulfur feeds like gluten and distillers grains in the face of high water sulfates.
- Consultation with a nutritionist to adjust rations.

—Roger W Ellis, CSU Extension Veterinarian

Veterinarians play an important role in investigating health problems related to water quality. CSU VDL personnel are available to assist you in water testing, diagnostic laboratory services and field investigations. Further information is also available through contact with CSU Beef Team extension specialists and at [www.CSUBeef.com](http://www.CSUBeef.com).



## HOW TO CONTACT US

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300 West Drake  
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Phone (970) 297-1281  
Fax (970) 297-0320  
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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

## Serology

## New and Better *B. ovis* ELISA

The Western Slope Veterinary Diagnostic Laboratory evaluation of the newly modified ELISA procedure for *Brucella ovis* serology has demonstrated it measures essentially the same *Brucella ovis* antibody in ovine sera as our current ELISA. As an added benefit, the new NVSL procedure reduces the effect of the nonspecific background material occasionally encountered, improving specificity. WSVDL began using the new procedure Nov. 1.

### VALIDATED AGAINST CURRENT PROTOCOL

To validate the new ELISA, we compared the new NVSL protocol to our current methodology utilizing five different sets of archived serum samples. The results from these procedures were compared using the statistical analyses known as correlation coefficient and paired t-test.

In all sets of sera the results from the new procedure strongly supported the hypothesis that the new procedure measures the same substance (*Brucella ovis* antibody) as our current procedure (high correlation coefficient and paired t-test results) with one notable exception. In a set of 36 sera previously determined to contain high background substance which interfered with our current ELISA procedure, the new NVSL procedure did not react with this high background substance. This was a very encouraging finding.

**Negative or noninfected flocks.** We compared the results from the different methods utilizing 268 sera, including many individual animals that were tested annually, from a flock that had repeatedly been negative for four consecutive years. This flock has never had any reported problems contributed to *Brucella ovis* infection. All 268 sera were negative on our current ELISA procedure and 265 were negative on the NVSL ELISA procedure. The 3 remaining sera were classified as low indeterminate or "IND" on the NVSL procedure.

**Positive or infected flock.** We next compared the effects of applying the cutoff ranges of these two different methods using 790 sera collected over multiple bleedings from a known infected flock during an intensive program to reduce and eliminate *Brucella ovis* infection. By our current procedure, 28 of the 790 sera were regarded as "Positive" or "Borderline." Using the NVSL procedure cutoff guidelines, all 28 of these animals would have been regarded as "Positive." An additional 16 test results from this flock previously regarded as "high negative SP ratios" would have become indeterminate under the NVSL

guidelines. Using the NVSL guidelines in this infected flock would likely have had a benefit in reducing the number of infected animals in a shorter time frame.

**2008 Sale Rams.** In a set of 183 sera from yearling virgin sale lambs received in 2008, applying the NVSL guidelines would not have identified any of these rams as positive, agreeing with our current methodology.

**High negative and borderline sera** and check test sera. In a set of 53 retained sera with high negative or borderline SP ratios and in a set of 96 sera from check tests, the new NVSL procedure resulted in SP ratios slightly lower than our current methodology, suggesting that the new NVSL procedure reduces nonspecific background reaction.

### SUMMARY

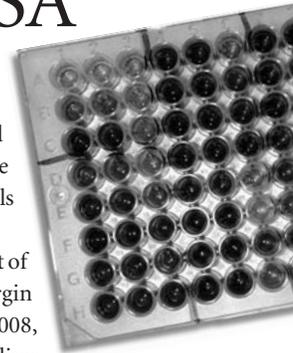
Using the new procedure and SP interpretation guidelines, all SP ratios previously regarded as borderline will now be regarded as positive. Animals with previous high negative values will now be regarded as indeterminate. In our experience, *Brucella ovis* infected animals with serology results in the indeterminate range will move to a positive status within four to six weeks and perhaps earlier. Non-infected animals with serology results in the indeterminate range will remain in this range or move to negative values within four to six weeks.

It is therefore our interpretation that any animal with an SP ratio regarded as positive has a high likelihood of being infected with *Brucella ovis* and that it should be removed from the flock.

Rams with SP ratios regarded as indeterminate should be separated from the flock and, if retained, retested four to six weeks later. Upon retesting, if these animals remain indeterminate or move to a negative status, they should be regarded as noninfected.

Culling any animal regarded as indeterminate may be useful in flock situations to control risk; however, in the case of valuable breeding animals retesting the animals four to six weeks later may establish a negative or noninfected status and allow these animals to be utilized with minimal risk.

We anticipate that, as in the past, a fraction (<1 percent) of noninfected yearling virgin rams may have test results regarded as indeterminate or positive. With the improved NVSL procedure the number of these animals regarded as positive should be drastically reduced.



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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 they are an integral part of the laboratory.

### SUBMISSION GUIDELINES FOR SUCCESS

- Submit X mL of serum  
\$XX per sample

**REGULAR COLUMNS**

- **PUBLIC HEALTH ISSUES** p. 4  
Testing for MRSA in small animal samples
- **VDL IN PRESS** p. 6-7  
Zinc toxicity in dogs, other recently published work
- **GET TO KNOW YOUR LAB** p. 8  
New residents join the CSU VDL team

**LAB LINES**

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The latest results on serum samples tested for Lepto titers through mid-2008
- WATER QUALITY** . . . . . p. 11  
Drought conditions and sub-standard forage create issues



**JOHNE'S TESTING** . . . . . p. 1  
How involving dairy producers in ongoing Johne's testing can make an immediate impact

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Why a fresh approach to equine strongyle control could control resistance spread

**SHIPPING REGS UPDATE** p. 5  
Guidelines to help avoid a costly shipping mistake

Nonprofit Organization  
US Postage  
**PAID**  
Fort Collins, Colorado 80523  
Permit Number 19

**Colorado State University**  
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# Update from the Director

**MISSED AN ISSUE OF LABLINES?**  
Read them all on-line, at [www.dlab.colostate.edu/webdocs/general/lablines.html](http://www.dlab.colostate.edu/webdocs/general/lablines.html)

Visit our website under 'News' for updated construction pictures of the new Diagnostic Medicine Center, scheduled for completion in mid-June.

At this time of year around the holidays, we should all remember the many things to be thankful for—family, friends and a healthy animal population. We at CSUVDL have an additional item to be thankful for—the nearing completion of our new Diagnostic Medicine Center, a plan and dream that has been in progress for over 10 years. The construction is well ahead of schedule with an expected move-in date of mid-June. Visit our website under “News” for updated pictures of the construction progress. We remain ever grateful for all the support we have had from many of you in getting this project funded and completed. We expect to have an open house ceremony at the end of June or early July.



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

Inside, we have many new updates of interest, including abstracts from a number of recent publications by our residents and faculty. On July 1 we welcomed our new residents, and on October 1 we welcomed our new case coordinator, John Maulsby, to our staff.

It was great seeing many of you at the recent meeting of the American Association of Laboratory Diagnosticians in North Carolina, at the annual Colorado Veterinary Medical Association meeting in Denver, and at the mid-winter Colorado Cattlemen's Association meeting in

Colorado Springs. An item of interest to all of you is the mid-October approval of the revision of USDA VS Memorandum 580.4. This document details the procedure for investigation of suspect foreign animal disease. The most important aspect of the revision is that it now allows samples from suspect cases to be split between approved National Animal Health Laboratory Network (NAHLN) laboratories and the USDA laboratories at Ames, Iowa, and Plum Island, N.Y. Although all foreign animal disease diagnoses must be confirmed by the USDA laboratories, the NAHLN

laboratories can now do testing to obtain preliminary results, which would save valuable time were a foreign animal disease ever be detected. Much effort by many state and federal personnel over many years went into making this revision. We applaud USDA for making this significant change.

I hope the holiday season finds you all well and prosperous and looking forward to 2009.

*Barbara E. Powers*

