

# Colorado State University Veterinary Diagnostic Laboratories



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## Letter from the Director

Welcome to this issue of LabLines! In this issue, in addition to the informational articles, we are very pleased to announce a number of awards that our faculty recently received. We also welcome our new pathology residents and new staff in the Avian Disease Section. We are saddened to see long-time pathologist Dan Gould retire and wish him well as we are sure he will be having fun!

Our fiscal year ended June 30, 2007, and showed a steady number of accessions with increases in pathology and steady to decreasing numbers of submissions in bacteriology, virology, chemistry and parasitology. The Transmissible Spongiform Encephalopathy section is in the midst of Chronic Wasting Disease testing for the hunting season, while BSE testing remains steady at the maintenance level. Of special note is the finding of rabies in skunks in Colorado (see inside for details). To cover ever-increasing medical supply costs and salaries, we had to institute fee increases effective July 1. We are undergoing significant changes in our computer system that will be visible to you within a year. We also are overhauling our website to conform to the newly established college guidelines.

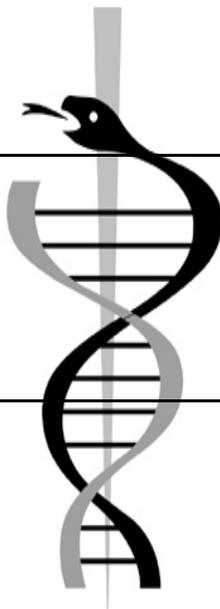
We are most pleased that we recently received renewal accreditation status (full-service, all species) from the American Association of Veterinary Laboratory Diagnosticians, following our site visit inspection in August. This is the first year of inspections at the new, more stringent standards based on ISO17025 and OIE.

It was good seeing many of you at the Colorado Veterinary Medical Associations annual meeting in September and Leadership Conference in November. This past October, I completed my term as President of the American Association of Veterinary Laboratory Diagnosticians. It was an extremely rewarding (and busy!) term that I immensely enjoyed. We had a great meeting in Reno and it was wonderful to visit with many colleagues.

Look forward to seeing many of you next year at various meetings and conferences.



**Barbara Powers, DVM/PhD/DACVP**

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## POOLED POLYMERASE CHAIN REACTION TO DETECT *TRITRICHOMONAS FOETUS* IN BEEF BULLS

--James A. Kennedy, Dayla Pearl, Linda Tomky, Jane Carman

(The following is a portion of an abstract accepted for publication in the *Journal of Veterinary Diagnostic Investigation*)

As part of a study to evaluate an alternate method to test for *Tritrichomonas foetus* infection, preputial scrapings from 305 mixed breed beef bulls were submitted to the Rocky Ford Veterinary Diagnostic Laboratory. All samples were collected by veterinarians and transported in commercial media to the laboratory as part of the Colorado *Tritrichomonas foetus* control program. Upon arrival, samples underwent microscopic examination for the presence of *Tritrichomonas foetus* and then were incubated until five days post collection before final microscopic examination. Culture detected 14 samples with *Tritrichomonad spp.*, all were confirmed to be *Tritrichomonas foetus* by polymerase chain reaction (PCR). After final examination, samples were randomly placed in groups of five samples; technicians were blinded as to culture results of the individual samples constituting each pool. From each sample within a group, a portion of the fluid sediment was removed and pooled with the other samples of the group to form 61 pools. From each of the formed pools, an aliquot was removed for PCR. PCR detected 16 positive pools (all culture positive animals were identified); an additional two positive samples were then identified on individual PCR on samples previously diagnosed as culture negative. Relative to culture, the 95% confidence intervals for sensitivity and specificity of PCR pools to detect *Tritrichomonas foetus* were 76.8% to 100% (mean value: 100%) and 85.5% to 99.5% (mean value: 93.4%), respectively.

The use of a pooled PCR test as part of a *T. foetus* control and/or eradication program is promising. The application of pooling to screen and monitor herds using pooled PCR will provide a high level of sensitivity with fewer collections required than traditional culture methods. In addition to its use to monitor herds considered free of *T. foetus* infection, it also will offer an improved method in cleaning herds considered to be infected without total bull depopulation. This could be accomplished by removing the positive animals and, after a complete herd test and isolation from all females, a follow-up pooled test could be used before releasing the herd from quarantine. This would provide a high degree of confidence that the animals were not infected. When samples are pooled, a portion of each pool constituent is saved for follow-up. If a pool is found positive, using the saved samples can identify the individual bull. Overall, the concept of pooling preputial scrapings can provide an improved test at less expense to the producer, less wear and tear on the veterinarian and less trauma to the bull. Although the cost of PCR is greater than the cost of culture, the owner would realize a significant savings by minimizing the number of collections required to achieve an equal amount of certainty that his herd was not infected.

PCR for *T. foetus*—Submit preputial scraping. Single PCR Fee=\$25.

### ALERT OUTBREAK OF TRICHOMONIASIS IN SOUTHEAST COLORADO

The Rocky Ford Branch Laboratory continues to help producers work through a major outbreak in *Tritrichomonas foetus*. In Colorado, Trichomoniasis was established as a reportable disease by 8CCR-1201-1 and herds diagnosed as positive are immediately placed under quarantine till follow-up testing is completed and an adequate amount of time has passed to insure that no subclinical infections exist. In the past four months, the Rocky Ford Laboratory has diagnosed 44 positive cases of Trichomoniasis from nine herds, 37 more individual cases and 7 herds more than diagnosed for the entire past year at the Rocky Ford Laboratory. It is estimated that the current outbreak has cost over \$450,000 with new cases continuing to be identified. The outbreak covers a wide area of Southeast Colorado from the Front Range to the Kansas border and south of US Highway 50.

**NOTICE:** Due to the increased costs of the In-Pouch TF system, we can no longer supply the pouches free of charge. Pouches are still available, however, a charge of \$3 each will be assessed. If returned to us for reading, the evaluation charge will be decreased by \$1.

## WHAT ARE WE SEEING IN ALPACAS?

--Pat Schultheiss

With the increase in popularity of alpaca ownership, there has been an increase in alpaca case submissions to the laboratory. These cases were reviewed prior to the onset of the current outbreak of a severe respiratory syndrome of unknown cause. During the time period that was reviewed, pathologists examined 243 cases in which the whole animal or tissues from a deceased animal were submitted. The types of cases are summarized in Table 1. Pathologists also evaluated 23 skin specimens from alpacas with non-fatal disease. Samples submitted only for microbiological evaluation are not reviewed here.

Table 1. Summary of the types of diseases causing death in alpacas.

Type of Disease Causing Death	Number of Cases
Abortion and neonatal death	86
Gastrointestinal disease	61
Neoplasia	16
Trauma	16
Pneumonia	11
Brain lesions	11
Liver disease	10
Myocardial degeneration	7
Urolithiasis	4
Rattlesnake envenomation	3
Tooth root abscess	3
Miscellaneous or no diagnosis	15
Total number of cases	243

The most numerous cases were abortions or neonatal deaths, with 86 cases evaluated. Among these cases, abnormalities of the placenta were the most common findings, occurring in 21 cases. Placental lesions included nine cases of inflammation, 10 of mineralization, and two of both inflammation and mineralization; 29 placentas had no lesions (total of 50 cases with placentas). The placenta lesions were poorly correlated with fetal lesions; only one-third of these cases had any detectable fetal abnormalities. Examination of feti identified 12 cases of developmental defects, five cases of chlamydia infection, four cases of BVD infection, four cases of fetal tissue mineralization, six cases of fetal pneumonia, two cases of fetal liver necrosis, three cases of hypoplasia of lymphoid elements, one case of encephalitis, and seven cases with inflammation suggesting an infectious cause. Three cases with a history of dystocia and one case with a history of twin births had no histologic lesions.

Gastrointestinal disease was the cause of death of 61 alpacas. Forestomach lesions include 18 cases of ulceration, three of impaction, and two of tympany. Intestinal lesions include 10 cases of hemorrhagic enteritis, four of clostridial enteritis, three of coccidial enteritis, and 19 of non-specific enteritis. There was one case of impaction of jejunum and one case of impaction of colon.

Neoplasia as the cause of death was found in 16 alpacas. There were nine cases of lymphoma and/or leukemia with an average age of 4 years, ranging from 5 months to 10 years. No underlying cause for the development of lymphoma in these young animals has been identified. There were seven other cases of malignant tumors, including one oral squamous cell carcinoma, two small intestinal adenocarcinomas, two mammary adenocarcinomas, a subcutaneous liposarcoma, and a urethral sarcoma. These animals averaged 10 years of age. The urethral sarcoma was first noted when the alpaca was only 7 weeks old and was treated with radiation therapy; the tumor recurred and the alpaca died at 4 years of age. Also, there were six cases of benign skin masses from animals averaging 7 years of age.

Seven cases of myocardial degeneration and mineralization were seen, five in feti or neonates and two in adults aged 8 and 12 years. Vitamin E and selenium levels were determined for two adults and two neonates and high selenium and low vitamin E were found.

There were 16 cases of trauma, 11 cases of pneumonia, six cases of bronchopneumonia, two of interstitial pneumonia, and three caused by aspiration. There were 11 cases of encephalitis; three had lesions of

polioencephalomalacia, eight had inflammatory lesions and these included two cases of *Listeria* infections, one case of fungal infection, and one case of a bacterial infection spreading from otitis media. There were 10 cases of liver disease identified as the cause of death; eight cases of cirrhosis or advanced inflammation, one case of liver fluke infection, and one case of adenovirus infection.

Seventeen skin biopsies were submitted for evaluation of dermatological disease. Twelve had pyoderma, three had hyperkeratosis or parakeratosis, and two had inflammation characteristic of hypersensitivity. Three of the pyoderma cases also had parakeratosis suggesting a concurrent zinc-responsive disease.

In summary, alpacas are affected by a wide variety of diseases. Abortion and neonatal death are the most common problems seen in this laboratory. Placental lesions and developmental defects appear to be common. Ulceration of the forestomach appears to be a more common problem in alpacas than in many other domestic animal species. Although there were only seven cases of myocardial degeneration and mineralization, this also may be a problem in alpacas worth further investigation.

### **CVMA HONORS DR. KRISTY PABILONIA**



The Colorado Veterinary Medical Association (CVMA) honored Kristy Pabilonia, DVM of Colorado State University's Veterinary Diagnostic Laboratory with its Outstanding Faculty service award at its 101<sup>st</sup> annual convention September 8-12, 2007. CVMA's Outstanding Faculty service award recognizes a CSU faculty member who has provided unselfish assistance to practitioners as a clinician, is a proficient and capable teacher, and/or has made significant contributions to continuing education.

Dr. Pabilonia is coordinator of the Colorado Avian Disease Surveillance Program, which was created after the first outbreaks of avian influenza in Southeast Asia caused serious concerns about the health of the global community. The program has tested thousands of birds for avian influenza within Colorado

Dr. Pabilonia's work outside of the state's and nation's borders reflects the connection between animal health and public health to help address global critical issues. She has traveled to Indonesia many times during the last year to help that nation develop training programs to manage its current highly pathogenic avian influenza outbreak. She also is a frequent presenter on poultry health topics, has provided compelling testimony in front of Colorado's State Legislature, and has helped attract talented students to projects and educational opportunities pertaining to poultry health.

### **DR. JIM KENNEDY RECEIVES CVMA VETERINARIAN OF THE YEAR SERVICE AWARD**

The Colorado Veterinary Medical Association (CVMA) honored Jim Kennedy, DVM, of the CSU Veterinary Diagnostic Laboratories, Rocky Ford, with the Veterinarian of the Year service award at its 101<sup>st</sup> annual convention September 8-12, 2007. The Veterinarian of the Year service award recognizes a distinguished CVMA member who has contributed to the advancement of veterinary medicine in the state of Colorado in the areas of organization, education, research, practice or regulatory service.

Dr. Kennedy is responsible for Colorado's successful voluntary program to control bovine viral diarrhea – an effort that is serving as a model for those in other states. As the Director of Colorado's Voluntary BVD Control and Eradication Program, Dr. Kennedy set out to establish Colorado as the nation's first to be certified as BVD free.



Dr. Kennedy also serves as the director of the Rocky Ford branch of CSU's Veterinary Diagnostic Laboratories. In that capacity, he actively assists veterinarians in the diagnosis of disease, and his special interest in diseases of cattle, horses and camelids are of particular value to the state's veterinary community.

A CVMA member who nominated Dr. Kennedy wrote, "He has always been accommodating and insightful with diagnoses, and his leadership in the BVD eradication program is outstanding."

## DR. PATRICIA SCHULTHEISS RECEIVES CARL NORDEN-PFIZER AWARD



**D**r. Patricia Schultheiss received the Carl Norden-Pfizer Distinguished Teacher Award for 2007. The award honors faculty who have contributed to the advancement of the profession through their teaching abilities. The recipient is chosen by veterinary students. It was presented to her at the 2007 Professional Veterinary Medicine Hooding Ceremony. Dr. Schultheiss is the leader of the sophomore pathology courses.

## THE SPINOSE EAR TICK--*Otobius megnini*

--Lora Ballweber

**R**ecently, several inquiries about the spinose ear tick have been received by our Parasitology section. Most have centered on horses and alpacas, and the most common question asked is how to get rid of these minute, annoying pests.

*Otobius megnini* is the only soft tick that commonly parasitizes domestic mammals in North America. Traditionally considered to be pests of livestock, particularly in the arid regions of the Western US, this parasite routinely pops up in other parts of the country in a wide variety of hosts including, on occasion, humans. Fortunately, unlike other ticks, *O. megnini* is not the primary vector of any pathogenic organism. However, they can cause considerable irritation and pain, secondary bacterial infections, destruction of the ear canal and tympanic membrane, and otitis interna. Death of cattle and severe muscle spasms in horses also have been reported, although these appear to be unusual manifestations of infestation.

The life cycle of *O. megnini* also is relatively unique among ticks. In general, soft ticks tend to be multiple-host ticks with all stages feeding on a different host. Adult females may produce multiple batches of eggs, ingesting a bloodmeal prior to the deposition of each clutch. In contrast, *O. megnini* is a one-host tick in which only the larvae and nymphs are parasitic. Larval ticks, which are about 0.5mm in size, hatch in three to eight weeks and climb anything vertical to await the passing of a potential host. They can survive in the environment for up to two months without feeding. When larvae encounter a suitable host they crawl to the ear and attach below the hair line. After feeding for about a week and growing to around 4mm, they molt to the first nymphal stage. There are two nymphal stages, each with a spiny cuticle from which the common name is derived. Second-stage nymphs reattach and feed for several weeks to months, growing to around 8mm. Engorged nymphs then leave the ear and drop to the ground where they hide in cracks, crevices, beneath rocks or under the bark of trees where they undergo the final molt to adults. Mating occurs in the environment. Adults do not feed, but adult females can survive for two years, depositing up to 1500 eggs in several clutches.



Control can be somewhat difficult and tends to focus on direct treatment of the infested animal. Acaricide treatment of pastures is generally impractical. In stables, kennels, and other housing situations, however, environmental treatment may be helpful. Eradication of protected sites also will go a long way towards helping to decrease tick populations. Animal ears should be regularly inspected and any ticks that are present should be manually removed. Manual removal is as for any other tick--grasp the tick as close to the skin as possible with fine forceps and extract using slow, steady pressure. Be cautious, though, as many animals resist manipulation of the ear particularly if it is sore as a result of the infestation. In these cases, as well as an adjunct to manual removal, a topical insecticide can be used. For cattle, insecticide-impregnated ear tags can help control infestations. For other animals, avermectins have been used but the introduction of phenylpyrazoles has essentially replaced the use of avermectin compounds.

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Parasite identification--Submit parasite. External or Internal--Fee=\$10; External Exotic or Internal Exotic--Fee=\$15.

## HELPFUL HINTS ON EQUINE HERPESVIRUS-1 SEROLOGY

--Hana Van Campen

You are called out to look at a horse with a sudden onset of fever, depression, purulent nasal discharge or neurologic disease, or to attend a mare who has aborted. Or, maybe you have a client who wants to know if her horse has protective antibodies to EHV-1 from prior vaccinations before deciding whether to vaccinate for "rhino" or not. The lab report returns showing a titer to EHV-1 ranging from 2 to 4096 (expressed as 1:2 to 1:4096 on the printed report).

*What does that titer really mean?*

Three-hundred and eighty serum samples were submitted to our Virology section for EHV-1 SN titers in a full calendar year. Reasons for submission included surveys of healthy horses, titers in lieu of vaccination, acute respiratory disease with fever, abortion and a variety of neurologic signs. The titers were tabulated according to the age of the horse and the disease stated in the history.

### Relationship Between Age and Titer:

- Most horses over 1 year of age (98%) had measurable EHV-1 SN antibody (range 2 to >4096) indicating previous infection or vaccination.
- Seventeen percent of foals less than 1 year of age were seronegative, and 65% had low (2 to 8) EHV-1 SN titers. The remaining 18% had EHV-1 titers from 16 to 256. The titers in this age group can reflect residual maternal antibodies as well as the foal's response to infection. In addition, EHV-4 infections are common in young horses and the antibodies they develop cross-react with the EHV-1 used in the SN test.
- Horses tended to develop higher EHV-1 SN titers with age up until about 10 years of age. There were no seronegative horses 5 years of age or older. EHV-1 titers ranged from 32 to >4096 in horses over 10 years of age with just 4% having low titers of 2 to 8. The observed increase in titers with age may be due to re-stimulation of a primed immune system over the lifetime of the horse. Sources of EHV-1 stimulation include multiple vaccinations or infections, or the recrudescence of latent herpesvirus from lymphoid or nervous tissues.

Conclusions: The EHV-1 SN seroprevalence of horses over 1 year of age is very high. Because many horses are previously infected or vaccinated with EHV-1, or have cross-reactive antibodies to EHV-4, an EHV-1 SN titer on a single serum sample is not interpretable.

### The Bottom Line:

- Collect a serum sample at the time you first examine a respiratory, neurologic or abortion case, and two weeks later (if the horse survives). A four-fold or greater increase between the EHV-1 SN titers indicates a recent infection and supports your clinical diagnosis. If this is a case of respiratory disease, request EHV-4 SN test, too, as both EHV-1 and EHV-4 titers will increase.
- Include the age of the horse and a history of recent vaccinations on the accession form.
- A single EHV-1 SN titer will tell you if the horse has antibodies to EHV-1, but not if the horse will be protected from disease in the face of a new infection.
- Herpesviruses recrudescence from their latent state particularly after stresses such as transport, parturition and other diseases. A rise in titer between acute and convalescent serum samples should be evaluated in light of the overall situation of the animal.

### Laboratory Information:

- EHV-1 SN tests are set up on Tuesday and reported on Friday evening, and set up on Friday and reported on Monday evening.
- Be sure to indicate on the accession form if you submitted the acute serum sample separately so that the technicians can perform the SN test on both samples on the same day.
- The EHV-1 PCR test (Test #787) detects a portion of the virus's DNA genome and a positive result indicates the presence of EHV-1 in the sample. If you want to determine if the horse is in the acute stages of EHV-1 infection or is shedding the virus, submit a nasal swab in a sterile tube (a red-topped tube or snap-cap tube) with 0.5ml of sterile water or sterile saline, and request an EHV-1 PCR test. The sample should be shipped overnight on ice. The test is routinely performed on Tuesdays and Thursdays.

EHV-1 SN: Submit serum. Fee (acute & convalescent)=\$11. EHV-1 PCR: Submit nasal swab. Fee=\$32/sample.

**PROTEIN ELECTROPHORESIS EQUIPMENT PURCHASE/IMPROVED REPORT QUALITY**

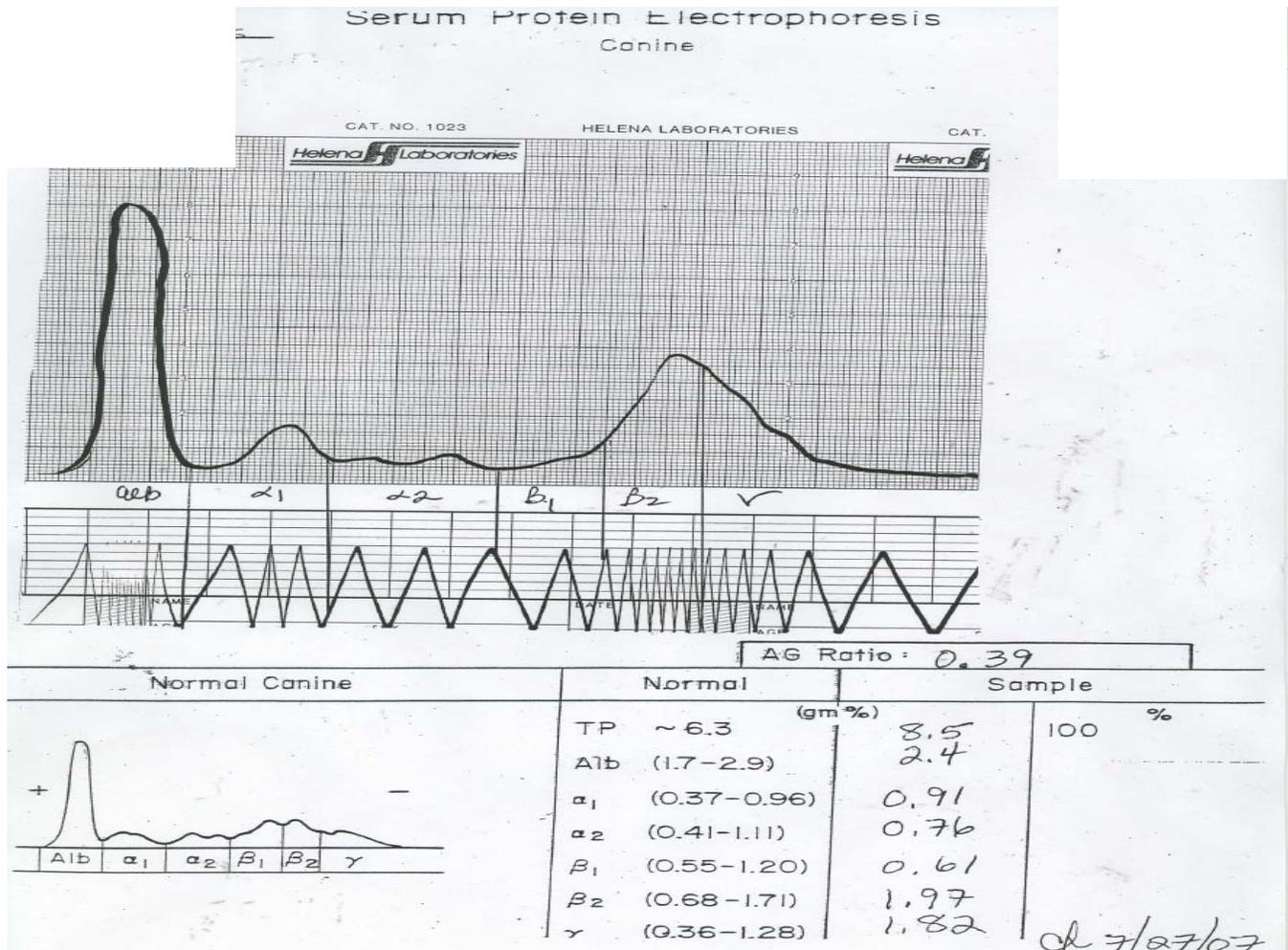
--Cindy Hirota and Doreene Hyatt

We are in the process of upgrading our protein electrophoresis capabilities by replacing our 30-year-old instrumentation with new technology. The new instrument is the Hydrasys LC Electrophoresis System from Sebia. The Sebia Hyrasys/Phoresis has walk-away automation of both the migration and staining portions of the technique. It is environmentally friendly and will greatly improve the quality of our electrophoresis reporting and future testing.

The most striking advances over the current method are found in the analysis software package and scanner. The Sebia system allows for digital storage of reports and images, provides tools to quantify protein regions and monoclonal peaks without manual calculations, and also gives the ability to easily create professional reports for our clients.

Attached are two reports from protein electrophoresis. The first is the tracing that was the result of the old system (along with calculations done by hand by the technician) and the second is a demonstration report from the new system (notice the inclusion of a picture of the actual gel).

A Sebia technical training representative trained technicians at the end of October and new reports became available in early November.

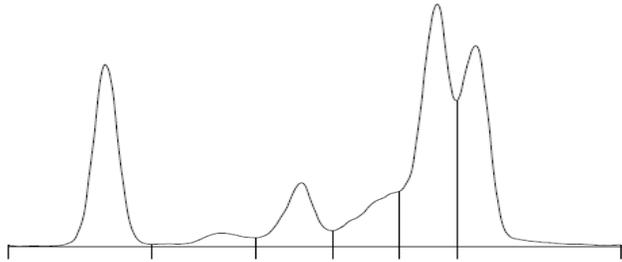


4146

Sample #: 3  
Depart.:

Date: 8/7/2007

ID:  
Birth:



**Serum protein electrophoresis**

Fractions	%		Ref. %	Conc.	Ref. Conc.
Albumin	19.4	<	60.0 - 71.0	1.92	43.00 - 51.00
Alpha 1	2.8	>	1.4 - 2.7	0.28	1.00 - 2.00
Alpha 2	9.2		7.0 - 11.0	0.91	5.00 - 8.00
Beta 1	9.4	>	6.0 - 9.0	0.93	4.00 - 6.00
Beta 2	33.7	>	2.0 - 5.0	3.34	1.00 - 4.00
Gamma	25.5	>	8.0 - 16.0	2.52	6.00 - 12.00

A/G0.24

T. P.: 9.9 g/dl

*Signature*

Protein Electrophoresis: Submit 1/2ml of serum. Fee=\$30.

### RECENT RABIES ACTIVITY—Christie Mayo

Throughout the year four skunks and one coyote have tested positive for Rabies within the state of Colorado. The strain obtained from three of the skunks was typed by the Centers for Disease Control and revealed they had been infected with a terrestrial strain, the South Central Skunk variant which is endemic in Oklahoma, Texas, and Kansas. These three skunks were from Prowers, Washington, and Las Animas counties. An inadequate sample was obtained for typing from the fourth skunk located in Mesa County, and the coyote which was located in Prowers County was also unable to be typed. Terrestrial Rabies cases are uncommon in the state of Colorado and can pose a risk for humans, domestic animals, and wildlife. It is important that Rabies remains as a differential when an animal expresses any abnormal neurological behavior.

### NEOPLASIA IN WILDLIFE

--Colleen Duncan, Laurie Baeten, Jenny Powers, Terry Spraker

“You miss more by not looking than not knowing”--Dr. Otto Radostits 1934-2006

**W**hile Dr. Radostits instilled this mantra into students of large animal medicine worldwide, nowhere is this philosophy more appropriate than in the study of wildlife disease. In general, tumors are an uncommon finding in wildlife; this is largely due to the fact that wild animals don't usually live as long as their domestic counterparts of humans. The probability of identifying these rare events is increased when examinations can be conducted on many animals.

We are fortunate to work with many of the regional wildlife agencies; most commonly the Colorado Division of Wildlife and the National Park Service. These relationships provide excellent learning opportunities for staff and students alike and have resulted in the identification of lesions not previously reported in some species. Here, we report three cases of “an old tumor in a new species” seen at the diagnostic lab this year. All three of these animals were suspected of having other diseases and the neoplastic processes observed were unexpected findings. These types of cases emphasize the importance of routine post-mortem examination of wild animals such that uncommon lesions can be identified.

#### **Case 1:**

An adult, male black-billed magpie (*Pica hudsonia*) was found dead and submitted for necropsy and West Nile virus (WNV) screening. On gross examination, the right testicle was enlarged, approximately twice the size of the left. Histologically, the normal parenchyma was effaced by an intratubular seminoma. Pooled brain, heart and kidney tissue was positive for West Nile virus on RT-PCR. While West Nile virus is a common cause of mortality in corvids of Colorado during summer months, identification of the testicular tumor in this bird was an unexpected finding. In domestic mammals, seminomas are most common in the dog but have been reported in numerous other species. This tumor has been reported in a pigeon, guinea and other domestic fowl, psittacines and ducks. However, there are no reports of this tumor in magpies.

#### **Case 2:**

An aged elk (*Cervus elaphus nelsoni*) in Colorado was suspected of having chronic wasting disease and humanely euthanized. At necropsy, field personnel identified a focal, 1cm diameter, subpleural mass in the right caudal lung lobe. Histologically, the mass was consistent with a multifocal, well-differentiated bronchioloalveolar carcinoma; a lesion not previously reported in elk. Primary lung tumors are uncommon in domestic animals with the exception of dogs and cats. The incidence of such lesions in wild animals is unknown but assumed to be even rarer. There are infrequent reports of pulmonary tumors in cervids. These include multifocal fibromas associated with papilloma virus in elk and pulmonary metastatic renal adenocarcinoma in a moose. Bronchioloalveolar carcinomas are thought to arise from either alveolar type II or Clara cells. Electron microscopy usually is required to elucidate the cell of origin. In wild and domestic sheep a retrovirus, Jaagsiekte sheep retrovirus, is associated with multi-focal, well-differentiated bronchioloalveolar carcinomas.

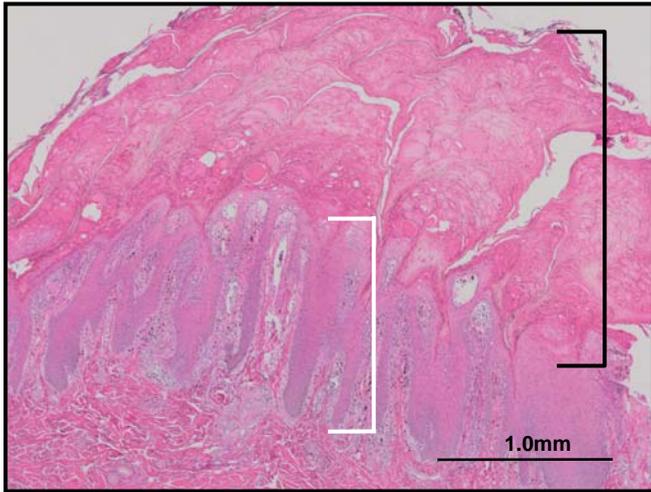
#### **Case 3:**

A captive, 20-year-old female elk (*Cervus elaphus nelsoni*) euthanized due to progressive lameness and weight loss was presented for post-mortem examination. Within the uterus there was a poorly demarcated, multilobulated mass measuring 10cm in diameter. Histologically, the tumor was an adenocarcinoma and unilateral metastases were present within the ovary. A focal, 1cm diameter adenocarcinoma was identified within the abomasum. This tumor was histologically distinct from the neoplasm found in the uterus and ovary. Although this elk had a history of experimental reproductive treatments including leuprolide, GnRH vaccine and *Brucella abortus* vaccination, it was most likely that both tumors represented spontaneous, independent neoplastic transformations and were unrelated. Uterine and abomasal adenocarcinomas are relatively uncommon tumors of domestic ruminants and previously unreported in elk. The incidence of these tumors in wild or captive elk and their biological significance is unknown.

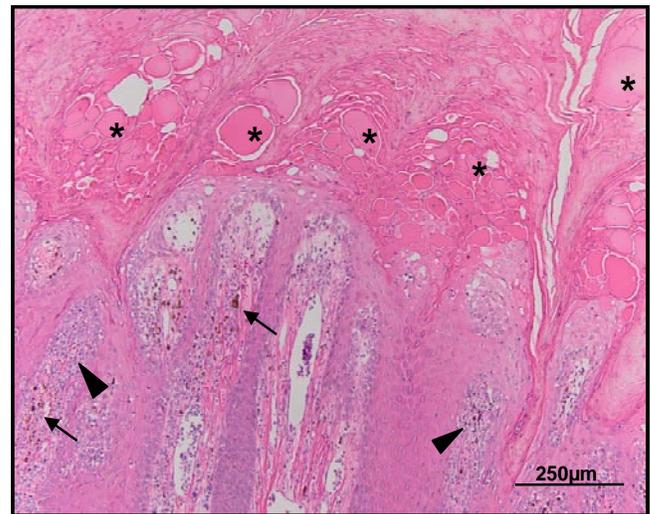
### **HAVE YOU EVER HEARD OF . . . HEREDITARY NASAL PARAKERATOSIS OF LABRADOR RETRIEVERS —Debra Kamstock and Jamie Bush**

**A** 1-year-old female spayed Labrador Retriever presented to the Veterinary Teaching Hospital Dermatology Service with a six-month-history of hyperkeratosis of the dorsal nasal planum. Lesions were not present in any other location and there was no history of pruritis or excoriation. Clinical examination revealed moderate to marked accumulations of heavy, adherent, dry, rough, proliferative keratin with the formation of keratin projections of the dorsal nasal planum. When crusts were removed, the underlying surface was moist with minimal depigmentation and retention of the cobblestone appearance. Based on the signalment and gross findings, clinical differentials included nasal parakeratosis of the Labrador Retriever, discoid lupus erythematosus, and idiopathic hyperkeratosis. To further evaluate the lesion, biopsy was recommended.

Three, 5mm diameter cutaneous biopsies were obtained and submitted for histopathological evaluation. Diffusely, there was marked parakeratotic hyperkeratosis measuring up to 2mm thick often arranged in papillary projections (peaks) and admixed with moderate to abundant amounts of variably sized accumulations of eosinophilic proteinaceous material (serum lakes) (Fig. 1 and 2). The epidermis was moderately-to-markedly hyperplastic (acanthosis) with variable keratinocyte cytoplasmic vacuolation. Infiltrating the superficial dermis at the dermal-epidermal junction and often perivascularly, was a moderate number of mixed inflammatory cells consisting predominantly of lymphocytes and plasma cells admixed with lesser numbers of macrophages, mast cells, eosinophils and neutrophils (lichenoid dermatitis and perivascularitis) (Fig. 2). Mild to moderate pigmentary incontinence also was noted.



**Figure 1.** Photomicrograph of a cutaneous biopsy from the nasal planum of a Labrador Retriever with hereditary nasal parakeratosis demonstrating marked parakeratotic hyperkeratosis admixed with abundant serum lakes (black bracket) and marked epidermal hyperplasia (acanthosis) with superficial dermal inflammation at the dermal-epidermal junction (white bracket). 40x magnification



**Figure 2.** Photomicrograph of a cutaneous biopsy from the nasal planum of a Labrador Retriever with hereditary nasal parakeratosis. Abundant variably sized intrakeratinic serum lakes (\*). Moderate mixed (predominantly lymphoplasmacytic) inflammation at the dermal-epidermal junction (arrow heads). Pigmentary incontinence (arrows). 100x magnification

These microscopic findings in conjunction with the signalment and gross lesions confirmed a diagnosis of hereditary nasal parakeratosis of Labrador Retrievers (HNPLR).

Hereditary nasal parakeratosis of Labrador Retrievers (HNPLR) is a rare condition characterized clinically by dry, rough, proliferative, keratinaceous debris of the nasal planum. Lesions are most severe on the dorsal surface of the nasal planum, may be associated with color change (depigmentation), and may result in fissuring with secondary bacterial infections in severe cases. The cobblestone appearance of the nasal planum is typically retained, but may be lost or obscured in late stages. Lesions typically are restricted to the nasal planum, although in two of 29 cases reported in the literature, lesions involving the footpads also were present. The condition has been seen exclusively in Labrador Retrievers and their crossbreeds with an onset between 6 and 12 months of age. No sex predilection is reported. Considering the strong breed predilection, an autosomal recessive mode of inheritance has been proposed and, based on electron microscopic findings, a primary defect in the cornification process has been suggested as the pathogenesis.

Differential diagnoses for hyperkeratosis of the nasal planum in the dog include but are not limited to: idiopathic nasodigital hyperkeratosis, zinc-responsive dermatosis, actinic keratosis, mucocutaneous pyoderma, *Malassezia* dermatitis, canine distemper, and immune-mediated dermatopathies such as discoid or systemic lupus erythematosus and pemphigus foliaceus. Distinguishing these entities from HNPLR is **not** considered to be challenging due to HNPLR's unique gross appearance of marked proliferative hyperkeratosis with keratin projections limited to the nasal planum. The condition exclusively affects young Labrador Retrievers and their crossbreeds, and microscopically demonstrates the unique presence of abundant, variably sized, intrakeratinic serum lakes.

Idiopathic nasodigital hyperkeratosis typically occurs in middle-to-older aged dogs and is seen in many breeds. Zinc-responsive dermatosis is rarely limited to the nasal planum and again occurs in various breeds, most commonly seen in northern breeds. Actinic keratosis demonstrates microscopic evidence of epidermal keratinocyte atypia and dysplasia while mucocutaneous pyoderma is typically associated microscopically with a greater degree of lichenoid inflammation and less severe, to absent, parakeratotic hyperkeratosis. *Malassezia* dermatitis will reveal the presence of yeast organisms upon cytological exam (impression smear, tape, scrape, or swab) and occasionally histopathologically, and will respond to appropriate therapy. Canine distemper typically involves the nasal planum and foot pads (hard pad disease), while immune-mediated dermatopathies (i.e. lupus erythematosus and pemphigus foliaceus) typically result in ablation of the nasal planum cobblestone appearance grossly and additionally demonstrate distinguishing features microscopically (ie., civatte bodies and acanthocytes, respectively).

Treatments for HNPLR reported in the literature include topical application of propylene glycol, petroleum jelly, and/or vitamin E; however, consultation with a veterinary dermatologist should be considered to determine optimal therapeutic intervention. Oral antibiotics also may be needed for concurrent secondary bacterial infections. The dog presented here was diagnosed in December 2006 and, as last reported, has been treated with topical 70% propylene glycol twice daily with waxing and waning results.

#### In Summary:

- If you are a clinician presented with a young Labrador Retriever or Labrador-cross with a history of marked, proliferative, adherent, keratinaceous debris (crusting) of the nasal planum think *Hereditary Nasal Parakeratosis of the Labrador Retriever*.
- If you are a pathologist presented with a biopsy from a “crusty” nasal planum of a young Labrador Retriever or Labrador-cross with microscopic evidence of marked parakeratosis with intrakeratinic serum lakes in conjunction with acanthosis, lichenoid inflammation, and pigmentary incontinence, think *Hereditary Nasal Parakeratosis of the Labrador Retriever*.
- If you hadn't already, now you *have* heard of **Hereditary Nasal Parakeratosis of the Labrador Retriever**.

We would like to thank Dr. Rod Rosychuck and the CSU-VTH Dermatology service for sharing and discussing this interesting case with us.

#### **Reference:**

Page N, Paradis M, Lapointe JM, Dunstan RW. Hereditary nasal parakeratosis in Labrador Retrievers. *Vet Dermatol*. 2003 Apr;14(2):103-10

Biopsy (histopathology)--Submit biopsies in 10% formalin. Fee=\$35. Dermatology consult--Additional \$25.

## **ABORTIONS IN SMALL RUMINANTS**

**--Christie Mayo and Hana Van Campen**

**T**here are many infectious and non-infectious causes of abortion in small ruminants, and a majority of the infectious diseases also cause clinical signs in people. These diseases are termed zoonotic and are of important concern during lambing or kidding when there is human contact with placental and fetal tissues. Protective clothing such as plastic gloves should be worn to reduce the risk of exposure. Consumption of unpasteurized milk or cheese by pregnant women is another route of exposure and should be avoided.

It is common to have abortion rates of 1.5%-2.0% in a flock. Abortion rates above these percentiles result in profit loss for the producer and it is important to identify the causative agent before isolated cases lead into an abortion “storm” comprising 20%-30% of the herds. Non-infectious causes also should be taken into consideration when investigating an abortion storm. These can include rough handling, fighting among animals, inadequate nutrition, and exposure to poisonous plants. The tables below list some of the most common infectious diseases and non-infectious toxic plants that cause abortions in sheep and goats.

Prevention of abortion storms involves good husbandry and vigilant observation of the flock during the breeding season and throughout gestation. In order to prevent some of the most common infectious causes of abortion, killed vaccines are available for vibrio (*Campylobacter fetus*) and chlamydia (*Chlamydia psittaci*) and can be used at the beginning of the breeding season. Unfortunately, a vaccine is not available in the United States for the prevention of

toxoplasmosis. The best alternative is to control cat populations which will limit exposure for pregnant ewes and reduce contamination of food and water sources.

It is crucial to identify the specific causative agent during an abortion storm as swiftly as possible. Expedient diagnosis is based on clinical signs, flock history, differentials, and diagnostic tests available to the practitioner. Each diagnostic test requires proper sample collection and packaging in order for a diagnostic laboratory to report adequate results. The tables below should aid in identification and reporting of infectious diseases. Immediate vaccination and the use of antibiotics may aid in treatment during an abortion storm but the best mode of protection is prevention.

### Most Common Infectious Agents That Cause Abortion in Sheep and Goats

Common Name	Causative Organism	Diagnostic Test
*Vibrio	<i>Campylobacter fetus</i>	Culture in Clarke's Media , FA, Dark-Field microscopy
*Enzootic Abortion in Ewes	<i>Chlamydia psitacci</i>	FA, PCR, giemsa slide prep of vaginal discharge or placenta
*Toxoplasmosis	<i>Toxoplasma gondii</i>	PCR, serology, FA, histopathology
*Leptospirosis	<i>L. hardjo, L. pomona, L. icterohaemorrhagiae, L.interrogans</i>	PCR, serology, dark-field microscopy
*Brucellosis	<i>Brucella ovis, * B. melitensis</i>	Serology, Culture
*Q-fever	<i>Coxiella burnetii</i>	PCR of placenta, fetus, tissues, serology, histopathology
Hairy-Shaker Disease	Border Disease (Pestivirus)	VI of tissues
Bluetongue	Bluetongue virus (Orbivirus)	PCR, AGID, VI
*Listeriosis	<i>Listeria monocytogenes</i>	Culture of fetus, nervous, other tissues
*Cache Valley Fever	Cache Valley Virus	Serology
*Salmonella	<i>Salmonella abortus sp. ovis</i>	Culture of fetus, placenta, uterine discharge
*Rift Valley Fever (not currently in the USA)	Rift Valley Fever Virus (Phlebovirus)	Serology

\* indicates diseases transmissible to humans (zoonotic diseases)

FA= Fluorescent Antibody; PCR= Polymerase Chain Reaction; VI=Virus Isolation; AGID= Agar Gel Immuno Diffusion

### Most Common Plants That Cause Abortion in Sheep and Goats

Plant Name	Environment/Location	Toxic principle
Broom Snakeweed, Perennial Broomweed ( <i>Gutierrezia microcephala</i> and <i>G. sarothrae</i> )	Dry ranges and deserts from California to Texas, south to Mexico and north to Idaho	Steroidal saponin, selenium
Carelessweed, Pigweed ( <i>Amaranthus</i> )	Barnyards with rich, moist soils throughout most of the United States	Nitrates
Locoweed, Peavine, Emory Loco ( <i>Astragalus emoryanus</i> )	Trans-Pecos region (Texas)	Myserotoxin and 3-nitro-1-propanol
Locoweed - Woolly Loco, Purple Loco ( <i>Astragalus mollissimus var. mollissimus</i> )	Southwestern South Dakota to Texas and New Mexico	Alkaloid swainsonine
Locoweed - Garboncillo, Wooton Loco, Rattleweed ( <i>Astragalus wootonii</i> )	Southern New Mexico, eastern Arizona and northern Mexico	Alkaloid swainsonine
Locoweed - Lambert Crazyweed, ( <i>Oxytropis lambertii</i> )	Extends into similar regions of New Mexico, Oklahoma, Colorado and Kansas.	Alkaloid swainsonine
Narrowleaf Sumpweed ( <i>Iva angustifolia</i> )	Vegetational areas of Texas	Unknown
Tobosagrass Ergot ( <i>Claviceps cinerea</i> )	Central Texas west to southern Arizona and south into Mexico	Alkaloids and tremorgens

Abortion Screen Food Animals--Submit entire fetus and placenta OR appropriate samples. Fee=\$65.

## **SAFETY PRECAUTIONS WHEN PERFORMING A NECROPSY OF AN ANIMAL WITH SUSPECTED ORGANIC PESTICIDE TOXICITY**

**--Dwayne Hamar, Cathy Bedwell and Dan Gould**

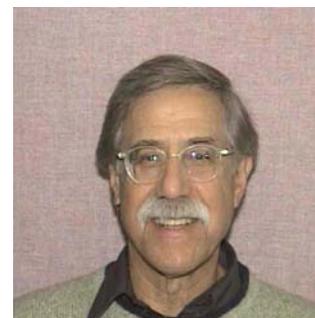
**W**hen performing a necropsy, we are generally concerned about the potential of being exposed to, or contaminating the environment with, an infectious agent, and obtaining uncontaminated samples. However, you should include other considerations when dealing with suspected organic pesticide toxicities.

Organic chemicals have one important property that must be considered when performing a necropsy. They are very lipid soluble and readily absorbed through the skin. Some of these chemicals have such a low LD<sub>50</sub> that they can result in clinical symptoms and potential death if they come in contact with unprotected skin. A good practice when performing a necropsy on an animal, especially with suspected organic pesticide toxicity, is to double-glove to prevent these compounds from coming in contact with your skin. Samples placed in plastic containers may be contaminated by organic compounds from the plastic which may interfere with the analytical methods used for pesticide analysis. To prevent contamination with plastic-derived chemicals, the samples should either be placed in a glass container or be wrapped in foil before being placed into a plastic bag. When shipping these samples, plenty of absorbent material should be placed in the box to contain any leaked material should the primary container fail. In veterinary medicine, these toxicants are commonly ingested by the animal, thus extra precautions should be observed in handling stomach contents as stomach contents often contain the highest concentration of toxicant.



***Gayle Thompson passed the NVSL Johne's fecal check test with 25/25 correct!***

**DAN GOULD RETIRES**--Dan Gould, Professor and Associate Department Head for Professional Veterinary Medicine and Clinical Service, has retired after a long and illustrious career. Dan received his BS and DVM (with high distinction) from CSU. He obtained his PhD from UC Davis in Comparative Pathology while studying brain lesions induced by *Clostridium perfringens* toxin. Board-certified by the ACVP in 1974, Dan was an Assistant Professor at the Ohio State University before joining the CSU faculty in 1980. He was promoted to Professor in 1992. Dan has truly made major contributions to the Department throughout his career. Over the years, Dan has provided effective service to the Department and Veterinary Diagnostic Laboratory, serving as Chief/Section Head of Necropsy since 1985 and Associate Department Head since 2002. To a person, everyone agrees that Dan's efforts in these capacities have been truly outstanding. In addition to his administrative duties, Dan also has been an outstanding pathologist, teacher and researcher throughout his career. Through his post-mortem investigation practicum course, Dan has helped innumerable young veterinarians develop skills in this area. Dan's research interests are extensive and varied, but primarily revolve around neuropathology, toxicology and metabolic/nutritional diseases. He has published over 70 refereed articles and book chapters, a body of work that has been cited well over 1200 times. Throughout his 27 years of service, Dan has clearly made major contributions to the department and to the pathology profession. Best wishes on a wonderful retirement. You've truly left a large pair of necropsy boots to fill.



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## GET TO KNOW THE LABORATORY

**AVIAN DIAGNOSTICS**—The Avian Diagnostics Section provides disease testing services for commercial poultry, exhibition/backyard poultry and waterfowl flocks, gamebirds, petbirds and wild avian species. This section also provides educational opportunities for bird owners, houses the Colorado State Agency for the National Poultry Improvement Plan and oversees the Colorado Avian Disease Surveillance Program. In addition, members of this section are also part of a CSU avian disease research group that works on domestic and international research projects. The section is staffed by Section Head Dr. Kristy Pabilonia, Laboratory Technicians Christina Gerhard and Angela Tucker, Program Specialists Nikki Malone, Rachel Nichols, Marshall Page and Sarah Millonig, graduate student Katie Slota and CSU veterinary and undergraduate students Albert Chai, Brandon Fraser, Jasmine Tom, Breeann Flores, Sadie Maybach, Kyran Cadmus, Ryan Henderson, Laura Backus and Ean Smith.



Front Row (L-R): Albert Chai, Sarah Millonig, Nikki Malone, Dr. Kristy Pabilonia, Jasmine Tom, Laura Backus. Middle Row (L-R): Breeann Flores, Angela Tucker, Rachel Nichols, Sadie Maybach, Katie Slota. Back Row (L-R): Ryan Henderson, Brandon Fraser, Marshall Page, Kyran Cadmus, Christina Gerhard, Ean Smith.

## NEW RESIDENTS--

**Brett Webb** grew up in Canon City, CO. He attended the University of Southern Colorado and earned a BS in biology. He received his DVM from Colorado State University. His professional interests are orthopedic pathology and infectious disease.

**Michael Wiseman** received his undergraduate and DVM degrees from UC/Davis, graduating most recently in 2001. He then worked two years at the San Francisco SPCA Animal Hospital before moving to Sydney, Australia. While in Sydney, Michael continued to practice small animal medicine at a few Sydney vet hospitals and had the opportunity to treat injured wildlife including parrots, possums, lizards, and the odd fruit bat. Michael's desire to pursue a career in Clinical Pathology drove him back Stateside, where he is now with CSU to complete a Master's degree and residency.

**Karen Fox** received her DVM from Colorado State University in May 2007. She earned her bachelor's in Animal Ecology at Iowa State University in 2002 and is originally from Cleveland, Ohio. She is interested in wildlife diseases and hopes to pursue a PhD in this area.

**Chuck Halsey** received a BA in Zoology from Connecticut College and an MS in Biochemistry and DVM from Auburn University. His Master's thesis involved studying the role of the cAMP-elevating agent Paylean™ on the transcriptional control of lipid metabolism in swine, a project funded by Eli Lilly. During vet school, Chuck's passion for research continued, which led him to pursue a career in pathology. He completed two summer internships at Pfizer Global Research and Development, where he developed in situ hybridization techniques to identify potential biomarkers involved in the pathogenesis of canine vascular lesions and to look at tissue distribution of mRNA expression for multiple drug targets. During his clinical year, he was awarded an ACVP externship scholarship, which he completed in the Comparative Molecular Pathology Unit at the NIH.

**Sofia Arthur**, born and raised in NYC, received her DVM from Colorado State University in 1995. She practiced small animal medicine for 12 years in Connecticut and Colorado. She returned to CSU to pursue her interests in cancer research.



Brett, Michael, Karen, Chuck and Sofia

**Groundbreaking for the new Diagnostic Medical Center was December 7<sup>th</sup>. The estimated completion date for this new 90,000 sq ft building is 2009. We had a wonderful event and thanks to the many attendees!**

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## WHAT'S IN THIS ISSUE

- PCR to Detect *T. foetus* in Beef Bulls
- What Are We Seeing in Alpacas
- Local Veterinarian Honored
- Veterinary of the Year Service Award
- Distinguished Teacher Award
- *Otobius megnini*—The Spinose Ear Tick
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- Hints on Equine Herpesvirus-1 Serology
- Protein Electrophoresis Equipment
- Neoplasia in Wildlife
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- Abortions in Small Ruminants
- Necropsy of Suspected Organic Pesticide Toxicity
- Who's New in the Lab

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