Colorado State University Veterinary Diagnostic Laboratories

✎ Letter from the Director

Certainly many events, both locally and nationally, have transpired since our last LabLines. Locally, the Veterinary Teaching Hospital has been renamed the James L. Voss Veterinary Teaching Hospital upon the retirement of Dr. Voss. We now have a new dean, Dr. Lance Perryman, who began October 1. New pathology residents—Drs. Jonathan Arzt and Karen Zaks—have begun their programs. Faculty pathologist Dr. Dale Baker has left and a search for his replacement is in progress. New staff include Steve Burns in Bacteriology, Joanne Hovland in the milk culture area, Larry Ludden in the pathology/biopsy section, Wade Clemons in the necropsy area, and Dan Erickson in the histology laboratory. This fiscal year we had 66,112 accessions and performed 213,866 tests, an increase of 12% and 7%, respectively. We had to increase prices of certain tests to adequately meet the increased workload. We recently installed our new WR2 tissue digester, an improved and safer method of disposing of deceased animals. Thanks to USDA and the Colorado Department of Agriculture for help with this purchase.

We enjoyed visiting with many of you at the Annual Colorado Veterinary Medical Association meeting in September. The tragic events of September 11 disrupted the meeting, but we continued and it was comforting to be among friends during this time of sorrow. Since that time, we have been on high alert for unusual disease outbreaks and we are members of the National Veterinary Medical Diagnostic Laboratory Network. The Network was formed last year in an effort to counter acts of bioterrorism.

We hope you enjoy this issue of LabLines and look forward to future interactions with you. We hope to see some of you at the Annual Conference in January at Colorado State University.

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FELINE INFECTIOUS PERITONITIS
—Hana Van Campen

Feline infectious peritonitis (FIP) is a fatal disease of cats caused by a coronavirus. FIP is characterized clinically by fever, depression, anorexia and weight loss. Abdominal and pleural effusions resulting in abdominal distension and dyspnea may develop in the "wet" form, or granulomas may develop in multiple organs in the "dry" form. There is now good evidence that FIP is a mutant of feline enteric coronavirus (FeCV) that arises in individual cats. FeCV is a common viral infection of cats that usually causes subclinical disease or mild diarrhea. Cats can develop chronic FeCV infections of the ileum and colon, shed the virus in feces for at least seven months, and be asymptomatic.

Factors that influence whether cats infected with FeCV will develop FIP include age of the cat at the time of infection, dose of virus, viral mutations and immunity. Infection early in life increases the risk of developing FIP. Experimentally, high doses of FeCV result in viremia, seroconversion and an increased percentage of kittens that develop FIP. Kittens that receive low doses of FeCV are less likely to develop viremia, detectable serum antibodies or FIP. A high initial dose of FeCV or FIPV increases the number of progeny viruses that are produced in the GI tract, increasing the chance that a mutant capable of systemic spread will occur. Experimentally, viruses isolated from cases of FIP will cause FIP in a higher percentage of cats than FeCV.

Suspected immune defects predisposing cats to FIP include ineffective cell-mediated immunity (CMI), and pre-existing infections with feline leukemia virus or feline immuno-deficiency virus. FIP primarily affects cats less than 1 year of age, possibly reflecting relative immaturity of the CMI response in kittens as compared to adult cats. The risk of developing FIP decreases in cats 5-to-10 years of age, but increases again after 13 years of age. A genetic pre-disposition to FIP has been demonstrated in Persian cats and may occur in other breeds.

Immune Enhancement. Researchers have shown that cats will respond to the spike (S) protein of FIPV/FeCV by making neutralizing antibodies and binding antibodies. The binding antibodies attach to the virus particles and enhance the uptake of the antibody-virus complexes by macrophages. The macrophages become infected and may spread the virus systemically. Immune enhancement has been shown to occur in experimentally immunized cats, but has not been demonstrated in naturally infected cats.

Vaccination. Currently, there is one FIPV vaccine, Primucell FIP (Pfizer), on the market. The modified live-FIPV vaccine is a temperature sensitive mutant that's replication is limited to the nasal passages. Stimulation of mucosal immunity in the oral and nasal passages should limit FeCV infection. The vaccine is labeled for administration to kittens greater than 16 weeks of age. In one study, 75% to 85% of vaccinated cats were protected against FIP when challenged with virulent FIPV, compared to 17% to 20% survival in unvaccinated controls. In a second experiment, 95% of vaccinated kittens and 75% of controls survived when challenged with a lower dose of virus.

The risks of immune enhancement of disease associated with the MLV-FIPV vaccine are uncertain. When tested by Cornell University, the MLV-FIPV vaccine showed evidence of immune enhancement or increased disease in immunized versus control cats. Subsequently, Louisiana State University tested this vaccine for efficacy and evidence of immune enhancement was not found. The difference between the results of the two studies may be due to the dose of challenge FIPV used; a higher dose of challenge virus was used in the Cornell study.

Clinical Pathology. Hematologic values for cats with FIP include neutrophilia with increased segmented and band neutrophils, lymphopenia, eosinopenia, and a decreased albumin/globulin ratio due to a hypoalbuminemia and hyperglobulinemia. Effusions usually have a protein content of $\geq$ 35 g/dl.

Serology:ELISA versus IFA Titers. There are two serology tests that detect antibodies to FIPV—an ELISA (qualitative) and an IFA (quantitative). Neither test distinguishes between antibodies to FeCV or FIPV. Antibodies to FIPV/FeCV are detected in a high percentage of free-roaming cats, cats in multiple cat households and vaccinated cats. Seropositive cats include vaccinates, normal (FeCV-exposed) cats and cats with FIP. The distribution of antibody titers in normal cats and cats with FIP is the same. Cats with clinical FIP may have low FIPV/FeCV titers. Because of this, serologic titers are poor predictors of the risk of developing FIP and are unreliable indicators of current disease. There is no correlation between antibody titer and FeCV shedding. Diagnosis of FIP still relies heavily on clinical and pathological findings.

Serology can be useful in determining whether a new kitten or cat can be safely introduced into a multi-cat household or cattery. If indoor cat(s) in a household or cattery are seronegative, then serology can be used to screen new cats to prevent introduction of FIPV/FeCV. The use of this approach is highly dependent on the "biosecurity" of the household. If the new kitten is seronegative for FIPV/FeCV and the household cats are seropositive, then immunization of the kitten prior to introduction into the household is advisable. As there is no apparent benefit to vaccinating seropositive cats for FIPV, serology can be used to determine whether to proceed with vaccination.

FIP FA and IHC. Fluorescent antibody (FA) and immuno-histochemical (IHC) procedures to detect FIPV or FeCV antigens can be performed on granulomas obtained by biopsy or necropsy. These procedures do not distinguish between FIPV or FeCV, but the detection of antigen systemically supports a diagnosis of FIP.

FIP PCR. We offer a PCR-based test to detect FIPV RNA. Similar to the case of FA and IHC methods, the PCR test does not distinguish between FeCV and FIPV. A PCR positive test on CSF, thoracic fluid, or tissues collected by biopsy or necropsy (other than intestine or intestinal lymph nodes), supports a diagnosis of FIP.

Refs: Gerber et al., 1990, Vaccine 8. Paltrinieri et al., 1998, Vet Immunol Immunopath 65. Poland et al., 1996, J Clin Microbiol 34.

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Serology—Submit 1cc serum, red-topped tube, refrigerate (ship on ice or chill pack overnight). Fee=$6.50
FIP/PCR—Submit fresh tissue, 1cc effusion or 2cc whole (EDIA) blood. Fee=$28.

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MILK MYCOPLASMOLOGY
—Doreene Hyatt and R. Page Dinsmore

As many of you might be aware, we have been analyzing our milk mycoplasma culturing techniques. We began this analysis because of some interesting results that were reported during a comparison study done by one of our clients. The client had split a few string samples to send to our laboratory and to another laboratory. When the results came in, the other laboratory had all 10 string/bulk milk tank samples positive for mycoplasma where we only had five positives. Although the clinical status of the herd did not correlate with having all 10 samples positive, we were concerned that the samples were not split correctly (since this was done at the farm), that the samples were contami-nated at the other laboratory, or that our sensitivity was too low to pick up the positives. Since we were unable to verify if either of the first two alternatives had occurred, we decided to recheck our sensitivity by doing a blind culture test.

For this test, we collected 211 samples that were from our laboratory or that had been shipped to us from other laboratories. We split all 211 samples into four separate samples and shipped them to three other laboratories for mycoplasma culture and cultured them ourselves. When the results were in, our sensitivity appeared to be lower than other laboratories. In other words, we found fewer positive samples than did other laboratories. One could conclude that other laboratories reported false-positive results due to inability to distinguish mycoplasmas from similar non-

pathogenic species. But we decided to assume we reported false-negative results and resolved to modify our techniques to improve our laboratory sensitivity.

We started with the media, since the media can have the largest impact on growth of microorganisms. We make our own media with fetal bovine serum (instead of the horse serum used by the other laboratories). According to all of the published literature, this is a preferred supplement but is rarely used because it is much more expensive. We did not believe our media was the problem, but decided to purchase some of the media used by other laboratories and re-culture samples used in the previous test.

To test the samples, we re-cultured samples most other laboratories had isolated mycoplasma from (and we had not), as well as some we had reported as positive for mycoplasma. We plated these samples onto the media the other laboratories were using. We did find a few more samples were positive. However, our most startling finding was samples that were enriched in broth media and then filtered before plating had a lower probability of being positive, both before and after enrichment. These findings seemed illogical. We are enriching the sample to encourage the growth of mycoplasma! Unfortunately, an occasional side effect of enrichment is increased growth of contaminating background organisms, so the enriched culture broth must be filtered before the final plating. It appears the mycoplasma organisms are being trapped in the filter along with other microorganisms and are not available for plating. This indicates to us it is not our media that is a concern, but perhaps the final filtering step.

Our plans at this stage are twofold. First, we will be changing our methods to mimic procedures used at other laboratories. Specifically, we will apply the milk from all individual cow samples directly to plates without the intervening broth enrichment procedure. A side benefit to this direct plating procedure is improved turnaround time for results – we will perform an initial reading at 72 hours and a final reading at seven days. Additionally, we will perform another blinded culture study on mycoplasma positive and negative samples in the coming month, comparing our sensitivity with the new and old methods to another laboratory's results.

Stay tuned for an update when we complete this new set of samples!

OTITIS INTERNA IN CALVES ASSOCIATED WITH *MYCOPLASMA* SPP.

—Randy Basaraba

Three approximately one-month-old Holstein calves with a history of a gradual onset of either unilateral or bilateral ear drop, facial nerve paralysis, epiphora and occasionally head tilt, were submitted for euthanasia and necropsy. Clinical signs had a gradual onset in calves ranging from 10 days-to-four weeks of age and were non-responsive to treatment with broad-spectrum antimicrobial therapy. Gross necropsy findings from all three calves were characterized by bilateral swelling and softening of the tympanic bullae with former air spaces filled with white to tan caseous exudate. There was replacement of bone with fibrous connective tissue characterized histologically as marked, bony remodeling with suppurative exudate (abscessation) and pyogranulomatous inflammation. This resulted in loss of normal architecture of the middle ear. Randomly, within the lesions, there were scattered bacterial colonies. One of three calves had mild, chronic con-solidation and abscessation of the cranioventral lung lobes. There were no other significant gross or histologic lesions of clinical significance. Aerobic cultures of middle ear exudates from all three calves and lung from one calf were positive for a heavy growth of *Mycoplasma* spp. and a light growth of non-hemolytic *Streptococcus* spp. and/or *Fusobacterium necrophorum*.

Otitis media has been reported in both pre-weaned and post-weaned calves with the most common bacterial isolates being *Haemophilus somnus*, *Pasteurella multicoda*, *Streptococcus* spp., *Actinomyces* spp. and, more recently, *Mycoplasma bovis*. Otitis media in pre-weaned calves can occur from 4 days-to-10 weeks old, and in post-weaned calves from 4 months to 18 months of age. The disease has been reported in both beef and dairy breeds with mortality that ranges from 1% up to 80% in individually housed dairy calves. The presenting clinical signs of ear drop, facial nerve paralysis, epiphora and occasionally head tilt are typical. These can progress to otitis interna and meningitis characterized by ataxia, recumbency, nystagmus, opisthotonos and eventually death. Differentials for signs of otitis interna include infestation by the bovine ear mite, *Raillietia auris*. This is differentiated from bacterial causes by normal tympanic bullae without signs of inflammation or rupture of the tympanic membrane.

M. bovis can be found worldwide and has been associated with polyarthritis, tenosynovitis, mastitis, pneumonia, genital infections and abortions in cattle. *M. bovis* is a common cause of mastitis, which results in shedding of organisms in milk which may serve as a source of infection of calves fed waste bulk tank milk. *M. bovis* often is recovered from the respiratory tract of cattle, particularly in herds with a history of mastitis due to *M. bovis*. Organisms may gain access to the middle ear by first colonizing the nasopharynx with subsequent migration through the auditory tube. *M. bovis,* however, is a common commensal and can be isolated from the respiratory mucosa of normal cattle. Therefore, other factors such as dose of organisms ingested, hygiene, and feeding practices such as bottle feeding, may contribute to the incidence of *M. bovis* otitis media. Spread of *M. bovis* to the middle ear also can be associated with bacteremia, particularly in severe infections such as tenosynovitis, arthritis or bronchopneumonia.

The current cases of otitis interna associated with *Mycoplasma* spp. in the dairy calves presented are similar to cases previously reported. While the pathogenesis is poorly understood, eliminating exposure of calves to contaminated milk should be considered as a management strategy in chronically infected herds.

FROM THE WESTERN SLOPE, *Haemonchus contortus*

—Darrell Schweitzer

Although sheep populations in western Colorado have been in decline in recent years due primarily to a decrease in large range herds, there has been a compensatory increase in the number of small farm flocks. As a result, we have seen an increase in manifestations of sheep parasitism by round-worms, principally *Haemonchus contortus*. This is a result of increased confinement of these smaller flocks to irrigated pastures, allowing for sometimes heavy infestations of these pastures. Many small flock owners are "hobby" farms, and often are not aware of the conditions that lead to this disease in their sheep and goats.

Haemonchus contortus is a nematode parasite which, as an adult, lives in the abomasum where it sucks blood. In heavy infestations, this can rapidly lead to ill thrift, depressed lacta-tion, life-threatening anemia and intermandibular edema. We have, for some reason, seen intermandibular edema less often, even when severe anemia has developed. The owners often only report sudden death, although sometimes they observe difficult respiration and assume the animal has pneumonia, until their treatment for pneumonia produces no favorable result.

You may find it useful to review for such owners the life cycle of this parasite. Adult females can produce 5,000-to-10,000 eggs per day. These eggs are expelled in feces where, with favorable temperature and humidity conditions, they may hatch within 24 hours to form L1 larvae. These molt twice more over a four to 10 day period to produce L3 larvae, which are infective. This stage is dispersed on forage

by hooves, rain and, to a limited extent, the larvae's own movement, where they are ingested by foraging animals. After ingestion, the prepatent period occurs while L4 and L5 larvae are developing, about 15-to-22 days. Recall, however, that because of the phenomenon of hypobiosis, development may be delayed by weeks to months, until more favorable conditions are present. This becomes an important consideration in planning for management of this disease.

Inexperienced owners have little knowledge of effective worming programs, often stating that the sheep or goats are "wormed regularly." We should be careful to point out that effective timing of worming programs must take into account hypobiosis, as well as development of resistance to any particular wormer. Some worm medications only are effective against adults while others, such as ivermectin, have some activity against larvae in arrested development.

We also should help them develop management strategies for effective worming programs and pasture rotation to lessen exposure by decreasing pasture contamination. Animals from situations where there is little exposure to intestinal parasites (ie., "bummer" lambs from range flocks) will have little immunity to these parasites and are highly susceptible.

BOVINE LEUKEMIA VIRUS (BLV): Transmission and Control
—Hana Van Campen

Bovine leukemia virus (BLV) is a retrovirus that infects cattle. BLV infects B-lymphocytes and is highly cell-associated. That is, little free virus is found in the serum of infected cattle. The biology of retroviruses is such that BLV infection is life long.

BLV Infection and Disease

- Over 60% of BLV-infected cattle do not display any clinical abnormalities. Some reports indicate a decrease in milk production in BLV+ cows.

- Approximately 30% of BLV-infected cattle develop a persistent lymphocytosis (PL). A diagnosis of PL is made when an animal has a high lymphocyte count (>12,000 cells/μl) in at least two blood samples collected three months apart. PL cattle do not display any clinical abnormalities. However, about 30% of the lymphocytes from PL animals contain BLV and they serve as a source of infection for other cattle.

- A small proportion (0.1% to 5%) of BLV-infected cattle develop lymphosarcoma (LS). These animals also have high levels of BLV and can serve as a source of infection for other cattle.

Transmission

- Horizontal—Transmission of BLV is largely horizontal. Prevalence differences between beef (low) and dairy herds (high), and between age groups in dairy herds suggest that BLV infection rates are related to management practices. The risk of infection is particularly increased during the period in which dairy heifers are bred, calved, or when they enter the milking herd.

- Blood—The main source of BLV is blood from a PL or LS cow. Transfer of as little as 50×10^{-6}cc of blood containing BLV-infected lymphocytes from a PL cow can transmit BLV. Procedures that transfer blood from animal to animal such as using needles to collect blood or IV treatments to multiple cows, obstetric (OB) sleeves, and dehorning give a high risk of BLV transmission.

- Oral route—Risk of infection via milk and colostrums is age dependent. Newborn calves are most susceptible and by $\geq$ 21 days of age are refractory to infection by this route. Ingesting colostrums containing antibodies to BLV is associated with protection of the calf from BLV infection.

- Vertical—In-utero transmission rates are low (approximately 4%). Risk of in-utero transmission is higher for cows with PL or LS, and negligible for BLV-infected cows that are not PL.

- Low risk—Procedures that represent a low risk of transmission include using a common needle for subcutaneous or intramuscular injections (eg., vaccines), natural breeding or artificial insemination, embryo transfer, ear tagging and tattooing. Biting insects are associated with a low risk of infection.

Susceptibility Factors

- Susceptibility to BLV infection is highly heritable (coefficient of heritability = 0.48).

- The risk of developing PL is dependent on the genetic background of cows.

- Susceptibility to LS also has a genetic basis independent of the risk of developing PL.

- Genetic susceptibility is responsible for the observation of cow families with LS.

Strategies

- Test and removal: Consider in herds with low prevalence of infection and high genetic value. Goal: BLV-free status.

- Test and separate: Consider in herds with capability of separating groups of cattle. Goal: Reduce prevalence by reducing new BLV infections.

- Test and institute measures to reduce BLV transmission. Goal: Reduce new BLVinfections.

- No control measures: Consider in herds where the economic losses due to LS are less than the cost of instituting control procedures. Goal: Cost-effective production.

Control Measures to Reduce Transmission

- Test calves from PL and LS cows using the BLV Polymerase Chain Reaction (PCR) test and remove BLV+ calves. Eliminates calves infected in utero.

- Feed colostrums from BLV-free cows or feed pasteurized colostrums/milk. Prevents neonatal infections.

- Change needles used in treatments and blood collection between individual cows. Prevents infection via transfer of blood from PL and LS cows.

- Change OB sleeves between cows. Prevents rectal inoculation with blood from PL and LS cows.

- Disinfect dehorning and surgical instruments between animals. Prevents iatrogenic transmission from PL and LS animals to others.

- Cull LS and PL cows as is economically feasible. Reduces chances of iatrogenic transmission and birth of BLV+ calves.

- Group LS and PL cows together and separate from BLV negative cows, as risk of horizontal transmission is greater in pens with a high percentage of LS and PL cows.

Diagnostic Tests Available

- Clinical Pathology—CBC large animal PL = >12,000 lymphocytes/µl. Test performed daily.

- Serology—BLV AGID or BLV ELISA = Detects antibodies to BLV. Does not distinguish between passively (colostral) or actively-acquired antibodies. AGID completed in 48 hours if received by 4:00PM.

- Virology—BLV-PCR. Detects BLV provirus. Test completed in 3-5 working days.

Interpretation

- Most BLV-infected cattle will be AGID/ ELISA+ and PCR+.

- Calves that are AGID/ELISA+ and PCR- have ingested BLV antibody + colostrums, but are not infected.

- Cattle (>3 months of age) that are AGID/ELISA+ and PCR- are infected. BLV may be present in lymph nodes and not in circulating lymphocytes.

- Cattle that are AGID/ELISA- and PCR+ are infected, but have not seroconverted.

Clinical Pathology—Submit 2cc EDTA blood, purple-topped tube and a blood smear slide. Fee=$15.
Serology—Submit 2cc serum, red-topped tube. AGID Fee=$5/test; ELISA Fee=$6/test.
Virology BLV PCR—Submit 5cc EDTA blood, purple-topped tube or tissue sample. Fee=$22.

CLOSTRIDIUM PERFRINGENS GENOTYPING OF ENTERIC ISOLATES
—Anita Dennison, Bob Ellis, Roberta Magnuson and Doreene Hyatt

Clostridium perfringens is a worldwide, ubiquitous organism. It exists in the environment, and as a commensal organism in the gastrointestinal tract of many mammals. Individual pathovars of *C. perfringens* are known to induce a myriad of diseases in humans and animals. Disease caused by *C. perfringens* is severe, and often fatal, resulting in substantial losses for owners or producers.

C. perfringens, in addition to causing disease in animals, also is known to cause several severe and debilitating diseases in humans. The most publicized of these are gas gangrene and enteritis necroticans. Thousands of soldiers died in the United States Civil War and in World War I due to *C. perfringens* Type A wound infections that resulted in gas gangrene.

Enteritis necroticans, caused by *C. perfringens* Type C, was defined following World War II. Many Europeans had gone through a period of decreased protein consumption during the war and a resulting decrease in production of proteases in these individuals. The beta toxin, produced by *C. perfringens* Type C, typically is very sensitive to degradation by pancreatic proteases and is readily degraded in their presence. A culmination of inadequate antibodies to the beta toxin, a relative lack of enzymes to inactivate beta toxin, and consumption of a contaminated, high protein meal resulted in a marked increase in cases of enteritis necroticans in postwar Europe. This syndrome, also termed Pigbel, is characterized by rapid proliferation of toxigenic *C. perfringens* in the intestine, which terminates in transmural inflammation and bowel necrosis. Surgical intervention is necessary to remove sections of the necrotic bowel, and may be the only successful treatment for the disease.

There are five known types of *C. perfringens*. Animals often are the victims of fatal intestinal disease based by Types B, C, D and E. Evidence is mounting rapidly to support the role of *C. perfringens* Type A as an entero-pathogen. Humans and animals also are victims of *C. perfringens* enterotoxemia, a food-borne disease caused by the enterotoxin produced by *C. perfringens*. The type of disease produced by individual genotypes is due to the mechanism of action of their specific toxins. *C. perfringens* types are differentiated according to the lethal toxins that they produce.

The five toxinotypes of *C. perfringens*, the major toxins they are capable of producing, and a few of their associated diseases are listed in the table below.

Beta 2 toxin is the latest *C. perfringens* toxin to be discovered and defined, first reported in 1997. The beta 2 toxin was first noted in conjunction with *C. perfringens* Type A from horses and piglets with enterocolitis. It has since been cited as a contributing factor to *C. perfringens* disease in horses, pigs and cattle in Europe and North America. The action of the beta 2 toxin--destruction of the cell membrane--is very similar to that of beta toxin, thus the newly defined toxin was named 'beta 2.' However, the gene responsible for production of beta 2 toxin is 85% different from the gene responsible for production of beta toxin. So beta 2 is not a derivative or mutant of beta but is a newly discovered toxin. It appears that Type A strains that possess the beta 2 toxin gene in addition to the *alpha* toxin gene, may be more likely to cause disease than strains without the beta 2 toxin gene.

Pathogenic mechanisms of the major toxins produced by *C. perfringens* types are listed at the top of the next page.

Type of *C. perfringens*	Major toxins produced by this type of *C. perfringens*	Representative diseases associated with this type of *C. perfringens*
A	Alpha (+/-) beta 2 and/or enterotoxin	Necrotic enteritis of fowls; food poisoning and gas gangrene in humans; cattle and sheep enterotoxemia; enterocolitis in horses and pigs; hemorrhagic gastroenteritis and chronic diarrhea in dogs
B	Alpha, beta 1, epsilon (+/- beta 2 and/or enterotoxin)	Hemorrhagic enteritis in calves and lambs; enterotoxemia in sheep; dysentery in lambs
C	Alpha, beta 1 (+/- beta 2 and/or enterotoxin)	Pigbel (Enteritis necroticans) of humans; necrotic enteritis of fowl; necrotic enteritis and enterotoxemia of lambs, calves, goats, pigs and foals
D	Alpha, epsilon (+/- enterotoxin)	Lamb enterotoxemia; enterocolitis in goats
E	Alpha, iota (+/- beta 2 and /or enterotoxin)	Enteritis in calves, lambs and foals

C. perfringens toxin	Activity or mechanism of action of this C. perfringens toxin
Alpha	Phospholipase (lyses cell membranes), hemolytic, necrotizing, lethal
Beta	Inflammatory, necrosis of intestinal mucosa, lethal
Epsilon	Increases intestinal permeability, toxic to central nervous system, lethal
Iota	Dermonecrotic, lethal
Enterotoxin	Gastroenteritis
Beta 2	Hemorrhage and necrosis of the intestinal wall

Differentiation of the types of *C. perfringens* enables identification of disease associated with that particular type, and appropriate treatment or vaccination modalities. In the past, toxin neutralization tests in mice have been used to determine the type of *C. perfringens* isolated. Currently, a multiplex PCR, developed by Dr. Glenn Songer at the University of Arizona, is used to identify these genotypes. The multiplex PCR uses six individual primer pairs for detection of genes encoding alpha, beta 1, beta 2, epsilon, iota and enterotoxin. The multiplex PCR is an excellent genotyping tool and is able to amplify and detect *C. perfringens* genes present. This enables accurate recognition of the type of *C. perfringens* isolated from a particular animal.

Confirmation of the presence of a gene encoding a particular toxin is a reflection of an isolate's capability to produce that toxin, but cannot be used as the sole means of diagnosis for *C. perfringens*-related enteritis in a particular animal. Presumptive diagnosis of *C. perfringens*-related disease is based on culture and positive identification of *C. perfringens* organisms in conjunction with a compatible history, clinical signs, and gross or histologic lesions.

Veterinary laboratory diagnosticians traditionally have been reluctant to consider *C. perfringens* Type A as an important enteropathogen because this organism has been proven to be a commensal organism in the intestinal tract of animals. Furthermore, this organism proliferates rapidly in the intestine post-mortem, often making isolation from necropsy specimens of questionable diagnostic significance. Isolation from necropsy specimens is considered significant if samples have been acquired from necropsy specimens less than two hours post-mortem and frozen immediately. A *C. perfringens* multiplex PCR with the amplified DNA segments seen in association with specific types of isolates is shown in the following figure.

We perform *C. perfringens* culturing and genotyping on samples acquired from presumed cases of Clostridia-associated disease in numerous animal species. This is hoped to improve diagnosis of *C. perfringens*-related enteritis and aid in effectiveness of treatment regimens. Varying levels of production of certain toxins has been

proposed as a determinant in the severity of disease induced by *C. perfringens*. We currently are developing assays to quantify the amount of toxin in specific samples. This will eventually assist in definitive assessment of the relevance of *C. perfringens* in certain diseases.

Species Affected by *C. perfringens*

Cattle	Songbirds
Sheep	Alpacas
Pigs	Llamas
Goats	Dolphins
Horses	Elk
Dogs	Mule deer
Poultry	Bighorn Sheep

Agarose Gel Amplicons
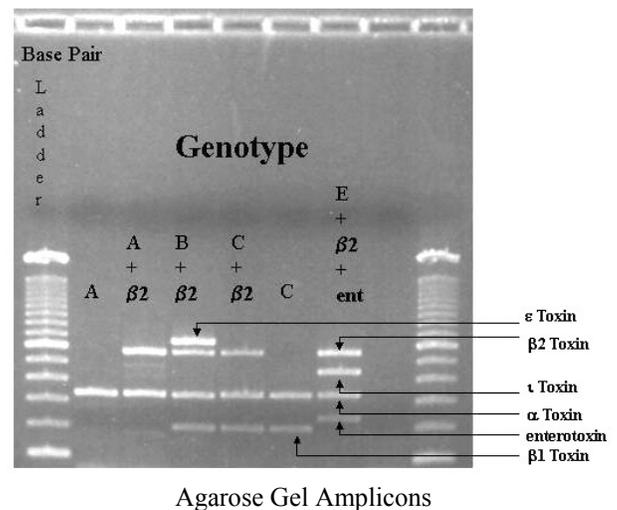
C. perfringens genotyping: Submit two fecal or intestinal content swabs in a refrigerated/cooled Copan tube, Port-a-cul tube, or other anaerobic transport media. Aerobic/anaerobic culture fee=$30. Genotyping fee=$30.

DIARRHEA IN FOALS: EQUINE NEONATAL ENTERIC CLOSTRIDIOSIS

—Charlie Dickinson, Kirsten Tillotson, Josie Traub-Dargatz, Robert Ellis, Doreene Hyatt, Paul Morley, and Mo Salman

Diarrhea in foals is a serious problem with a number of potential causes including bacteria, viruses and parasites. A common and very serious type of diarrhea caused by *Clostridium perfringens* and *Clostridium difficile* occurs in newborn foals. Both of these organisms can cause acute gastrointestinal disease in young foals characterized by signs of malaise, bloating, colic and diarrhea, which often is bloody. The disease tends to be rapidly progressive and often fatal. Early recognition, prompt medical attention, and methods of management aimed at prevention are essential.

The disease typically occurs on the second or third day of life, often in foals that are particularly vigorous. The initial signs may include malaise, diarrhea and/or colic. Since the progression of the disease can be very rapid, foals showing signs such as drooping ears, head hanging and not getting up to suckle should be observed very closely. With progression of the disease, signs of colic, with or without diarrhea, become apparent. Signs include teeth grinding, abdominal distension, getting up and down more than usual, rolling onto the back, or rolling over repeatedly. Often, the affected foal will be seen to suckle the mare, then drop to the ground and roll on its back. Diarrhea may be watery, thick and orange-colored, black, or contain frank (bright red) blood, and may have a strong odor. Bloody diarrhea is characteristic of the disease and is a serious sign. The presence of colic signs and/or bloody diarrhea indicates a need for prompt medical attention.

Foals with colic and diarrhea due to enteric clostridiosis often require intensive veterinary medical care. Dietary restriction, intravenous fluid support, systemic and oral antibiotics, pain management, and good nursing care are basic elements of the treatment plan. Anti-ulcer drugs, gastrointestinal protectants, and probiotics also may be employed. Additional medical interventions may include plasma transfusions and intravenous feeding. Such treatment interventions generally require continuous care in the hospital setting. In one study of foals affected with enteric clostridiosis due to *Clostridium perfringens*, 54 percent of the foals died despite such intensive care measures.

The high mortality associated with neonatal enteric clostridiosis underscores the need for preventive strategies. Definitive recommendations for prevention have not been developed because of lack of understanding of the epidemiology of the disease. Currently, we recommend appropriate perinatal husbandry and hygiene practices such as washing the udder, inner thighs, and backside of the mare prior to the foal suckling. Foaling stalls constructed of non-porous materials, which are easily cleaned, are best. Artificial surfaces are preferred over dirt floors. The walls and floor of the foaling stalls should be thoroughly cleaned with soap and water, and thoroughly rinsed and dried prior to and between foalings. The organisms produce spores which are not killed by disinfectants, so thorough cleaning is necessary to physically remove spores. Stalls in which there has been an affected foal should not be used for subsequent foaling unless they can be effectively cleaned. After the birth, the stall should be kept as free of manure as is possible and bedding changed often. Foaling mares on grassy pastures where the manure load is low may be an effective alternative to foaling under stall confinement.

Special precautions may be appropriate on farms with a history of neonatal enteric clostridiosis. Veterinarians may recommend the use of probiotics, oral antiserum preparations, or oral antibiotics in the neonate. While there is no vaccine specifically intended for horses available, specialized immunization strategies for brood mares can be developed under the direction of a veterinarian for farms with a clostridiosis problem.

The research team at CSU is investigating the epidemiology of equine enteric clostridiosis with an ultimate goal of developing effective and safe prevention measures. We have received funding from multiple sources including a donation from a private horse farm owner, USDA:CSREES funded Center for Economically Important Infectious Animal Diseases, the College Research Council for Horse Racing funds, and a pharmaceutical company. The first goal was to determine the frequency of shedding of *C. perfringens* in the normal broodmare and foal population. We investigated the optimal methods to culture the organism and then used these methods to identify *C. perfringens* in fecal samples from broodmares and foals. We further determined the types of *C. perfringens* using polymerase chain reaction (PCR). We determined that *C. perfringens* type C appears to cause disease whenever it infects neonatal foals. We also determined that the majority of normal foals shed *C. perfringens* type A in their feces. Further work will be necessary to determine why foals are so permissive for this organism and why some foals are ill if they have type A *C. perfringens* in their intestine and others remain healthy. Our current activities involve developing a test to detect the toxins produced by *C. perfringens* in a fecal or intestinal sample. We also plan to develop a test for detection of antibodies to the toxin in mare's blood and milk. We hope with these tests we will be better able to make a diagnosis of type A *C. perfringens* enterocolitis and potentially assist us in determining which specific type A isolates are causing disease.

ANTICOAGULANT RODENTICIDES
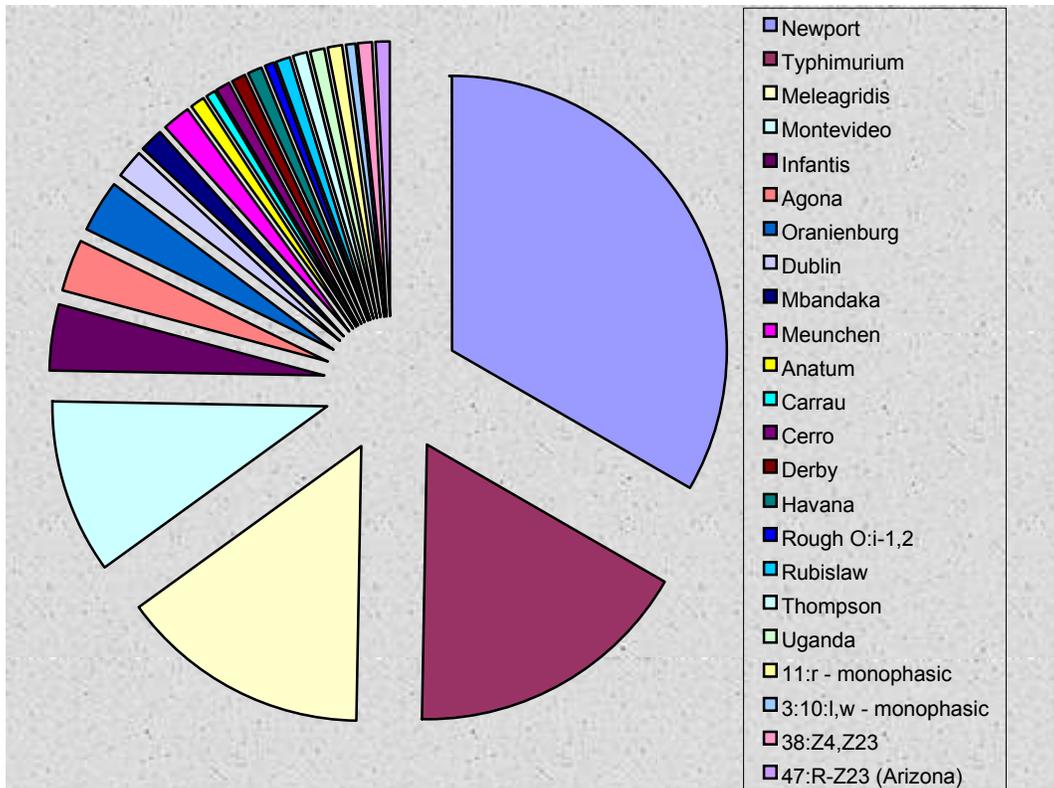### —Dwayne Hamar, Dale Baker, and Cathy Bedwell

Anticoagulant rodenticides were developed following the investigations of moldy sweet clover toxicity in cattle. Fungi form dicoumarol, the toxic agent, from coumarin found naturally in sweet clover. Dicoumarol's mechanism of action is the inhibition of vitamin K 2,3 epoxide reductase, resulting in defective clotting factor synthesis and clinical hemorrhage, or possibly death. After this discovery, warfarin, an analog of dicoumarol, was synthesized. Warfarin was the first anticoagulant rodenticide marketed. Because of its extensive use for many years, many rodent species became resistant to the effects of warfarin. Compounds effective against warfarin-resistant rodents have been developed as a consequence. These compounds are called second-generation anticoagulant rodenticides. Second-generation anticoagulant rodenticides are generally more toxic and have a longer tissue half-life than warfarin.

We visited farm stores, feed stores, grocery stores and agricultural chemical suppliers. Anticoagulant rodenticides were by far the most common rodenticides available and only two businesses carried warfarin. Second generation anticoagulant rodenticides are the most likely rodenticide poisonings you will encounter in clinical practice. Some stores carried three to four brand names of the same active ingredient. Warfarin was sold with the label, Ft. Dodge Bar Bait. Second-generation anticoagulant rodenticides sold in these stores consisted of brodifacoum, difethialone, diphacinone, bromadiolone and chlorophacinone. Brodifacoum sold as Just One Bite, Havoc, Hawk Rodenticide, D-Con, Farm Gard and Jaguar Bait. Difethialone sold as Mouse Die-ner. Diphacinone sold as Ramik, JT Eaton Bait Block and Tom Cat liquid concentrate. Bromadiolone sold as Boot Hill, and chlorophacininone sold as Rozol Pocket Gopher.
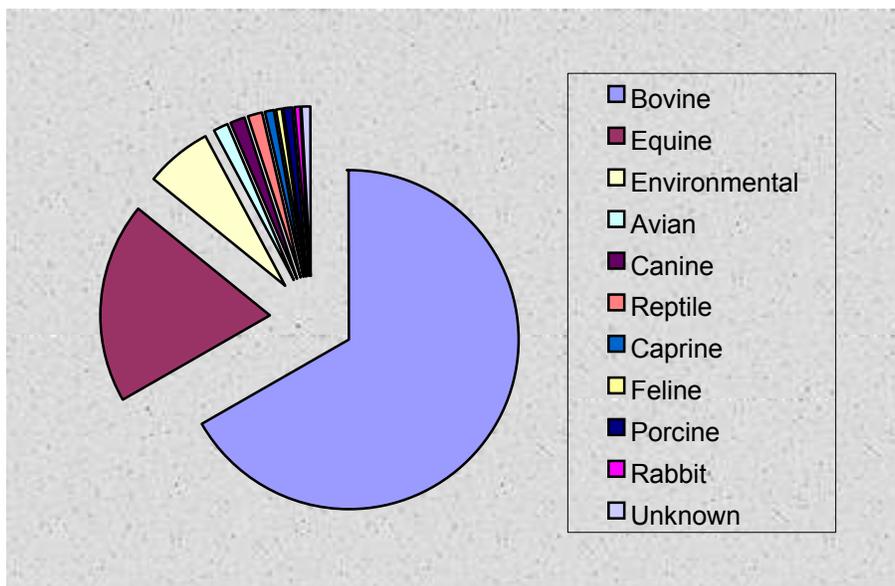
Clients that observe their pet consuming any of these compounds should be advised to have them evaluated for potential intoxication. The lack of clinical signs, even for prolonged periods of time following consumption, does not mean the pet is safe. After consumption, it is possible the animal may not bleed for several days. But they may have a prolonged coagulation time (OSPT or PT) in as little as 8-to-12 hours after consumption. Animals intoxicated by anticoagulant rodenticides should have forced cage rest to prevent bleeding from developing. Animals may not have clinical bleeding observed by the owner, and may first show with sudden, unexpected death. These animals most commonly bleed into the thoracic, pericardial or peritoneal cavity with death caused by hypovolemic shock or cardiac tamponade. Therapy for clinically-affected anticoagulant intoxicated animals depends on the type of compound the animal consumed and the specific clinical signs observed. Hypovolemic shock may require a blood transfusion and vitamin K administration for recovery, whereas a dog with just bruising may simply require vitamin K administration and forced cage rest. Vitamin K1 is the most effective form of vitamin K for therapy and should be administered daily for up to four weeks if a long-acting anticoagulant was consumed, or for several days if warfarin was consumed. Alternatively, animals may be administered vitamin K1 parenterally, followed by daily oral administration of vitamin K2. Clients with animals they suspect to have been intoxicated by Vitamin K antagonist rodenticides should always consult their veterinarian for proper diagnosis and treatment. If the source of the suspected anticoagulant toxicant is available, they should also take that to their veterinarian.

Anticoagulant rodenticide toxicity must be differentiated from other causes of coagulopathy such as liver disease, canine erlichiosis, congenital factor deficiencies, and platelet deficiencies or malfunction. Confirming the diagnosis of the coagulopathy as a result of anticoagulant rodenticide requires identification of the specific compound. A determination of the specific compound involved easily can be made by examining the ingredients on the label of the bait. If that is not available, then whole blood or liver can be used for analytical analysis. Knowing the anticoagulant rodenticide involved may affect the choice of therapeutic regimen. However, the concentration of the anticoagulant rodenticide found in blood or liver does not indicate the severity of the clinical disease. The availability of analytical results may take several days to a week. We presently send samples for anticoagulant rodenticide analyses to a referral laboratory.

Salmonella Serotypes isolated at Colorado State University Diagnostic Laboratories from January 1, 2001 to August 29, 2001

Legend:
- Newport
- Typhimurium
- Meleagridis
- Montevideo
- Infantis
- Agona
- Oranienburg
- Dublin
- Mbandaka
- Meunchen
- Anatum
- Carrau
- Cerro
- Derby
- Havana
- Rough O:i-1,2
- Rubislaw
- Thompson
- Uganda
- 11:r - monophasic
- 3:10:l,w - monophasic
- 38:Z4,Z23
- 47:R-Z23 (Arizona)

Animal Species from which Salmonella was recovered at the Colorado State University Diagnostic Laboratories From January 1, 2001 to August 29, 2001

Legend:
- Bovine
- Equine
- Environmental
- Avian
- Canine
- Reptile
- Caprine
- Feline
- Porcine
- Rabbit
- Unknown

DIAGNOSTIC TESTING FOR EQUINE HERPES VIRUS-1 IN CASES OF NEUROLOGIC DISEASE
--Hana Van Campen

Case History: In July 2001, approximately 40 horses in a dude string of 180 horses and mules developed neurologic signs acutely. Clinical presentation included circling, penile prolapse, urinary incontinence (dribbling), ataxia, paralysis of hindlimbs and recumbency. Other findings were distended urinary bladders, decubital ulcers and oral ulcers. Respiratory signs were not a major feature in these cases. A few horses had fevers before the onset of neurologic signs. Horses with neurologic signs had normal temperatures. Eventually, more than 50 horses developed neurologic signs.

Diagnosis: Whole blood samples collected in EDTA vacutainer tubes from 11 horses, CSF from two horses with neurologic signs, and a nasal swab were assayed for EHV-1 and EHV-4 by a polymerase chain reaction (PCR). A positive test was found in one of the two whole-blood samples obtained from horses early in the course of their disease. The remaining 10 blood samples, CSF, and nasal swab were negative for EHV-1 or EHV-4 primer specific products. Serum neutralizing (SN) antibody titers to EHV-1 were determined for seven horses. The PCR-positive serum sample had an EHV-1 SN titer of 1:128. Two horses had low SN titers (1:8 and 1:16), and four horses had elevated EHV-1 SN titers (1:512 and 1:1024).

Diagnostic Tests: EHV PCR is a rapid test that detects the presence of viral DNA. The best samples for this test appear to be nasopharyngeal swabs or whole blood (EDTA) collected during the acute phase (days three to 14) of the infection. Neurologic signs develop 6-to-10 days post-infection and may overlap the period of viremia in the early stages of neurologic disease. Although EHV-1 causes vasculitis and hemorrhage in the brain and spinal cord, these tissues and CSF appear to have relatively little detectable virus and are rarely positive by PCR or virus isolation (VI).

Serology is still a very useful diagnostic tool. Infected horses seroconvert rapidly, particularly if they have been previously exposed to or vaccinated for EHV-1. A four-fold increase in SN titers between the acute and convalescent phase often can be demonstrated. Elevated SN titers frequently are detectable in the acute phase and titers of $\geq 1:256$ in clinically affected horses supports a diagnosis of EHV-1. SN titers $\geq 1:4$ may be detected in the CSF of affected horses due to the vasculitis and leakage of blood in the spinal cord. However, contamination of the CSF sample with blood during collection also may result in an elevated titer.

Histopathology and immunohistochemistry are valuable in confirming a diagnosis of EHV-1 neurologic disease. Characteristic lesions in the brain and spinal cord (vasculitis, vascular degeneration and necrosis, and non-suppurative encephalomyelitis) are strongly suggestive of EHV-1 infection, and immunohistochemistry sometimes can be used to demonstrate viral antigen within affected blood vessels.

Continuing Investigation: Virus isolation also was performed with buffy coat cells from the EHV-PCR+sample. Cell cultures developed cytopathology characteristics typical of EHV-1. The virus isolated has been sent to Dr. George Allen, Gluck Equine Center, University of Kentucky, for further analysis. We hope Dr. Allen's research will give us some insight into why certain EHV-1 isolates cause neurologic disease. We'd like to thank Dr. Todd Cornish, Wyoming State Veterinary Laboratory, for his help with this case.

EHV PCR—Submit 5cc EDTA whole blood, refrigerate ship on ice overnight; nasal swab in PCR transport media available from the laboratory on request, or CSF in a sterile tube or RTT. Fee=$24.

EHV Serology—Submit 1ml of serum or CSF, acute and convalescent serum samples, refrigerate, send on ice or chill sample. Fee=$6.

RESULTS OF A SURVEY ABOUT *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* INFECTION IN COLORADO EQUIDS
--Andrea N. Torres, Wayne Cunningham, Josie Traub-Dargatz, Paul Morley, Doreene Hyatt, and Barb Powers

We thank all those veterinarians who participated in this survey.

Corynebacterium pseudotuberculosis infections cause a disease in equids commonly referred to as "pigeon fever," "pigeon breast," or "dryland distemper" and are the most common cause of ulcerative lymphangitis in equids. In the western United States, mainly California, the most common presentation in equids is external abscessation, which can occur anywhere along the lymphatic system. The infection can range from mild, small, localized abscesses, to a severe disease with multiple, massive abscesses containing liters of tan, odorless, purulent exudate.

The three most common sites for abscesses are the inguinal area, ventral abdomen, and the pectoral region (hence, the name pigeon breast). Internal abscesses also can occur. Abscessation and ulceration of one distal hindlimb starting

at the fetlock and progressing proximally via the lymphatic system is a common manifestation of ulcerative lymphangitis. A different biotype *C. pseudotuberculosis* is the etiologic agent of caseous lymphadenitis in small ruminants (sheep and goats). Although the risk factors for transmission of *C. pseudotuberculosis* in equids have not been absolutely defined, it is believed to have an insect vector. A previous wound or other trauma also may predispose equids to a *C. pseudotuberculosis* infection. This disease is contagious and usually affects multiple horses on the premise. *C. pseudotuberculosis* can be differentiated from strangles (*Streptococcus equi* ssp. *equi*) or other bacterial infections by routine aerobic culture or visualization of pleomorphic Gram-positive rods on a Gram-stained smear of the purulent exudate.

Prior to 1997, we annually reported approximately one isolation of *C. pseudotuberculosis* from horses statewide. During the fall of 1997, the Western Slope experienced an outbreak of *C. pseudotuberculosis* infections in horses concurrent with the vesicular stomatitis virus outbreak. Although only four isolates of *C. pseudotuberculosis* were submitted to us during that time, we determined through a survey and questionnaire that approximately 100 horses developed clinical signs of *C. pseudotuberculosis* infection. From September 1999 to January 2000, *C. pseudotuberculosis* was isolated from samples obtained from 18 horses in Larimer County alone. To gain a better understanding of this disease, we worked with the State Veterinarian's Office to determine the extent of *C. pseudotuberculosis* infection in Colorado equids. A questionnaire was mailed to those veterinarians currently practicing equine medicine in Colorado to determine the number of equids in Colorado that have had this infection and when these infections were observed.

The list of 550 large animal accredited veterinarians practicing in Colorado was obtained from the State Veterinarian's Office. The survey consisted of 16 questions. The case definition for the purposes of the survey was an equid with infection, caused by *C. pseudotuberculosis*, presenting with external and/or internal abscesses and/or ulcerative lymphangitis of the extremities, whereby confirmatory culture may or may not have been performed. A cover letter detailing the perceived increase in equine cases of *C. pseudotuberculosis* in Colorado, as well as descriptions of the disease, was enclosed with the survey and mailed to veterinarians in February 2001.

Out of 550 veterinarians, 196 responded for a 36% response rate. Of the 196 responses, 141 (72%) were usable. The other 55 veterinarians were not currently practicing equine medicine in Colorado. Of the 141 veterinarians with usable responses, 33 veterinarians (23%) had seen an equid matching the case definition (a suspected case) sometime between January 1996 and December 2000 (the study

period). Of the 33 veterinarians who had seen a suspected case within the study period, 23 veterinarians (70%) had seen a suspected case within the past two years, between January 1999 and December 2000. June to October were the most commonly reported months that suspected cases were seen. Almost every county in the state was represented, with the majority of suspected cases reported by veterinarians from the Western Slope, South Central Colorado, and the Front Range. A total of 208 suspected cases were reported during the study period with 78 of these occurring between January 1999 and December 2000. Details were obtained from only those 78 suspected cases that were reported by 23 veterinarians.

Unfortunately, only 26% (20/78) of these 78 suspected cases were confirmed as *C. pseudotuberculosis* infections by aerobic bacterial culture. The reported equids matching the case definition had a variety of clinical signs including edema (78%), lameness (40%), ventral dermatitis (35%), fever (33%), depression (28%), anorexia (26%), non-healing wounds (18%), weight loss (13%), and mastitis (4%). As expected, the abscesses most commonly were located in the three sites typical for *C. pseudotuberculosis* infections – the pectoral area (45%), the inguinal area (35%), and the ventral abdomen (32%). Abscesses also were reported on the extremities (17%) and the head and/or neck (15%). Internal abscesses were reported in 5% of the suspected cases. Most of the suspected cases were treated by lancing (77%) and flushing (78%) the abscess and most of the suspected cases received systemic antibiotics (83%) and systemic anti-inflammatory drugs (68%). Approximately 5% of suspected cases received topical antibiotics. Veterinarians reported 68% of suspected cases had resolution of their disease with lancing and flushing the abscess, systemic antibiotics and anti-inflammatory drugs. Another 18% of suspected cases did not have resolution of their disease with this treatment but did have resolution after a repeat of this same treatment. A few suspected cases (5%) did not have resolution of their disease with this treatment and did not receive a repeat treatment. Another 4% of suspected cases did not resolve with repeat treatment. Three percent of equids matching the case definition died of their disease.

This survey has helped us and the State Veterinarian's Office gain a better understanding of *C. pseudotuberculosis* infections in Colorado equids. Only 26% of suspected cases were confirmed by aerobic bacterial culture because many veterinarians (10/23, 43%) managed suspected cases symptomatically as a non-specific/undifferentiated abscess and/or bcause veterinarians were limited by the owners' financial constraints (9/23, 39%). Approximately 22% of veterinarians (5/23) did not culture because of confidence in their clinical diagnosis. Unfortunately, without bacterial culture, there is no way to definitively rule-out strangles or other non-contagious infections causing abscesses. In fact, 15% of suspected cases had abscesses along the head and/or

neck, a common location for abscesses associated with *S. equi* spp. *equi* infections. Because *C. pseudotuberculosis* is contagious, it is crucial for veterinarians to accurately diagnose these cases in order to tailor treatment and control. Infected equids need to be isolsated and once the abscess is opened and draining purulent exudate, the pus needs to be collected into a disposable container and then discarded. If the abscess is draining pus onto a concrete floor, the floor needs to be cleaned and disinfected. Veterinarians should avoid having the abscess drain onto the dirt. Although not definitively determined, an insect vector is suspected in transmission of this bacterium. Interestingly, most suspected cases were seen during summer and fall months when insects are most active. Therefore, insect control is important especially for those horses that are currently infected. Although a 36% response rate for a survey is quite good, it can be assumed that the extent of this disease is underestimated. What was once considered a disease of California horses is now a growing concern for the Colorado equine population.

GET TO KNOW YOUR LABORATORY/Computer and Business Staff

The computer and business areas of the laboratory are managed by Jay Kammerzell, Carrie Schmer and Lisa Monzingo.

Carrie, a Colorado State University graduate with a degree in Animal Science, has been with the us since 1985. Carrie began as the sample/case entry person routing samples and entering case information onto our computer system. She then became our Patient Service Coordinator supervising the client services area. In 1993, she moved to Office Manager where she remained for seven years. Carrie is currently working as an Applications Programmer assisting with a myriad of computer projects. Carrie misses the daily contact with clients, but is thoroughly enjoying her new responsibilities.

Lisa Monzingo is another long time, dedicated employee who joined us in 1982. She started doing case billing and answering phones, and later performed medical transcription of necropsy and biopsy reports for several years. Lisa is currently responsible for accounting, purchasing and student and hourly employment.

Jay Kammerzell has been the business and computer manager for us the past 12 years. He has an MBA from Colorado State University and his responsibilities include monitoring the budgets for all three labs, designing and maintaining the billing/records database, and tackling all the

"other duties as assigned." Jay is also the person to contact if you want to access your results on-line.

Carrie Schmer, Lisa Monzingo and Jay Kammerzell

COLORADO PROPOSED TRICHOMONIASIS RULES

—John Cheney and Jim Grady

At the 2000 winter meeting of the Colorado Cattlemen's Association (CCA), the Animal Health and Welfare Committee received several draft resolutions directing the State Veterinarian to develop rules for controlling bovine trichomoniasis in Colorado. The State Veterinarian's office proposed rules which went to a trichomoniasis advisory committee (veterinarians, sale barn operators, and Colorado Department of Agriculture veterinarians). The proposed rules were presented to the CCA Animal Health and Welfare Committee at its June meeting. The committee voted to accept the proposed rules and combined all the resolutions into one resolution. The CCA membership attending the summer meeting voted to accept the proposed resolution.

These proposed rules were taken to CCA affiliates, producers, and veterinarians throughout the state for their comments and input. Fifteen meetings were held across the state, most organized by local livestock extension agents. Many changes resulted from the dialogue at the meetings, and approximately 700 producers now have had direct input into the proposal.

Some of the important points of the proposed rules follow. A complete set of the proposed rules are available from the State Veterinarian's office, or at the Colorado Department of Agriculture website—http://www.ag.state.co.us. The proposal will soon begin a journey through the Administrative

Procedures Act which involves a formal public comment period. This will be announced in the press.

Import

- All bulls entering Colorado must be accompanied by a Certificate of Veterinary Inspection (CVI).
- All non-virgin bulls shall have an import permit and a negative *T. fetus* test within 30 days of entry.
- Exceptions to rules are exhibition, slaughter, or virgin bulls to feed.
- No bulls, which have ever tested positive for *T. fetus*, shall enter Colorado unless consigned directly to slaughter.
- No bulls from known positive herds shall enter Colorado unless the bull tested had three negative *T. fetus* tests one-week apart and within 30 days prior to entry.
- No female bovine originating from a known positive *T. fetus* herd shall enter Colorado except in herds where all bulls have had three negative tests for *T. fetus* and the cows either have a calf at side, the cows are at least 120-days pregnant, or they are known to be virgin heifers. (NOTE: Virgin bull means a sexually intact male bovine less than 12 months of age or a sexually intact male bovine 12 to 24 months that is accompanied by a signed affidavit from the owner/manager as having had no potential breeding contact with females.)

In-State Cattle

- Any change of ownership or lease of non-virgin bulls requires a negative *T. fetus* test within 30 days of such change.

Commuter Permitted Cattle

- Bulls must be tested annually after separation of at least one week from all female bovine. All bulls must test negative for *T. fetus* prior to receiving a permit for the next year.

Public Grazing and Grazing Associations

- All bulls co-mingling in grazing associations and/or public lands, regardless if private or multiple user permits, shall be tested annually for *T. fetus*.

Voluntary Colorado *T. fetus* Free-Herd Certification

- The State Veterinarian has made a provision for producers or a group of producers to have their herds certified as *T. fetus*-free. The detail of this program can be obtained from the State Veterinarian's Office.

Sample Submission and Collection

- In Colorado, samples must be submitted and tested by one of the three CSU Diagnostic Laboratories or the Rocky Mountain Regional Animal Health Laboratory (RMRAHL) in Denver. The State Veterinarian must approve all other laboratories whether in-state or out-of-state.
- Veterinarians collecting samples must receive training in sample location approved by the State Veterinarian.

For additional information, a copy of the complete proposed rules, or to comment on the proposed rules, contact:

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970-216-0799
970-858-8842/Fax

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Diagnostic Laboratory
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CHRONIC WASTING DISEASE (CWD) UPDATE

There recently have been a number of captive elk herds put on quarantine by the State Veterinarian's Office following positive CWD testing. Many of these herds will be depopulated and necropsies performed either on-site or at the laboratory. In addition, we have been testing hunter-killed deer and elk for CWD and to-date, we have had 12 positive deer and one positive elk. This test takes five (5) working days to complete due to the unexpected volume of testing already being requested. The fee is $25.

WHAT'S IN THIS ISSUE--

Colorado State University