

Colorado State University Veterinary Diagnostic Laboratories



Volume 2, Number 2 Spring 1998

Letter from the Director

Spring is here, along with another edition of LabLines! We hope you enjoy the interesting articles inside.

Since our last newsletter, many things have happened. Of special importance and pride to us is that we passed our accreditation site visit by the American Association of Veterinary Laboratory Diagnosticians (AAVLD). This accreditation occurs every five years. The site visit team inspected our laboratory to ensure that we meet all the quality control/quality assurance criteria insisted upon by State Veterinary Diagnostic Laboratories across the country. We did so well, in fact, that I was asked to become a member of this accreditation committee to inspect other veterinary diagnostic laboratories. This will allow me to bring home new ideas to further improve our laboratory.

In January, we met with our external advisory committee, composed of veterinarians and producers who routinely use the laboratory. They were complimentary of our service and brought us new ideas to improve service and direct our efforts in disease investigations. We greatly appreciate their time and effort. Those of you involved with poultry will be pleased that Charles Dickie at the Rocky Ford Laboratory is taking the initiative to improve our service to the poultry industry.

On a sad note, Bob Glock has left us to become Director of the State Veterinary Diagnostic Laboratory in Arizona. However, he leaves us well-trained in swine diagnostics, and we'd like to acknowledge the great improvements he has made in our laboratory over the past years. We also will miss Gary Cockerell, who is off to new adventures with the UpJohn company in Michigan. We wish them both the best.

Look for us this September in Snowmass at the Annual Colorado Veterinary Medical Association Conference. We are pleased to have been invited to provide a half-day session in the food animal section on food animal pathology/diseases. Hope to see you there!

Barb Powers, DVM/PhD

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LOCOWEED TOXICITY

Dwayne Hamar/Cathy Bedwell/Duane Lassen

Consumption of certain species of *Astragalus* and *Oxytropis* (commonly referred to as locoweeds) may result in "locoism". *Astragalus* locoweeds are short-lived perennials that flourish when growing conditions are right, and may result in large livestock losses. *Oxytropis* locoweeds are more stable and usually result in fewer, yet more consistent livestock losses. Common clinical signs are decreased feed conversion (with decreased weight gains), nervousness, proprioceptive deficits, cardiovascular disease, hydrops amnia, and reproductive problems (abortions and/or birth defects). Animals may die or become severely debilitated and emaciated. Intoxication is normally chronic, since animals must graze the plants for several weeks before developing clinical signs.

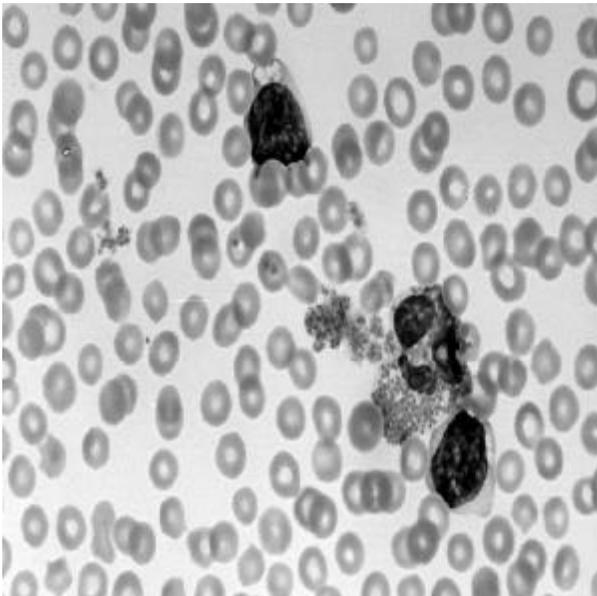
The locoweed toxin is swainsonine, an indolizidine alkaloid, which is a competitive inhibitor of α -mannosidase, resulting in accumulation of mannose-containing oligosaccharides in lysosomes. Vacuoles containing oligosaccharides form in lymphocytes, neurons, and epithelial cells, especially epithelial cells of the thyroid gland and kidney. These lesions are similar to those of heritable mannosidosis, a lysosomal storage disease.

Examination of blood lymphocyte morphology may be used as a screening test for locoism. **BLOOD SMEARS SHOULD BE PREPARED IMMEDIATELY AND AIR-DRIED.** Transportation of liquid EDTA blood can result in artifactual vacuolation of leukocytes, including lymphocytes. Presence of large, distinct vacuoles in lymphocytes of animals with clinical signs suggesting locoism is presumptive evidence of disease. However, the sensitivity of this observation (ie., the percentage of locoweed affected animals with vacuolated lymphocytes) is not certain. In addition, vacuoles in blood lymphocytes are not specific for locoism. Small vacuoles in the lymphocytes of ruminants are common, however, large, distinct vacuoles in lymphocytes are not.

Locoism in live animals can be confirmed by measuring the serum swainsonine concentration and/or α -mannosidase activity. **SINCE THE HALF-LIFE OF SWAINSONINE IN THE ANIMAL IS ABOUT 20 HOURS, SAMPLES SHOULD BE OBTAINED FROM ANIMALS BEFORE THEY ARE MOVED FROM LOCO-INFESTED PASTURES.** Serum swainsonine and α -mannosidase are stable for several days when refrigerated or frozen.

Swainsonine test: Submit at least 4ml of serum on ice. Fee=\$10.

α -mannosidase test: Submit at least 1ml of serum on ice. Fee=\$10. Both tests, Fee=\$15.



Cytology of vacuolated lymphocytes.

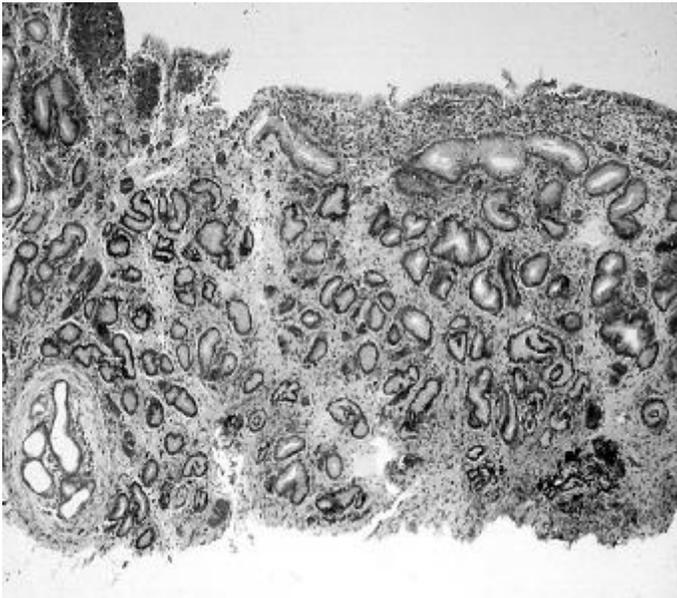
UTERINE BIOPSIES IN MARES

Barb Powers

Do you have a problem breeder mare? Is she failing to conceive or does she conceive, then come up open? A uterine biopsy is a valuable tool for diagnosing reproductive problems in mares. Endometritis, gland fibrosis, gland clumping, and lymphatic cysts can be observed in biopsy samples. Glandular changes and the depth and degree of inflammation cannot be evaluated by cytology, culture, or ultrasound, and require histopathologic evaluation.

We grade uterine biopsy samples using a modification of a grading system established by Drs. Al McChesney, Bob Shiedler, and Jim Voss. Grade 1A mares are normal. Grade 1B mares have mild abnormalities such as post-foaling changes, minor surface irritation, clumped glands, or a few fibrotic glands. Grade 2A mares have varying degrees of endometritis warranting treatment while grade 2B mares have gland fibrosis in addition to endometritis. In the Grade 2 category, inflammatory changes are further characterized as to extent (mild/moderate/severe), depth (superficial/deep), and type (active/chronic/both). Grade 3A mares have gland fibrosis permanently lowering fertility to 40% to 60% while grade 3B mares have gland fibrosis lowering fertility to below 40%. Grade 3 mares may have endometritis in addition to fibrosis.

Endometritis causes conception failure or early embryonic death, but with appropriate treatment, a grade 2A mare can return to grade 1A normal. A grade 2B mare can become a grade 1B if inflammation is cleared, however, the fibrosed glands usually do not respond to treatment. Grade 3A or 3B mares with fibrosed glands usually do not improve and are predisposed to early embryonic loss or abortion. Clumped glands can occur at certain stages of cycle, during transitional times, secondary to inflammation, or can indicate hormonal imbalance. Clumped glands can return to normal. Mares with clumped glands may be given a grade 1B or 2A depending on their number, time of year, or other relevant findings. Lymphatic cysts (or lacunae) are seen histologically as collapsed spaces. These cysts may be evident on ultrasound and, if numerous, can interfere with fertility.



Grade 2B uterine sample with inflammation superficially and a fibrosed gland at lower left.

In addition to evaluating the problem mare, we evaluate uterine biopsy samples from potential embryo transfer recipient mares and mares as part of the pre-breeding or pre-purchase examination. We also evaluate uterine biopsies from llamas. Last year, we performed 1686 uterine biopsies and grades were distributed as follows:

GRADE					
1A	1B	2A	2B	3A	3B
18.8%	27.7%	24.7%	24.4%	3.6%	0.8%

Uterine biopsy: Submit sample in 10% formalin. Fee=\$21; plus \$1.50 regular postage, \$6 FedEx courier, or \$5 local courier.

"SAYS WHO?" A SECOND OPINION ON SECOND OPINIONS

Tony Frank/Barb Powers

The phrase "second opinion" does not traditionally have the best connotation for medical personnel. Images of doubting clients, missed diagnoses, being retrospectively second-guessed by others with a more complete set of facts and data, and the spectre of legal action all spring to mind. But second opinions can and should play a valuable role in diagnostics, including diagnostic surgical pathology. Just as accurate and complete communication between clinicians and pathologists regarding clinical aspects of cases is critical to obtaining an accurate histopathologic diagnosis, communication after the diagnosis is critical to quality control. A diagnosis that does not match the clinical picture of the patient should be questioned by the clinician and no pathologist should be offended by such a request. If the diagnosis stands, perhaps completely redirecting case management, the clinician will at least have greater confidence that the diagnosis is correct and the client/patient's best interests will have been served. If the diagnosis is reversed, either because the case was particularly difficult or because of simple human error, possibilities such as unnecessary surgery or euthanasia may be avoided. While all our users should always feel free to request second opinions, we feel you should be aware of several "behind the scenes" aspects of second opinions at the Diagnostic Laboratory.

Informal second opinions happen invisibly on numerous pathology cases. An advantage of having more than ten board-certified veterinary pathologists with different areas of expertise and decades of experience is that any troublesome slide can easily be reviewed. This usually means a quick walk down the hallway and a few minutes of dialogue regarding a case and its slides. Although such second opinions are not apparent on a pathology report, they are invaluable to diagnostic quality. This is a tremendous advantage of having a large laboratory with multiple pathologists.

Second opinions also can be generated by pathology residents. Surgical biopsies from non-Veterinary Teaching Hospital (VTH) cases are diagnosed by a board-certified pathologist, then we use these cases to train the next generation of veterinary pathologists. If a pathology resident is unclear on why a diagnosis was made, they can obtain additional opinions which may either confirm or change the original diagnosis. Second opinions also are routinely obtained via our laboratory's quality control system of peer review. Each VTH-derived surgical pathology case is reviewed by a second board-certified pathologist. This system provides unparalleled quality control of diagnoses.

Formal second opinions, whether initiated by clinicians, pathologists, or residents, or any aspect of our quality control system, result in two or three written opinions that are summarized and communicated to the clinician. In some cases, these opinions are contradictory and consensus cannot be obtained, but the clinician will have full knowledge of the degree of confidence in the diagnosis allowing case management decisions to be made in an informed manner.

Because of our expertise in oncology, we also serve as a center for providing second opinions on difficult oncologic cases from all across the United States and Canada. In the realm of dermatology, where clinical presentation is an essential feature of diagnostics, we frequently obtain opinions from the dermatology clinicians at the VTH, who also provide input on case management.

We believe our quality control and "second opinion" status, whether formal or behind the scenes, is second to none and markedly enhances our case diagnostics and management; certainly offering you a more positive perspective on "second opinions."

Call any time a second opinion is desired. Fee=No charge for an existing case, \$10 for slide referral from another laboratory.

NEW PAYMENT OPTIONS

You can now check your account balance and make payments over the phone using Visa, MasterCard, and Discover. Colorado State University Accounts Receivable phone number is 970-491-6466. The automated system will guide you through payment options.

SPECIAL NOTE

There have been many duplicate payments made by Diagnostic Laboratory clients. You receive our invoice with your results and you also receive a monthly statement from Commercial Accounts. Please pay from one or the other, but not both. If you wait for the statement, you have until the end of the month to make your payments without facing late charges.

CHRONIC WASTING DISEASE IN NORTHEASTERN COLORADO

Terry Spraker

Chronic Wasting Disease (CWD) is a specific transmissible spongiform encephalopathy affecting free-ranging and captive mule deer, white-tailed deer, and elk. This disease was first observed by biologists with the Colorado Division of Wildlife in captive mule deer in the late 1960s and was diagnosed as a spongiform encephalopathy in captive deer and elk in 1978. In 1981, a free-ranging elk from Rocky Mountain National Park was found with CWD and the first free-ranging mule deer with CWD was found northwest of Fort Collins in 1984. Presently, CWD is found in free-ranging deer and elk in northeastern Colorado and southeastern Wyoming, and in captive deer and elk at wild animal facilities at Fort Collins, Colorado, and at Sybille, Wyoming. Recently, several elk were positively diagnosed with CWD in a game farm in South Dakota.

CWD is a neurological disease characterized by a spongiform degeneration of the brain, primarily affecting the thalamus and brain stem. Clinical signs of CWD are excessive salivation, emaciation or wasting, behavior changes, and weakness. At necropsy, few changes besides emaciation, ulcers, and secondary pneumonia are seen. CWD is believed to be caused by an altered prion protein. This prion protein is antigenically similar to the prion protein thought to be the cause of scrapie in domestic sheep and goats.

Over the last several years, the Colorado Division of Wildlife has collected deer and elk heads from hunted and road-killed animals throughout Colorado to delineate the regional distribution of CWD in cervids. We remove the brains from these heads and examine them both histologically and immunohistochemically to determine



Elk with chronic wasting disease.

if they are affected by CWD. To date, we have examined approximately 3,500 cervids. The prevalence of CWD in deer is highest (approximately 5%) in a relatively small area bounded by the Wyoming border, Fort Collins, Rocky Mountain National Park, and Estes Park. The prevalence of CWD in elk in the same area is less than 1%. CWD has been diagnosed in deer east of Fort Collins, but the number of animals found positive in this area is extremely low.

CWD has been known to occur around the Fort Collins area for over 30 years and during this time there have been no cases of a spongiform encephalopathy in cattle here, or anywhere else in the United States. The few cases of scrapie in domestic sheep from the Fort Collins area were from sheep recently brought into the state or were from scrapie-infected flocks and were not associated with deer or elk. Scrapie has not been linked to any disease in humans, however, bovine spongiform encephalopathy (mad cow disease) has been linked to new variant Creutzfeldt-Jacob disease in humans in Great Britain.

The only method to diagnose CWD is to examine several specific areas of the brain histologically and immunohistochemically. The immunohistochemical stain was developed for diagnosis of scrapie but also stains the prion protein in deer and elk brains and is used for confirmation of diagnosis for all cases of CWD.

SOMETHING OLD, SOMETHING NEW

Darrel Schweitzer

Horse populations on the Western Slope have been experiencing an unusually high incidence of *Corynebacterium pseudotuberculosis* abscesses. This disease, often locally referred to as "pigeon breast" was first described in 1893. During this recent outbreak, cases have generally fit the classic description as far as signs, lesions, and pathogenesis (Knight, H.D., Other bacterial diseases. In: Equine Medicine and Surgery, 1972:90-92).

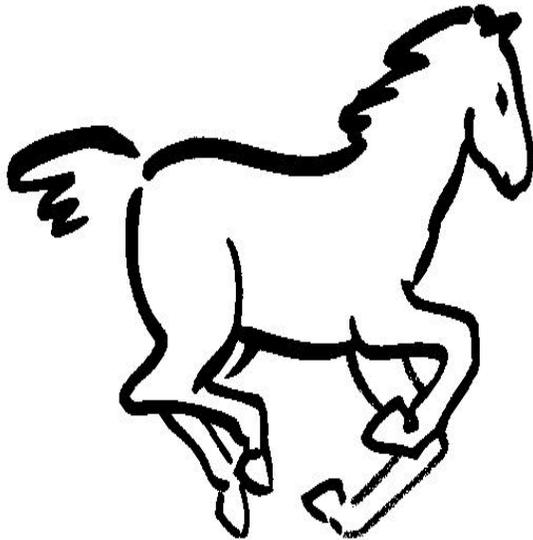
Horses have generally presented with lameness or swelling, the latter usually on the ventral portion of the body, and frequently in the pectoral area and less commonly on the neck, around the head (especially ears), in mammary glands, and on upper legs. Aspiration of these swellings varies from reddened fluid to thick, yellowish-tan pus. If unattended, these swellings may break and drain on their own, often at the site of a small scab presumed to be from fly bites. Interestingly, on one horse, an abscess developed within a

melanoma with resulting exudation of black pus. We have isolated pure cultures of *C. pseudotuberculosis* in all cases. While antibiotic susceptibilities have varied somewhat, the organism is not greatly resistant. Clinically, these abscesses can be confused with *Streptococcus equi* infections, so cultured identification is important.

Treatments by veterinarians have varied but have generally been handled like any abscess. The consensus is that antibiotic therapy does not greatly alter the course of disease. Reports indicate that the lesion(s) can clear, only to recur in another location within days to weeks. We began receiving reports of this condition in mid-summer of 1997, with the incidence steadily increasing as fall approached. Culture submissions to us tapered off by November, but during December and January, we still saw new cases.

The question remains, what caused such a dramatic increase in incidence of this disease in western Colorado? Dr. John Harris, who has been practicing in Grand Junction for many years, has seen this condition sporadically, but never an outbreak of this magnitude. The Spring of 1997 was not typical, with early spring being much wetter than usual. Animal diseases in Western Colorado are often influenced by life zones which roughly correspond to amount of precipitation and altitude. Numerous cases were reported from Montrose, Olathe, Delta, Grand Junction and Cortez. Fewer cases were reported from Norwood and Parachute. One was reported from Hotchkiss. None were reported from Carbondale, Glenwood Springs, Rifle, Aspen, Meeker, or Paonia. A quick look at a map reveals the former communities to be in lower elevations of major drainages, and the latter in higher areas.

Fly bites are thought to be the means by which this infection is spread. Is there a specific species of fly that is able to transmit the disease, but does not live in higher life zones? Could the wet spring have influenced the life cycle of this species and thereby increased its numbers? However, presumably, horses pastured together are all being bitten, but usually only a few develop lesions. Horses kept in corrals around buildings (thus greater biting fly populations) are apparently less likely to contract the disease than pastured horses. Since November, several new cases have been reported, and flies are not present now. Horses developing this disease are reported to be frequently positive serologically for vesicular stomatitis virus (VSV). However, we have experienced VSV outbreaks in other years without a concurrent rise in *C. pseudotuberculosis* abscesses. There also does not appear to be any association between horses pastured with sheep or around sheep corrals. Many questions remain unanswered about this curious condition.



ABORTION DIAGNOSIS

Gary Mason

Abortions constitute a significant proportion of our diagnostic caseload in late winter and spring. We have had several recent abortion cases in beef cattle with relatively rare or unusual etiologies. We diagnosed an abortion due to locoweed intoxication by identifying cytoplasmic vacuoles in fetal thyroid cells. We identified an abortion due to *Listeria monocytogenes* with lesions of placentitis and hepatic necrosis, and confirmatory bacterial culture. We also identified an abortion caused by infection with the anaerobe, *Bacteroides fragilis*, which resulted in a placentitis and fetal infection of the lung and liver. Additionally, a calf that aborted with muscle necrosis and mineralization, typical of Vitamin E and Se deficiency, led to identification of a management problem at the ranch.

Many abortions remain undiagnosed; however, the absence of a demonstrable etiology is often useful information for producers, by eliminating infectious causes such as BVD, IBR, brucellosis, leptospirosis, campylobacter, neospora or toxoplasmosis. Aborted fetuses have soft tissues with a high water content, undergo rapid post-mortem autolysis and seldom contain gross lesions, however, gross and histopathologic assessment are essential components of abortion diagnosis. Bacteriology, virology and toxicology findings often heavily influence the process of arriving at a diagnosis. Last year we performed 191 abortion screens with the following results; in some cases, there was more than one etiology.

We perform abortion screens including histopathology, and all laboratory testing including bacterial and viral cultures, fluorescent antibody tests, nitrate determination, serology, and other tests appropriate to the species. We prefer to receive whole fetuses with the placenta and the dam's serum, however, many times, submission of an entire fetus is not possible. Appropriate sample selection and submission increase the odds of arriving at a diagnosis from aborted fetuses necropsied in the field. These samples are listed in our User's Guide and are fresh fetal lung, liver, kidney, spleen, stomach contents, and placenta. Ideally,

Species	Number Examined	Viral	Bacterial	Other	Underdetermined
Bovine	115	15 all BVD	37	21*	49 (43%)
Ovine	15	0	6	7**	5 (33%)
Equine	39	6	6	5***	24(62%)
Porcine	9	1	2	0	6(67%)
Other	13	0	3	0	10(77%)
Total	191	22	54	33	94(49%)

*=14 suspect nitrate toxicity, 4 fungal, 2 Neospora, 1 developmental

**=6 chlamydial, 1 developmental

***=developmental or umbilical

place these tissues in separate sterile containers (ie, whirlpaks), but samples which are large enough to aseptically incise and culture (at least 1cc) can be shipped in standard ziplock plastic bags. Formalin-fixed tissues should include lung, liver, kidney, thymus, heart, brain, and placenta. Additional samples include a fresh eyeball for nitrate determination, formalin-fixed thyroid if locoweed poisoning is suspected, and the dams serum in a red top tube for serology. Ship fresh samples with ice packs by overnight courier.

Abortion screen: Submit samples as described above. Fee=\$60.



Aborted bovine fetus with placenta.

MILKWEED POISONING

Charles W. Dickie

Six Hereford cows were found dead, strung out over approximately a mile, up a grassy swale in southeastern Colorado on rangeland near the Huerfano River. Samples of liver, kidney, lung and muscle were sent to the Rocky Ford Laboratory. No significant bacteria were grown, and no significant histopathology was seen. We inspected the site; starting at the bottom end of the swale at the first carcass, we

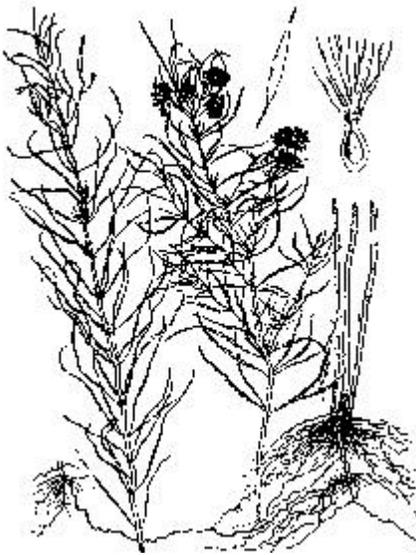
walked slowly to the top and the last carcass. Since it was mid-January, we were surprised to find green, sprouting, western whorled milkweed (*Asclepias subverticillata*). No snow was on the ground and there had been approximately three weeks of 50F days. Abundant palatable dry range grasses were present, with blue and side oats grama predominating higher up on the swale, and western wheat grass with little bluestem in the wetter bottom area. The bottom area contained the majority of the milkweed, which showed abundant signs of grazing.

Milkweed is quite palatable, especially when it is green in a dry range environment. Milkweed is extremely toxic; as little as 0.8 ounce of *Asclepias labrifomis* is lethal to a 100-pound sheep. *A. subverticillata* is only slightly less potent. Cardiac glycosides (cardenolides) are considered the toxic principles. These compounds inhibit the Na⁺-K⁺ATPase enzyme system in cardiac muscle. Clinical signs are a slow, labored respiration, and frequently rales can be heard. Cyanosis develops as respiration is depressed. Animals are lethargic, weak, and eventually go down. Survivors of the acute phase are blind (presumably from cerebral anoxia), grind their teeth, and stay down; they usually do not respond to supportive care and are euthanized.

SAMPLE COLLECTION FOR IONIZED CALCIUM

(I-Ca) DETERMINATION--Veterinarians may submit samples collected anaerobically in a heparinized syringe (with all heparin expelled) and maintained for up to 3 hours in an ice-water bath. Remove air bubbles prior to sealing the syringe. For mailed samples, serum must be collected and allowed to clot anaerobically. Do not use serum separator tubes as the gel leaches calcium into the sample. Remove air bubbles and seal the syringe. Serum stored anaerobically is stable at 4EC for three days or -10EC for 7 days. Mixing of room air with the sample will falsely decrease the I-Ca and increase the pH.

Fee for I-Ca determination=\$13.



Milkweed (*Asclepias* sp.)

The mysteries of milkweed poisoning are manifest in equines to an even greater extent than in bovines. A typical history is of a normal, frisky horse being fed hay in the evening and being found dead in the morning. Usually nothing definitive is found; but pulmonary and renal congestion, along with possible mild gastroenteritis, might suggest milkweed poisoning. If stomach contents are submitted, along with tissues, we may be able to identify milkweed parts in the usually well-masticated equine ingesta. This insidious killer also grows in patches in the hayfield, so numerous bales of hay may be found to have no milkweed present, and the next bale may be the lethal one.

A particular owner had five horses, all of which eventually succumbed to milkweed poisoning. Initially, two died overnight, and we suggested that milkweed might be involved and that every hay bale be checked before feeding. Another horse died about a week later, and a fourth died about a week after that. After an evening ride on the last horse, the owner cooled her out and threw her some hay. The next morning she, too, was dead. This is the hard way to realize that milkweed in hay is palatable, extremely dangerous, and frequently difficult to find.

When milkweed poisoning is suspected, submit ingesta, heart, lung, kidney, liver, stomach, small intestine, and any portion of hay left from the last feeding.

BOVINE VIRUS DIARRHEA (BVD)

TYPING

We have implemented a new polymerase chain reaction (PCR) test to type BVD isolates. For the next six months, we will routinely type all BVD isolates free of charge to learn more about the prevalence of BVD types. Look for a report of our findings in future issues of LabLines.

SWINE CORNER: SEROLOGY-- WHAT DO ALL THOSE "TITERS" MEAN?

Bob Glock

We perform a number of serologic tests including brucellosis, pseudorabies (PRV), swine influenza (SIV), transmissible gastroenteritis (TGE), and porcine reproductive and respiratory syndrome (PRRS). We refer to other laboratories *Mycoplasma hyopneumoniae* (mycoplasma), *Actinobacillus pleuropneumoniae* (APP), *Haemophilus parasuis* (HPS), and *Streptococcus suis* (strep). Most of these tests are of little value unless there are clear objectives for the use of results. Some thoughts follow:

Brucellosis-Positive or negative; self-explanatory; very few infected herds in the United States and none in Colorado.

PRV Positive or negative; Latex test used produces rare false positives which must be confirmed by SN or ELISA. Additional testing to differentiate vaccine titers may be necessary but vaccine use is illegal in Colorado.

SIV Titers > 100 may indicate recent infection. Practically all swine populations have SIV infection which circulates in different populations so titers tend to fluctuate in different age groups regardless of any clinical disease. Vaccine titers tend to be quite low (<100).

TGE Any titer indicates past infection and titers >100 usually mean infection within the past several months. Titers do not determine carrier/shedder status. CAUTION Most swine populations carry porcine respiratory coronavirus titers which cross-react and require differentiation. Results may be equivocal. Therefore, negative TGE titers may be quite useful but positive titers need cautious interpretation.

PRRSThe most common test and the one we use is an ELISA. Results are reported as S/P ratios, which are not the same as titers. Positive is generally an S/P ratio over 0.4 and some feel that average S/P ratios over 2.0 or 2.5 indicate recent infection. This interpretation may be over-simplification. Field strains and some vaccine strains of this highly mutable virus may be endemic in some herds. There is no serologic test to differentiate between strains, but polymerase chain reaction (PCR) tests on isolated virus are available for this.

Mycoplasma Positive or negative, most swine populations are infected but negative results in a group of animals may identify those few negative populations. This test may be useful to identify age of infection as an aid in planning control strategies. It is of little value in determining status of individual animals.

APP Positive or negative, there are numerous cross-reactions with related non-pathogens so interpretation is difficult. Evaluation on a herd basis may be useful but testing individual animals is not. This is a good example of a test not to run unless there is a specific plan for use of the results.

HPS and Strep Nearly all swine populations carry these organisms and there are many strains of each. Interpretation of results is very difficult.

TIPS:

1. Never do a serologic test unless there is a plan for use of the results. (Example = Positive APP titers in a breeding herd may preclude sales even though there is no significant pathogen present.)
2. "Chasing" titers is a waste of time and money unless the results are part of a clinical plan.

Swine serologic testing: Submit 1ml serum. Fee=\$1.25 to \$5, depending on number of samples.

HISTOPATHOLOGY AND SEROLOGY PREPAID MAILERS

We have available for your convenience prepaid mailers for histopathology and serology submissions. Our histopathology mailers contain formalin-filled bottles, submission forms, and prepaid postage labels in a plastic returnable mailer. Our serology mailers contain empty redtop tubes, submission forms, and a prepaid postage label in a returnable box. Need a mailer? Call us at 970-491-1281. We will automatically send new mailers back to you after samples are received.

NEW DIAGNOSTIC TEST FOR HEARTWORMS IN CATS

Glenda Taton-Allen

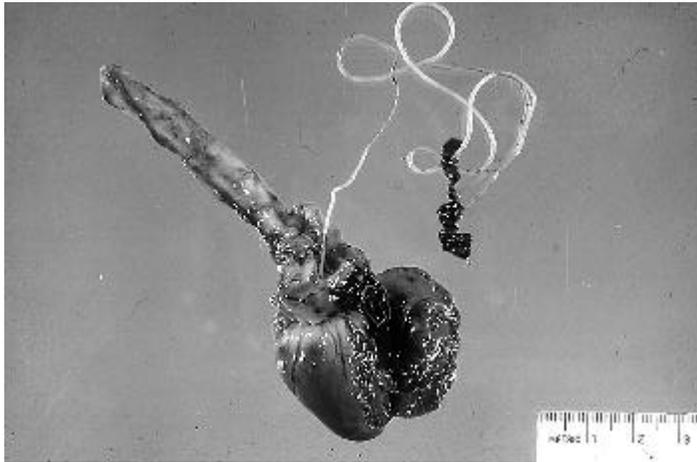
We are now able to test cats for heartworms. This new ELISA antibody test is specifically designed for cats and should be utilized when a cat has signs associated with heartworm infection. Clinical signs include coughing, abnormal lung sounds, listlessness, tachypnea and dyspnea. Heartworm disease in cats is difficult to diagnose because the worm burden is often very low (1 to 2 worms), and circulating microfilaria are rarely seen. Heartworm disease in cats is very rare in Colorado.

Since this ELISA test is an antibody test, a positive result indicates the presence of circulating antibody due to exposure to infective larvae or adult heartworms. A positive antibody test does not necessarily indicate adult heartworms are present, however, it does indicate further testing with an antigen test should be done.

Antigen tests are very diagnostic when two or more adult female heartworms are present. Antigen tests are not reliable when only male heartworms are present. Antigen tests detect proteins shed by adult heartworms (primarily the female), so if the antigen test is positive, it is a reliable indication of a mature heartworm infection. However, if the antigen test is negative, it is less reliable because the level of circulating antigen may be undetectable.

If a cat is going to be placed on preventive medicine, we recommend the cat be tested first. Our recommendation is to initially perform an antibody test. If it is negative, no further testing needs to be done and preventive medication may be given. If the antibody test is positive, an antigen test should be performed.

Feline heartworm test: Submit 0.5ml serum or plasma on ice. Fee=\$11 for antibody test; \$18 for both antibody and antigen test.



Cat heart with adult heartworms.

NEW TESTS AVAILABLE

- Johne=s disease ELISA Serology
- Immunohistochemistry for identification of tumor types/subtypes

PCR for *Actinobacillus pleuropneumonia*

PCR for ovine herpes virus type 2 (presumptive agent for MCF)

PRRS ELISA Serology, FA test and virus

isolation

Locoweed toxicity serum test

Heartworm Serology for Cats

Blood Lead by graphite furnace

PCR for equine and bovine herpes virus (including types)

PCR for Mycoplasma speciation, detection of *M. bovis* in milk and *M. hypopneumonia* in pig lungs.

PCR for genotype of *Clostridium perfringens*.

???WEEKEND NECROPSY???

Did you know that we do necropsies on Saturday and Sunday mornings? If you have an animal for necropsy on the weekend, call us at 970-491-1281 to obtain the message system of the pathologist on duty or report to the front desk of the Veterinary Teaching Hospital, or report to the Diagnostic Laboratory entrance at the north side of the building and follow instructions posted by the office door.

MULTIDRUG RESISTANT *Escherichia coli* FROM CANINE URINE

Claudia Gentry-Weeks

We have isolated four multidrug resistant *E. coli* from canine urine specimens since November 1997. These isolates were resistant to tetracycline, ampicillin, amoxicillin/clavulanic acid, cefoxitin, cephalothin, chloramphenicol, enrofloxacin, erythromycin, and gentamicin. All isolates remained sensitive to amikacin. Two isolates were sensitive to trimethoprim/sulfamethoxazole, one was intermediate, and the fourth isolate was resistant.

Several mechanisms exist for the generation of resistant bacteria, including 1) the acquisition of plasmids which contain enzymes that modify and inactivate the antibiotic, 2) mutation of the gene encoding the antibiotic target, thereby altering the target and making it no longer susceptible to the action of the antibiotic, and 3) the activation of multidrug efflux pumps which literally pump multiple types of antibiotics out of the cell. At this time, it is unclear which of these mechanisms is responsible for the appearance of the multidrug resistant *E. coli* isolates.

Many of the resistances specified above are mediated by plasmids (eg., tetracycline, ampicillin, chloramphenicol), however, fluoroquinolone resistance is usually due to a chromosomal mutation that effects the affinity of the target (DNA gyrase) for the antibiotic. *E. coli* has at least seven distinct multidrug efflux pumps and one of these pumps, encoded by the *marRAB* locus, regulates susceptibility to multiple antibiotics, including quinolones. In one study of human urinary tract isolates with multidrug resistance, the *marRAB* operon was overexpressed in two-thirds of fifteen isolates tested (Cohen, SP, Yan, W, and Levy, SB, 1993). In addition, a multidrug resistance pump, designated MdfA, was recently reported for *E. coli* and was found to confer resistance to rifampin, tetracycline, puromycin, and other clinically important antibiotics such as chloramphenicol, erythromycin, and certain aminoglycosides and fluoroquinolones (Edgar, R, and Bibi, E, J. Bacteriology 179:2274, 1997). One or more of these mechanisms may be responsible for the antibiotic resistance patterns associated with the multidrug resistant *E. coli* we isolated.

Aerobic culture and antimicrobial susceptibility: Submit urine specimen. Fee=\$20.

INTEGRATED LIVESTOCK MANAGEMENT PROGRAM

Frank Garry/Dan Goul

The Integrated Livestock Management Program is providing a stimulus for change in numerous aspects of the University's focus. Some of these changes are likely to be of great interest to you. The specific mission of the Integrated Livestock Management (ILM) initiative is to train animal agriculture specialists with not just a focused expertise, but also with a perspective and understanding of the complex nature of modern agriculture. This includes food safety, economics, animal well-being, land use, and environmental health.

The ILM mission is accomplished via research, teaching and service activities. Trainees from the ILM Program will be better equipped to work across disciplines with other specialists to cope with production problems. Our challenge is to modify these activities as we adapt to the changing circumstances and needs of the food animal production industries. The ILM team works on specific projects initially presented by the affected producer and/or their veterinarian. The four components of each ILM developed project are:

- Graduate student involvement in all phases of a project.
- Producer/veterinarian involvement in identification of problems.
- Outreach education and direct communication with affected producers and veterinarians.
- Focus on issues of broad concern with multiple effects.

Our ILM Program follows the successful course set by the Integrated Resource Management (IRM) program, but targets an investment of university-level student training in the process. The agricultural community benefits from ILM by having current, relevant problems investigated by a multidisciplinary group, which can then disseminate the results of research projects to all producers. Animal production areas include dairy, cow-calf, feedlot, sheep, and wildlife. Students develop expertise in a specific

discipline, within the context of a broad array of related fields that are important in animal agriculture. Projects are designed to incorporate field work and veterinarian/producer communication so that students include the production setting in their training.

Our ILM Program maintains a strong commitment to communicate with veterinarians and producers as a means to prioritize activities, target student training opportunities, focus on problems of real importance, and direct our outreach activities. We publish newsletters and trade journal articles, and conduct field-based seminars for producer education, in addition to the publication of refereed scientific articles.

Collaboration of a multidisciplinary faculty group, animal health and production specialists, Diagnostic Laboratory personnel, and Cooperative Extension resources, generates a well-rounded approach to animal agriculture problem-solving. Historically, Colorado State University's outreach activities have included advice, information, and service for individual problems, plus farm and ranch visits in collaboration with local specialists for more complex problems. The ILM Program complements and adds to these efforts by developing funded research protocols for problems that are frequently or regionally encountered. Examples of ILM projects are the investigations of rumen-generated toxins causing polioencephalomalacia in cattle (see articles in our last two newsletters) and locoweed poisoning (see article this issue). Look for upcoming articles on other ILM projects, including coliform mastitis, Johnes disease, cow-calf economics, and neonatal calf survival.

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