Wyoming State Veterinary Laboratory Newsletter – April 2007

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MESSAGE FROM THE DIRECTOR

Pet food withdrawal

In March the FDA and Menu Foods announced the largest withdrawal of pet food ever announced in the United States. This came in the wake of a palatability study in which several of the feeding subjects died. The following month, a laboratory in New York announced the suspected toxicant was aminopterin. *This has not been confirmed by other laboratories.* At present, there is quite a bit of controversy as to what the toxic component is, but several labs have settled on 1,3,5-Triazine-2,4,6-triamine ("melamine") as a marker of the suspected raw materials in completed feedstuffs, and in urine and kidney of affected animals. Melamine is a feedstock used in the synthesis of several commercial products including Formica and may have been deliberately introduced into wheat gluten in order to artificially inflate its protein analysis.

We have yet to see a case of this condition in Wyoming although we have examined several suspects. Suspected cases are reported from accredited laboratories in other states and provinces (NY, GA, IA, CA, KY, CO, IL, TX, NM, TN and ON).

Cases present as acute renal failure. Most are in cats. Affected animals were fed Menu Foods wet cat or dog food approximately 1 week earlier. Changes in kidneys include distal tubular degeneration and necrosis, and a modest amount of intra-tubular crystals that are quite distinct from oxalate.

If you think you have a case of this and it ends up as a post-mortem, please flag it in the history sheet as food-associated. As always, the best sample to submit is: the entire carcass. If it is a necropsy in a bag, at a minimum we need:

- Kidney - fresh and fixed
- Liver - fresh and fixed
- Brain - fresh and fixed
- Urine – as much as you can get

A good clinical history, exposure to any of the affected brands of food (manufactured between Dec 06 and early March 07), acute tubular nephrosis, and the presence of distinctive crystals in renal tubules may allow a tentative diagnosis until the exact nature of the toxicant is confirmed.

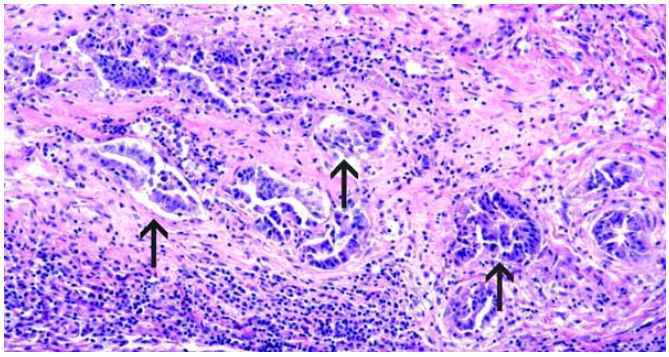
Donal O'Toole & Merl Raisbeck
March 27, 2007

DIAGNOSTIC CASES OF INTEREST

Intractable digital disease in a cat associated with metastatic carcinoma

A 15 year old spayed longhaired calico cat was treated for 6 months for intermittent non-weight bearing lameness affecting digits of both forelimbs. Digits were painful and swollen. The cat's nails periodically became discolored and were shed. The veterinarian tried a range of antibiotic and antifungal agents. The condition was non-responsive and the veterinarian suspected the disease might be autoimmune. The cat's condition did not improve and she was euthanized. Fixed samples of soft tissue from digits of the forelimbs were submitted.

Histologically Dr. Montgomery found multifocal clusters of neoplastic cells, many surrounded by a scirrhous response, in the dermis. Some carcinoma cells were in blood vessels.



Metastatic carcinoma in the digit of a cat with recurrent onychopathy. Some of the aggregates are in blood vessels

His tentative diagnosis was nailbed and digital injury secondary to metastatic adenocarcinoma. Unfortunately we did not have samples of lung to corroborate the diagnosis. The veterinarian is checking to see whether he has the cat's remains, and if the owners would give permission for us to do a necropsy.

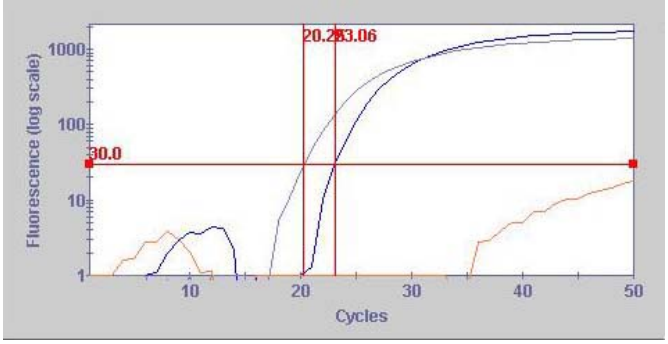
Metastatic disease in the digits of cats with pulmonary carcinoma is well recognized. The overall metastatic rate is 75%. The presentation is as here: the pulmonary carcinoma is occult, so cats present with lameness and one or more swollen toes. Toes are erythematous and ulcerated, and nails may detach. The average age of occurrence is 12 years.

Dr. Don Montgomery

Johne's in a goat

A goat was presented to a veterinary clinic in SE Wyoming. No history was provided, but the veterinarian requested the goat be tested for Johne's disease. The test we use is an ELISA, which is licensed for use in cattle but not other species. The goat tested positive on the test. We wanted to see if we could corroborate the serological diagnosis. The goat was euthanized and a range of tissues samples were collected for histology and PCR for Mycobacterium avium paratuberculosis (MAP).

Dr. Cornish saw severe granulomatous enteritis and lymphadenitis, consistent with Johne's disease. Acid-fast bacteria were demonstrated in epithelioid macrophages in large numbers. DNA was extracted from tissues and processed for real-time PCR for MAP. It was a strong positive - indeed, stronger than the control we us as the test. This confirmed the goat had Johne's, and that the ELISA can detect the disease in goats, even though it is not USDA-licensed for this purpose.



Real-time PCR read-out of a control and a test sample from the goat. If amplification is seen before 40 cycles, that establishes the agent (in this case MAP) was present

Abortion and stillbirth in cattle associated with isolation of RB51 (vaccine strain) *Brucella abortus*

As you may know, heifers and - with the permission of the state veterinarian - adult cattle are vaccinated with RB51, particularly in the western part of the state to reduce the chances of brucellosis. Approximately 1600 pregnant adult were vaccinated with a full dose of RB51 over a short period in early November 2007 in Sublette County. Little is published on losses associated with RB51 when it is given to pregnant cattle. The producers were advised by USDA APHIS personnel that some losses could be expected as a result of vaccination.

One producer with 280 adult cows noted one cow had aborted on February 11. The animal retained its placenta for one week, during which time there were no more losses. The carcass was submitted to the WSVL. Dr. Mills' laboratory cultured *Brucella abortus* from multiple tissues. It had the cultural characteristics of RB51. Amanda Fluegel, a graduate student who is developing a PCR test for *B. abortus*, confirmed it was RB51, and not wildtype. Losses peaked

between March 6 - 15, during which 10 cows aborted or delivered premature live weak calves. At this time it appears as though losses have stopped. RB51 was also isolated from a calf that was born alive and weak. It had lesions consistent with a bacterial infection in umbilicus and in a small piece of chorioallantois that came with the fetus. We are still doing PCR and attempted bacterial culture on additional calves. Tissues from other calves on the property were submitted to NVSL - we do not yet know those results. Losses to date in this 280 herd are 7 abortions, 4 cows open and presumed to have aborted, 2 premature calves that died, and 3 premature calves that were weak but still alive (5.7% loss). There have been abortions and one open animal in the heifer group, but this may be unrelated since they were not vaccinated. Animals to date are negative for BVDV.

Since RB51 has been isolated now from two calves, we assume it was contributory to the losses.

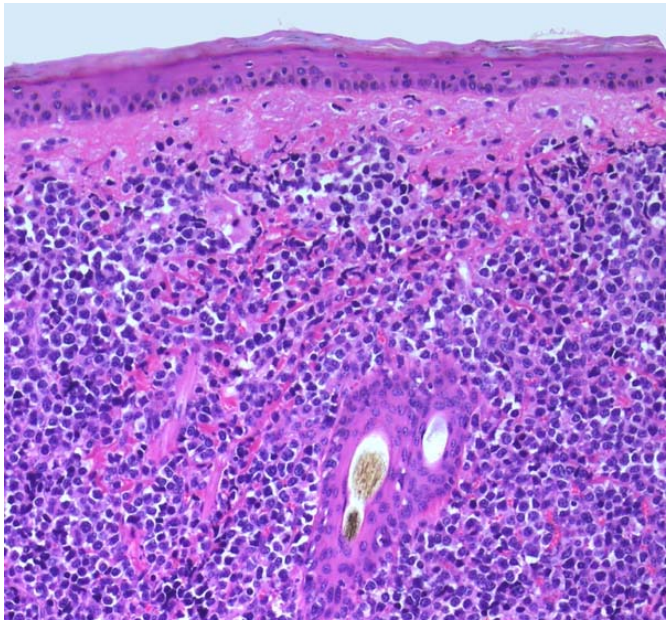
There is not much published on RB51-associated abortion, premature birth and weak calves. Recently Dr. Don Montgomery did a study with a researcher at NADC in Ames, Iowa to look at the safety and immunological response when cows at different stages of gestation were vaccinated with low and high doses of RB51. None of the cattle in that study aborted due to RB51. In South Korea in 1996-1997, full adult vaccination dosages of RB51 were given to adult dairy cattle and induced 1abortions in 1%. The cows had not been vaccinated as calves. In 1997 an RB51 abortion occurred at Kansas after an approximately 10-month old crossbred heifer was given a calfhood vaccine during pregnancy. The case was chronicled in Morbidity and Mortality Reports by the CDC as they were in the process of evaluating the human risk posed by RB51. Several veterinary students were exposed. In field trials for vaccine approval, the Colorado Serum Company vaccinated approximately 620 cattle at various stages of gestation. One known RB51 abortion occurred. There were 2 abortions in which the etiologic agent was not determined. The most susceptible period appears to be the middle third of pregnancy. We will present a more complete account of this episode at the Jackson Hole Veterinary Rendezvous this June.

Donal O'Toole

Cutaneous lymphosarcoma in a yearling Angus heifer

A one year old Angus heifer developed acute anasarca with raised lesions in skin around the anus, eyes and vulva. There were lesions in skin of the neck, thorax, abdomen, as well as in the oral cavity. The heifer's skin tore and hemorrhaged when she was being processed in the chute. She had a temperature of 104.4 F, was alert and continued to eat. Tissues were submitted to the WSVL for examination. She had cutaneous lymphosarcoma.

Cutaneous lymphosarcoma affects cattle 1-3 years of age. There is no associated etiologic agent. During the initial phase of 1-3 months, cutaneous swellings that tend to wax and wane in size occur around the anus, vulva, shoulders and flank. Lesions are typically 2-3 cm and have necrotic centers. They may be painful on palpation. Other signs occur, depending on other organ systems affected. At necropsy a variety of organs are involved. These may include heart, brain, spinal cord, liver, lung kidney and abomasum.



Cutaneous lymphosarcoma in skin of a yearling Angus
Dr. Leslie Woods

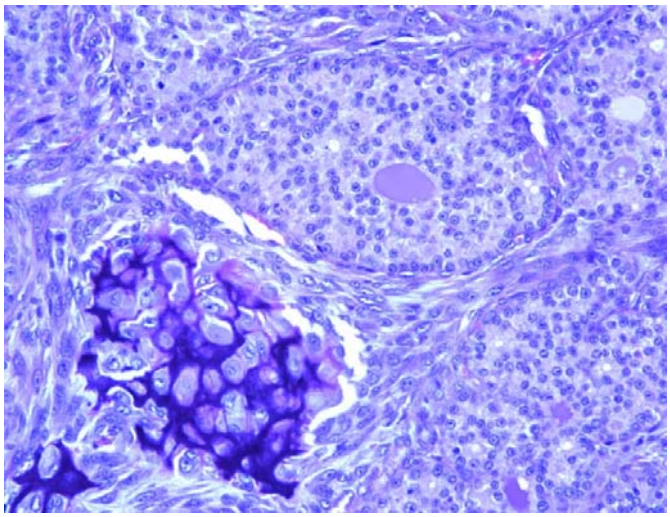
Cutaneous lymphosarcoma in an aborted fetus

A 27 kg male polled Hereford calf died of dystocia in western Wyoming. The calf had multiple cutaneous nodules, as well as a broken back. Necropsy revealed both lungs had multiple nodules throughout. Similar nodules were in the heart, liver and spleen. Histologically Dr. Cornish found multiple nonencapsulated, infiltrative masses of neoplastic lymphocytes in skin, liver, spleen and kidney. It graded as a high grade large cell, diffuse lymphosarcoma. There are some reports of congenital lymphosarcoma in cattle - but they are rare. There is no established association with enzootic bovine leukosis.

Dr. Todd Cornish/Dr. Donal O'Toole

Malignant mixed thyroid tumor in a dog with pulmonary metastasis

Mixed tumors of the thyroid gland in dogs are uncommon compared to other neoplasms. Recently tissue samples (thyroid and lung) from a 9-year-old male Husky crossbred were received for examination. The sample from the thyroid consisted solely of an osteosarcoma with no appreciable thyroid epithelial component. Multiple metastatic nodules were in the sections of lung. All consisted of a neoplastic mass consistent with follicular-compact cellular carcinoma. In two of the lung nodules, osteosarcoma was mixed with the carcinoma.



Malignant mixed tumor of thyroid origin in the lung of a dog; two components are present: an osteosarcoma and a follicular-compact carcinoma.

In mixed tumors of the thyroid gland, both epithelial and mesenchymal components are malignant and can metastasize, usually to the lung as in this case.

Dr. Don Montgomery

Neospora abortion in cattle originating in Wyoming

I teach a disease in food animals and horses class to upper division pre-veterinary students and animal science production majors. Until now I've always told them that neosporosis has never been diagnosed as a cause of abortion in Wyoming cattle.

The Montana diagnostic laboratory has just confirmed an outbreak of neosporosis in a group of cattle originating in Wyoming. Seventy six pregnant breed heifers were purchased in Torrington, WY in fall of 2006. They were held in a feedlot for an undetermined length of time in the Torrington area. The heifers were shipped to a ranch in the Baker/Broadus MT area sometime during the fall, along with ~25 older pregnant cows (presumably purchased in Torrington). They were put on a large pasture. Pregnant cattle from other sources were also put in the MT pasture. In December 2006 the ranch owner in MT noted one heifer had aborted. Diagnostic workup on one fetus indicated that the cause of death was *Neospora caninum.* Samples collected at the MT laboratory were shipped to the CAHFS laboratory in CA. The diagnosis was corroborated by finding positive staining by IHC for the protozoan. To date, 32 of 76 heifers have aborted. None of the older cows have aborted. They also had weak calves and perinatal deaths. The cattle that aborted had high titers to *Neospora caninum*. Other commingled cattle did not have titers to Neospora.

The history suggests that the heifer group may have acquired infection in Wyoming. We are uncertain of the source. In similar episodes in other states in the past, the presumed source was dogs, since they serve as the definitive host and excrete infective oocysts in feces. There is a lot of published information on *N. caninum* abortions in dairy cattle but the literature on beef breeds is sparse.

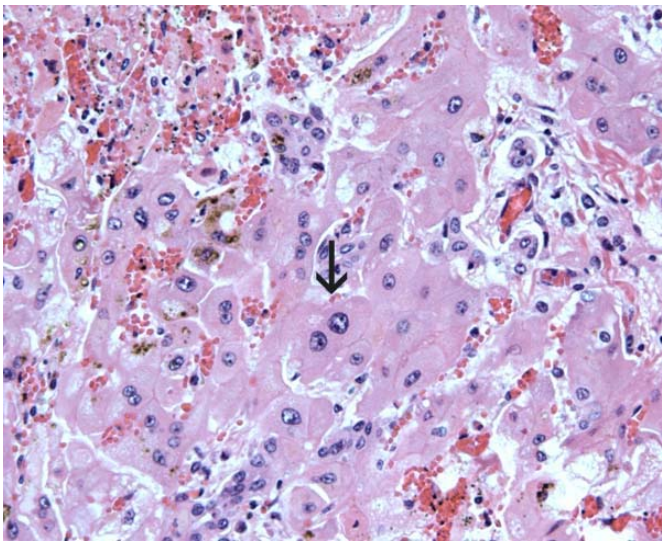
We are grateful to Dr. Randy Hunter for bringing this case to our attention.

Dr. Don Montgomery/Dr. Donal O'Toole

Severe hepatic disease with nephrosis in a group of bulls fed moldy beet pulp - suspect mycotoxicosis

We've seen an unusual hepatotoxicity syndrome associated with feeding moldy beet pulp to bulls in a feedlot in NW Wyoming. These owners buy bulls as yearlings and hold them 1 – 2 years. Due to the high price of hay, this year the owners have been feeding beet pulp, much of which is moldy. In mid-March 2007, 42 of 50 bulls were found with swollen heads, drooping ears, slobbering, photophobia, corneal ulcers, ocular discharge, and a watery brown diarrhea. They were on beet pulp – and no hay. Some calves on the beet pulp were showing similar but milder signs. Tissues from one affected bull were submitted. The veterinarian noted marked jaundice in the carcass at necropsy. Dr. Montgomery examined the tissues. In addition to serous atrophy of fat, the principal changes were in the liver and kidney, including chronic and acute zonal hepatic necrosis gigantism, anisokaryosis, and polykaryons, and bile duct proliferation. In the kidney there was tubular nephrosis.

We suspect this may be a mycotoxicosis. The owners have continued to offer the feed, but have added hay. Dr. Raisbeck's laboratory has been unable to confirm our suspicion that the moldy beet pulp is the culprit. Diagnosis of mycotoxicosis is difficult because the toxin(s) is very unevenly distributed throughout spoiled feedstuffs and a typical "grab" sample has a high probability of missing the contaminated feedstuffs.



Liver from a bull fed moldy beet pulp. There is focal necrosis, along with necrosis gigantism (arrow), anisokaryosis, and polykaryon formation

We'd be interested in knowing whether anyone else has associated a hepatic syndrome with feeding moldy beet pulp to cattle.

Dr. Don Montgomery/Dr. Merl Raisbeck/Dr. Donal O'Toole

Canine influenza - back again

The Cheyenne Animal Shelter has had another episode with CIV. Most of the dogs made it, but one died. Dr. Woods and our necropsy technician went to the shelter to bleed recovered dogs to use as control serum for the hemagglutination serological test. The principal gross finding in the dog that died was a firm hemorrhagic, wet, non-collapsed left lung. The right lung was minimally affected. The affected lung had a severe diffuse hemorrhagic bronchopneumonia with vasculitis and intralesional bacteria. Influenza virus was detected in lung by PCR but no virus was grown.

Please keep CIV as a differential if you see an outbreak of respiratory disease, particularly where large numbers of dogs are in contact - as in shelters or kennels. Dogs will generally spike a fever and you may see some deaths. We can either attempt to culture the virus, or you can send us nasal and oro-pharyngeal swabs for PCR.

Hemagglutination inhibition test for canine influenza

We now have a working HI test based on a CIV isolate from a dog in Thermopolis. If you want this run, Joan Edwards can do the assay here, and on the first sets of samples she will send duplicates to Cornell to make sure our titers are comparable to theirs. You will be charged only for the samples run here. We would appreciate getting serum from cases of suspect canine influenza so that we can validate the test. We need 1 ml of serum with minimal hemolysis. It is ideal if we receive paired sera 3 weeks apart but this is not always practical. Please contact Joan if you have questions about the assay: 307 742 6681 ext. 163.

We are grateful to the Cheyenne Animal Shelter, which allowed us to collect serum from dogs that were recently infected.

Specimen Mailing Regulations Update

Recently there have been significant changes to the regulations for shipping diagnostic, clinical, or patient specimens. In short, all clinical or diagnostic samples now must be ***triple*** packaged, with an outer package of sturdy rigid construction (cooler, corrugated fiberboard, rigid plastic, or wood) and with leak-proof primary and secondary inner containers with absorbent material between the primary and secondary container. Some carriers also require that the secondary container be marked with the internationally accepted Biohazard markings (use of our available Biohazard bags will suffice), and all require that the outer packaging be marked with appropriate labeling ("Biological Substance, Category B" and the appropriate UN code within a diamond mark "UN 3373"). Furthermore, the name, address, and telephone number of the shipper must be marked on the outer container or enclosed in an envelope on the outer container. We receive quite a few clinical or diagnostic samples from clients (usually swabs or blood tubes) with outer packaging composed of brown paper envelopes, plastic carrier envelopes (i.e., UPS or FedEx envelopes), and Styrofoam containers, and these are NOT ACCEPTABLE. All three major carriers in Wyoming (USPS, UPS, and FedEx) have warned us that improperly prepared or labeled packages are subject to return or disposal in the near future, and we strongly encourage you begin following the proper regulations now to avoid such problems.

These changes can be found on the websites for the major carriers:

http://www.ups.com/content/us/en/resources/prepare/hazardous/responsible/diagnostic.html (UPS)
http://pe.usps.com/cpim/ftp/manuals/dmm300/full/mailingStandards.pdf (USPS)
http://images.fedex.com/us/services/pdf/PKG_Pointers_Specimens_2007.pdf (FedEx)

Or, as always, you can call our Necropsy/Shipping & Receiving Technician (BreAnna Bonner) or her supervisor (Dr. Cornish) with shipping questions.

Dr. Todd Cornish/BreAnna Bonner

West Nile virus testing

This year the Wyoming Department of Health's West Nile virus grant from CDC was cut. As a result and with great regret we no longer have Terry Creekmore in the laboratory to offer WNV serology or PCR, or to speak to interested audiences around the state about the disease. Terry was a huge asset to the laboratory. We'd like to see him back in the state working in some diagnostic or wildlife capacity in the near future.

WNV serological testing will now be done in diagnostic serology by Joan Edwards. We've had to amend our prices, since the PCR and serology was partly subsidized by the CDC grant which has now ended.

The new prices for serology are:

IgM ELISA (single sample):	$15 each
(two or more samples):	$10 each

EVA SN Test

Joan Edwards is currently working up this assay so we can offer it in-house. We anticipate it will be up and running within a month, and until then we will be forwarding samples to another laboratory. Please contact Joan if you need this test run. Samples we need are 1 ml of non-hemolyzed serum.