

Wyoming State Veterinary Laboratory Newsletter

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Dr. Jim Briddle	Mr. John Morris
Dr. J.D. Fox	Mr. Bill Lambert

MESSAGE FROM THE DIRECTOR

The WSVL will soon convert to a new laboratory data management system. This will have little impact on you, our client, with one exception: for a period of 1 – 2 months, after we go live, you will not be able to access the system for on-line client reports. We have to make sure that the security system works and that it is functioning well internally. I will write to those of you who use the on-line function separately to let you know the timeline.

Todd Bleifuss, our information technology person, spent the past year preparing for migration to the new system. We are making the switch for technical reasons, some of it related to flaws with the original laboratory data management system obtained 2000. The new system is home-grown, developed by Todd and a Laramie software company called MicroChrome. The system will be more searchable and allow for retrospective epidemiological studies.

I am grateful to Drs. Jane Udem, Bill Williams, and Mark Schreiber for working with diagnosticians from the laboratory in presenting a series of interesting and challenging cases at the recent Jackson Hole Veterinary Rendezvous. Some are described in the Noteworthy Cases section of this newsletter.

The Department of Veterinary Sciences is home to the Wyoming State Veterinary Laboratory. Along with the rest of the university, which is wont to engage in periodic umbilical contemplation, we planning for the next five years (2004 – 2009). A draft plan for the department's intentions over the next five years is on our web site http://www.uwyo.edu/AgCollege/Strategic_Issues_files/Dept_VetSci_plan_draft.htm I welcome your comments and criticisms as they pertain to the laboratory. Some changes I would like to put in place are:

- Endowed chair for a professor specializing in diseases at the wildlife -livestock interface.
- Expanding from 3 to 4 pathologists, due to the rise in caseload.
- Hiring a veterinary epidemiologist to serve as extension veterinarian/field investigator.
- Resolve whether the laboratory should have a board-certified clinical pathologist. Clinical diagnosis is increasingly moving toward the live animal.
- Seek line-item funding for either replacement of laboratory equipment or for full-time "soft money" technicians.
- Formal quality assurance/quality control for all tests done at the laboratory.
- Formal membership of the National Animal Health Reporting Network, so that the laboratory can assist the

USDA in testing during foreign animal disease emergencies.

Our search for a veterinary pathologist continues. Normally, the laboratory has three pathologists reading cases. Although we had 10 applicants for this position, we did not identify a person with the qualities we want. The market for PhD, board-certified veterinary pathologists is tight and we must compete with pharmaceutical companies and veterinary schools. We recently re-advertised to fill the position. I hope to have a new person on board soon, but recognize that the turnaround of our pathology cases is affected. I apologize for this. Drs. Cornish and Williams are doing the work of three people, helped by sending out some cases to Dr. Quist in Montana. Please be patient with us! When you have an urgent biopsy, please phone us so that case can be read first. The slower turnaround will be fixed once we have the new pathologist on board.

Donal O'Toole
Director, WSVL

NOTEWORTHY CASES

PANLEUKOPENIA IN MUNICIPAL ANIMAL SHELTER

A litter of 4-week old kittens from a feral queen was brought to a municipal animal shelter in Wyoming. The history stated that the queen was found dead. Some kittens were sick or dying, and the shelter began rehydration therapy. A kitten bit a staff member and the laboratory received a request to test the cat's head for rabies. After discussion we encouraged the shelter to euthanize the litter, and submit all kittens for diagnosis. Samples of bowel were positive for parvoviral particles on EM, and also positive on fluorescent antibody. The shelter immediately disinfected the area where the kittens were held. Three kittens had classical lesions of acute panleukopenia (cryptal necrosis and enteritis).

In the past few years there have been several large outbreaks of panleukopenia in Wyoming animal shelters. As those of you who have had the misfortune to have FPV in your clinics will know, outbreaks can be difficult to control even in well run shelters. Parvoviruses are small, hardy viruses and acutely infected cats shed enormous infectious loads into the environment. Repeated cycles of disinfection and decontamination may not completely control contamination. In an outbreak in Laramie two years ago, infected asymptomatic cats were released to new owners, only to come down with acute disease and spread it to other cats in households.

The shelter in question was contained infection and no more cases occurred following diagnosis.

MICROCYSTIN TOXICOSIS IN BEEF HERD

On March 23 2003, 3 beef cows were discovered dead in their pasture at the morning feeding. Of the remaining 51 cattle, only a handful ran up to the feed truck in their usual manner. Clinical signs in affected cows were ataxia, star-gazing, lassitude, recumbency and brief agonal

convulsions. Post-mortem examination by the referring veterinarian did not reveal noteworthy changes beyond thistle-like seed heads in the rumen. Thirty five sheep in the same pasture were clinically normal. Six affected cows were treated empirically with activated charcoal and mineral oil without effect. By the following morning, another 6 cattle were dead. Forty two cows died during the ensuing 2 weeks. No sheep were clinically affected. A serum chemistry panel revealed moderately elevated alanine aminotransferase and markedly elevated lactate dehydrogenase. The principal histological finding was massive acute necrosis of the liver

An affected live cow was submitted for necropsy. Massive centrilobular hepatic necrosis was the principal histological finding. Liver and rumen from the index case contained 15 ppb microcystin by ELISA. The result was confirmed by mass spectrometry.

This case is unusual in that blue-green algal toxicoses are commonly a warm-weather problem. In this case, onset of signs was preceded by three years of drought, and unusually warm weather which precipitated a sudden runoff which flooded the pasture, leaving many puddles of warm, stagnant water. We hypothesize that either a dormant mat of a benthic cyanophyte such as *Oscillatoria* was dislodged and washed into the pasture via the irrigation system, or that unseasonably warm weather permitted a bloom to occur in standing water in the pasture. In the past, microcystin diagnosis was sufficiently cumbersome that it was seldom attempted in any but the most typical cases. However, with the advent of sensitive methods capable of detecting the toxin in tissue, microcystin toxicosis should be included in the differential diagnosis for any case of severe, acute massive hepatocellular necrosis in outdoor animals.

Dr. Raisbeck is presenting this unusual episode at the AAVLD/US Animal Health Association meeting this autumn.

JOHNE'S DISEASE IN A BEEF HERD

Johne's disease is a chronic progressive intestinal disease caused by *Mycobacterium avium* subspecies *paratuberculosis* (*Map*). We do not diagnose many cases of Johne's disease in Wyoming since it is most commonly identified in dairy herds. Nevertheless, cases occur in Wyoming beef herds. Drs. Cornish and Mills recently confirmed a case in a bull.

A 4-year old Angus bull was submitted alive with a history of chronic watery green diarrhea and weight loss. The bull originated in Montana and had been in the Wyoming herd for 3 years. An AGID test for Johne's disease was positive, and the owner agreed to confirm the diagnosis by a necropsy. The animal was bright and alert with a good appetite. The lining mucosa of the ileum, spiral colon and descending colon was thick with corrugated folds. Intestinal lymph nodes were mildly enlarged. Lesions were subtle and could have been overlooked in a field necropsy. Histologically there was mild segmental enterocolitis with acid-fast bacteria. The presence of *Map* was confirmed in gut..

Due to the "iceberg effect," for each one clinical case there are many pre-clinical cases in a herd. In

this case, the owner may have lucked out. He had 69 high risk animals blood-tested for JD and all were negative. Test and slaughter is the best way to control the disease. JD is not regulated in Wyoming at present.

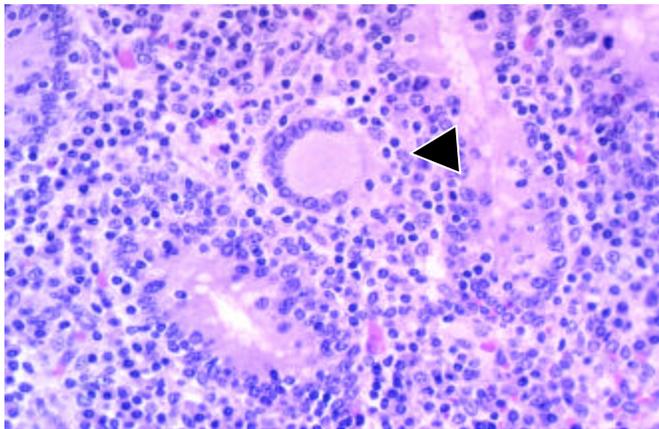


Figure: Giant cell in lamina propria of a young Wyoming bull with John's disease (arrowhead).

JD is a concern for several reasons. Confirmed cases increased in recent years in the US. Diagnostic assays are adequate for clinically affected cattle, but we are some way from a test that will pick up all subclinically affected animals. The gold standard for diagnosis – a positive culture from feces – takes months due to the growth properties of the organism. The *Map* organism has been linked to some cases of Crohn's disease in people, although the importance of *Map* in that disease is subject to debate. It is for these reasons the USDA has been considering an eradication program for JD.

In February the U.S. National Academy of Sciences issued an excellent report on the diagnosis and control of JD. The entire report is available free and on line at:

<http://www.nap.edu/books/0309086116/html/>

The WSVL is offering an ELISA test for John's disease testing (see New Tests for details)

RABIES ON PLATTE RIVER DRAINAGE

Rabies cases are increasing in Nebraska and at a 20-year high. Most positive cases are skunks in the central part of the state along the Platte drainage. Since skunks prefer riparian habitat, we commonly refer to rabies spread as "following rivers" which just means that there is a sufficient population to support continued passage of the virus from one animal to another.

With these high numbers in central Nebraska, it would not be surprising to see cases occur further west on the Platte and eventually reaching the eastern border of Wyoming around Torrington. In the past we have had skunk rabies in Wyoming on the Platte but these were limited to an area from Casper to Douglas. Cases did not occur in skunks east of that area. Be aware that with a robust skunk

population the rabies virus can make significant geographic movements into previously uninfected areas.

With the closure of the two western Nebraska laboratories, we have picked up clients in the panhandle area. We do NOT charge our regular clients for rabies exams, including those from western Nebraska.



Figure: Nebraska counties (in white) where rabies is diagnosed.

CAMPYLOBACTER ABORTION IN EWES

Campylobacteriosis is still a problem in Wyoming, particularly in large unvaccinated flocks. We see anywhere between one and five abortion storms per year – and some are doozies. In a recent episode beginning in late April some 20 days before lambing was due to start, an abortion storm occurred in a large commercial flock in the central part of the state. The producer lost 5 - 10 lambs daily. An aborted lamb was submitted. No distinctive gross lesions were found. Histological examination revealed suppurative pneumonia and leptomeningitis, suggestive of fetal septicemia. *Campylobacter jejuni* was isolated in pure culture from lung, liver, abomasal fluid and placenta.

The owner wanted to try to control the abortion storm by a single injection of procaine penicillin G-dihydrostreptomycin, but his veterinarian advised him this was unlikely to be successful. The flock was treated with 75 mg/head of oxytetracycline/day, along with a killed *Campylobacter jejuni* vaccine.

BIG HEAD IN SHEEP (PHOTOSENSITIZATION)

In late May a producer in western Wyoming who was moving a flock of 1,000 sheep in the Farson area noticed that 80 yearling ewes had swollen heads. Samples were submitted to establish whether this might be bottlejaw due to parasites with hypoproteinemia, or big head due to photosensitization. The sheep had high serum enzymes (12,000 - 24,000 U/l) and total total bilirubin (2.8 - 3.8 mg/dl; reference <0.5 mg/dl), with no evidence of significant parasitism, anemia or hypoproteinemia. The diagnosis is secondary photosensitization due to hepatic damage.

The cause of most photosensitization cases we hear about are never resolved. There are just too many possibilities in a typical Wyoming pasture and since animals usually respond to a change in diet and symptomatic

treatment, most owners are reluctant to allow more aggressive testing and investigation. I personally suspect that there are as yet unrecognized etiologies of big head in the state, but unless we're fortunate enough to investigate an episode that involves exposure to a small number of plant species, there isn't a practical way to identify them. **St John's wort** is the only known primary photosensitizer that grows in Wyoming. This plant is fairly rare and I doubt that it's really a problem. Secondary (cholestatic) photosensitizers are much commoner. **Kochia** is probably the most common cause in cases where we have a good idea of what happened. I don't know why, but it doesn't seem to cause photosensitization nearly as often in Wyoming as in southeastern Colorado. **Horsebush** is also fairly widely distributed around the state and is widely recognized as a photosensitizer. We had an unusual case east of Cheyenne which involved cattle ingesting nearly 100% of **yucca** roots for approximately 24 hours. Approximately 15% developed hepatogenous photosensitization within the following week. None died and we never got any follow up beyond running tests for liver enzymes. Since we didn't do a controlled feeding study this link has to be considered anecdotal, but many of the other Agavaceae are widely recognized as causing photo. **Senecio** (groundsel), **panicum** (witchgrass, proso millet), **ranunculus** (buttercup spp), **Brassica** (rape, kale, mustards) and the hepatotoxic **blue-green algae** have all been reported to cause photosensitization and each grows in some part of Wyoming. We have never recognized the association at the laboratory.

Dr. Merl Raisbeck

FATAL FELINE DYSAUTONOMIA

Dysautonomia in cats is an idiopathic disease affected the autonomic nervous system. It was first recognized in England in 1982 by Key and Gaskill, hence the eponym Key-Gaskill syndrome. During the early 1980s it was a common, epidemic disease of cats in the United Kingdom and Ireland. It was subsequently diagnosed elsewhere in Europe and, on rare occasions, in cats in North America. The incidence of the disease has declined. Its etiology is unknown.

A previously healthy, 4.5-kg 4-year old castrated domestic shorthair cat from northern Wyoming was presented to a veterinary clinic after 2 weeks of illness. Presenting signs were lethargy, mydriasis with no response to light, prolapsed third eyelids, regurgitation of food, and constipation. This was the only cat in the household, which was a predominantly indoor animal. For four days the veterinarians administered enemas, antibiotics and fluids, and force-fed the cat. There was no response. The presumptive diagnosis was feline dysautonomia. The cat was euthanized and the carcass submitted to the WSVL for necropsy.

The carcass was in adequate nutritional condition. The most evident changes at necropsy were megaesophagus (x3) with malodorous feed, megacolon (x2) with dry feces, and red mottled lungs. Microbiology and other tests were unrewarding: no bacteria or viruses were isolated from samples of lung, which was also negative on FA for *Chlamydia*, no viral particles were observed in gut contents,

and enteric parasites were inconsequential (38 EPG *Toxocara cati*).

There were severe, widespread histological lesions in the autonomic nervous system. These consisted of neuronal degeneration and necrosis in celiac, mesenteric, submucosal and myenteric plexus. Other changes were diffuse megaesophagus with erosive esophagitis, fibrinopurulent glossitis, and bilaterally symmetrical neuronal degeneration in select nuclei in brain stem, particularly dorsal vagal nuclei.

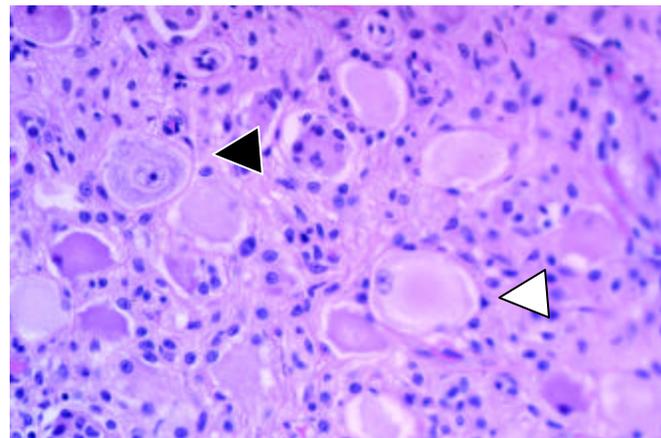


Figure: High power photomicrograph of celiac ganglion from Wyoming cat with dysautonomia. There is only one normal neuron in the field (black arrowhead). All other neurons show signs of severe degeneration with pallor, loss of Nissl and swelling (white arrowhead).

Laboratory confirmation the basis for disease in this instance was facilitated by the clinical diagnosis of dysautonomia. Confirmatory clinical testing was not done (prompt papillary constriction in response to pilocarpine solution; urination after subcutaneous administration of bethanechol). Clinicians should suspect dysautonomia when presented with cats with a history of acute onset lethargy, anorexia, depression, weight loss, vomiting/regurgitation, and dysuria, particularly if the one or more of the following clinical signs is present: papillary dilation, dry mucus membranes, loss of anal tone, bradycardia, postural reaction deficits, and prolapsed third eyelids. Approximately 25% of such cats survive with good supportive care, but they remain autonomic cripples. Most affected cats are euthanized within a year of clinical diagnosis. Canine dysautonomia also occurs in Wyoming. Both diseases are readily diagnosed on the basis of clinical signs.

This case will be presented by Dr. Cornish at the AAVLD meeting this autumn.

RABIES IN PALLID BAT COLONY

A researcher who studies the auditory system of pallid bats trapped 18 in the SW United States, including two non-pregnant females, for his research. The two females were kept in the same cage. One displayed atypical

threatening signs, such as hissing, and shortly thereafter died. The carcass was submitted for rabies examination. The brain was positive for rabies. The companion in the cage was euthanized and examined. She was rabies negative. The researcher was vaccinated for rabies. Subsequently, a second bat in the group displayed abnormal clinical signs. It too positive for rabies. We are waiting to see if more of the colony will come down with clinical signs.

Each year we see cases of rabies in bats. Bat-rabies is of particular interest to public health authorities since most human cases of rabies are of bat origin (24 of 32 cases; 1990 – 2000). Just two bat species (silver-haired (*Lasionycterus noctivagans*) and the eastern pipistrelle (*Pipistrellus subflavus*) bats) are responsible two-thirds of the human cases. Only the former occurs naturally in Wyoming – it present across the state during the warm months. The species that have the highest incidence of rabies are the solitary species or ones that form small roosting colonies.

Each bat species appears to have its own rabies strain. These can be identified by monoclonal antibodies, even when the strain crops up in terrestrial species. Of the 39 species of bat in the US, at least 30 have tested positive for rabies.



Figure: Pallid bat – *Antrozous pallidus pallidus*

A carrier state for rabies does not occur in bats, and they are little different from carnivores in terms of incubation periods, and clinical outcome. One difference is that rabies virus can exploit hibernation as a way to over-winter in bats. Bat rabies can get into terrestrial species, such as an episode in skunks in the Star Valley some years ago, but these outbreaks are not sustained.

When you are submitting bats to us for necropsy, please make sure they are dead first. We occasionally get shipping in live, rabid bats. We'd rather we didn't.

Dr. Ken Mills

IONOPHORE TOXICOSIS IN LAMBS

For six years an experienced sheep producer in Wyoming fed a special order protein supplement containing lasalocid to feeder lambs. In 2003 he fed 600, 20 – 30-kg home-raised Targhee and Finn-Targhee lambs using alfalfa grass hay (~5 bales/day), shell corn, live and tank water, special order pellets (22% protein; 120 g lasalocid/ton feed; target intake 15 – 70 mg/head/day), and free choice salt mineral mix containing lasalocid. The owner injected lambs

with a sodium selenite-vitamin E mixture. The mineral mix contained selenium (>12 ppm) and vitamin E (605 IU/kg). The private label feed was used by three producers in the area.

On 11 May the owner fed 200 lambs with one bag from the bottom of the pallet. She noticed that the pellets were mixed with a granular material that she interpreted as fines. All of the bag's contents were offered to the lambs. The following day, two lambs were dead and approximately 50% of pen mates were "down in their pasterns." Two adjacent pens of lambs, fed from other bags on the same pallet, were healthy. The owner suspected there was a problem with the feed. Samples were collected from the feed bunks for analysis.



Figure: Powder in a pellet ration that owner correctly suspected was responsible for losses among lambs.

Over the next 3 weeks, lambs displayed weakness and had difficulty walking. Ten lambs died. The remaining 90 affected lambs recovered. Neither of the other two producers using this lot of feed reported a problem.

Observations at necropsy were pale or variegated thigh muscles, full abomasums, blanched or pale cardiac septum, epicardial petechiation and, in lambs recumbent for extended periods, anteroventral pneumonia. Serum chemistry analysis revealed high AST (5,000 -25,000 IU), CK (91,000 – 427,000 IU) and LDH activities (15,000 - 99,000 IU). Lambs had mild neutrophilia, and granular casts in urine.

Tissues from 5 lambs were submitted. Lesions were severe acute or subacute myonecrosis of skeletal muscle (submitted slide), inhalational bacterial pneumonia, and mild multifocal myocardial degeneration.

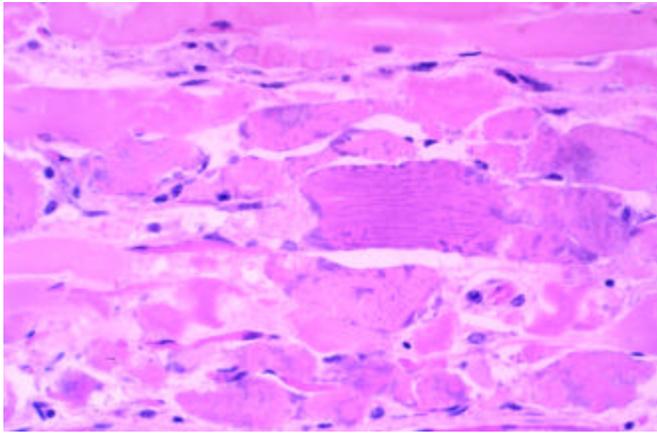


Figure: Severe acute myodegeneration in thigh muscle of affected lamb.

The presumptive diagnosis was ionophore toxicosis related to granular material present in one bag of feed. A manufacturer-commissioned analysis for lasalocid was done by a commercial laboratory. It reported 110.7 g/T (pellets) and 9 g/T (granular material). These were within the label claim (120 g/T). The feed company contended that the feed could therefore not be the basis of the problem and that the episode might be nutritional myodegeneration. It declined to pay for additional laboratory testing to resolve the cause of losses. The WSVL performed these tests.

Hepatic vitamin E (2 ppm) and selenium (0.24 ppm) were adequate. The granular material collected from the bunk were analyzed for ionophores by thin layer chromatograph and HPLC-MS. Results were <1 ppm naracin, <1 ppm salinomycin, 90 ppm lasalocid, and 500 ppm monensin in one bunk, and <1 ppm naracin, <1 ppm salinomycin, 80 ppm lasalocid and 150 ppm monensin in a second. The cause of death was monensin contamination, probably carryover from a previous batch of feed, restricted to one bag in the pallet, presumably the first in the production sequence of the owner's special order. Hepatic vitamin E (2 ppm) and selenium (0.24 ppm) were adequate.

In cases of feed contamination, testing for a range of potential contaminants associated with particular lesion profiles (in this case, severe skeletal myonecrosis with mild myocardial necrosis; ionophores) is essential to define the problem. After results were disclosed, the feed company confirmed that it recently recognized that carryover of monensin from a preceding feed batch had occurred.

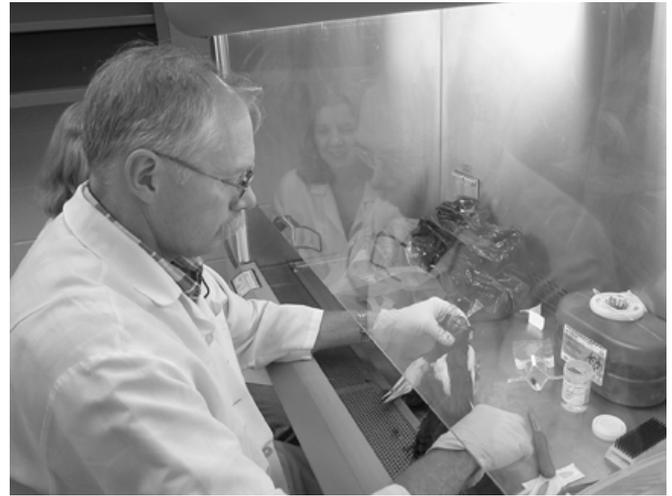
This case will be presented at the AAVLD national meeting this autumn

BACK IN TOWN FIRST WY CASES OF WNV IN 2003

Dr. Cornish confirmed the first West Nile virus case in a bird from Cheyenne that died on April 29.th There are EIGHT positive horses to date this year in Goshen, Fremont (2), Sheridan, Sublette, Albany, Big Horn and Sweetwater, and by the time this newsletter goes out these numbers will

be out of date. This will be a much bigger WNV year than 2002.

If you need to report a DEAD bird, please phone 1 800 WYO BITE. Alternatively, phone the WSVL.



Terry Creekmore, West Nile virus surveillance coordinator for Wyoming Department of Health, opens a dead magpie at the Wyoming State Veterinary Laboratory

WEST NILE VIRUS - REQUEST FOR COOPERATION

Horses in Wyoming are proving to be useful sentinels for West Nile virus (WNV) activity. In fact, we diagnosed WNV-positive horses **before** positive birds in most Wyoming counties. Positive horses appear to be the best early warning of local WNV activity in our neck of the woods.

We have been requested to provide pertinent information about WNV-positive horse (and other) cases to state/local public health and mosquito control officials, so that local citizens can be educated about risks, and mosquito control programs (where available) can be implemented or modified. This distribution of information will NOT circumvent normal laboratory-client confidentiality.

IN SUCH CASES, DR. CORNISH OR TERRY CREEKMORE WILL CONTACT YOU TO OBTAIN YOUR CONSENT AND THE ANIMAL OWNER'S CONSENT BEFORE RELEASE OF SPECIFIC INFORMATION.

While we realize that sharing of such information is not obligatory, we strongly encourage you and your clients to cooperate and help protect human and animal health in our state.

Dr. Todd Cornish
11 July 2003

NEWS ABOUT TESTS

1 - JOHNE'S DISEASE SEROLOGY

Diagnostic serology is offering a Mycobacterium paratuberculosis (Johne's) ELISA test as well as an AGID assay. Fees are as follows:

ELISA <= 20 samples	\$5/sample
ELISA >20 samples	\$4/sample (all samples)
AGID	\$8/sample

The ELISA kit is expensive and has a set expiration date. We will only continue to run this test if there is sufficient demand to run it in-house.

2 - BRUCELLA OVIS SEROLOGY

A reminder: we offer the *B. ovis* ELISA at a cost of \$4/sample. The CAE AGID test should only be requested for diagnostic or herd status. It is not licensed for use on health certificates for sales and fair purposes. The OPP/CAE AGID test is licensed for this purpose. Please request this test on the accession if you want it for health certification purposes. And PLEASE: Bang's tubes should not be used to collect serum for the above tests since soap residues in the tubes can affect the accuracy of results. Please submit 0.5 to 1 ml of serum. Serum can be sent in on the clot but keep in mind that if the environmental conditions are bad the sample may be ruined.

Contact person: Joan Edwards, Diagnostic Serology

3 - CORRECT FORMS!

For regulatory testing, please record the accession on the *Brucellosis Federal Test Record Forms* for brucellosis, pseudorabies and bluetongue.

But if you need regulatory testing done for anaplasmosis, submit it on the *Anaplasmosis Federal Test Record Form*.

SAMPLING FOR EQUINE NEUROLOGICAL DISEASES DURING WNV SEASON

Clinical signs of all significant infectious causes of neurological disease in horses can be similar and may overlap. Some clinical signs are suggestive of certain diseases. For example, muscle fasciculations are suggestive of WNV, whereas loss of tail tone, anal sphincter tone, and bladder control in a horse with hind limb paresis/paralysis is suggestive of EHV-1. But all of the common neurological diseases share enough clinical signs to make differentiation problematic.

Veterinarians with clients whose horses have neurological signs should call the laboratory to discuss the case before submitting samples. If this is not possible, in generic cases we suggest follow the sampling protocol below.

Always consider RABIES as a rule-out in horses with neurological signs

Live horses: Take serum for WNV IgM testing. If WNV serology comes back negative, but you remain suspicious of WNV, consider taking a second sample 7 days later. If WNV is ruled out, proceed with EHV-1 testing (done in-house at WSVL) and WEE/EEE (referred to another laboratory). Both of these tests are done on serum. If clinical signs warrant, please take cerebrospinal fluid for EPM (referred to another laboratory).

Dead horses: **TRY** to get post-mortem serum for the WNV IgM test and for EHV-1 serology. **ALWAYS** collect serum from horses with neurological signs when they are about to be euthanized. Serum is one of the most useful samples for WNV and EHV-1, even when an entire carcass is available for sampling. This is because postmortem tests for both diseases in horses are less than satisfactory.

Samples we need for a neurological workup are:

1. CSF fluid, collected at the atlas-C1 junction using needle-syringe or vacutainer through unincised dura mater.
2. Swab of meningeal surface before other brain samples are taken (to minimize contamination).
3. Whole brain, split longitudinally. Submit one half fixed, one half fresh.
4. Spinal cord: ideally, the whole thing **OR** segments of cervical, thoracic and lumbar cord. Less optimally, collect a segment of thoracic cord spinal cord. Submit both fresh and fixed samples. Please keep in mind that some diseases affect only certain portions of the spinal cord. Submission of the entire cord or multiple samples increases the chances of the laboratory making an appropriate diagnosis. If you are in a hurry, consider submitting a segment of spinal cord in intact 1 – 3 vertebrae, and trim off muscle to reduce the cost of shipping. We will take out the cord segment here.
5. PLEASE collect all other routine samples (lung, liver, spleen, kidney, heart, GI tract, etc.) both fresh and fixed. Some "neurologic" horses will have primary disease elsewhere (e.g., hepatic encephalopathy) and we will miss these if the sample is just brain.
6. If you suspect **BOTULISM**: examine feed for contamination (carcasses). Send feed and gut content samples for toxicology.
7. If you suspect **EHV-1 MYELITIS**: collect and send a purple top tube and nasal swabs.
8. If you suspect **EPM**: CSF is very helpful. It should be free of RBC contamination and collected promptly (<4 hours) after death.
9. If you suspect **INTOXICATION**: Collect 10 ml heparinized whole blood, serum, 100 ml urine, several hundred grams of fresh liver, kidney, ingesta, urine, heart blood, and any suspicious environmental samples (feedstuffs, water, drugs). Toxicology is expensive, so please specify what tests you have in mind. If you want

us to "screen" for poisons, please provide an upper price limit for toxicology testing.

WSVL will run rabies tests first, and then proceed with other tests as appropriate. We will NOT routinely attempt virus isolation from WNV-suspects due to the health risk to laboratory personnel.

A complete history of clinical signs, duration of illness, vaccination status (WNV, rabies, EEE/WEE, other), travel history, other horses affected/sick/dead, etc., is CRITICAL.

If you have questions about a difficult neurological case, please call the laboratory at 307 742 6638 before submitting samples.

Dr. Todd Cornish

DR. WOODARD'S RETIREMENT

Dr. Lynn Woodard plans to retire from the Department of Veterinary Sciences at the end of this year, although he graciously agreed to teach his popular Diseases in Food Animals and Horses course to final year undergraduates in Spring 2004. Lynn has been a fixture of the laboratory since he came to the University of Wyoming in 1986. For 13 years he was head of the department and director of the Wyoming State Veterinary laboratory. He was responsible getting the laboratory accredited by the American Association of Veterinary Laboratory Diagnosticians, which sets the national standard for veterinary diagnostic laboratories.



To celebrate and recognize Dr. Woodard, the Department will have a barbecue on Thursday 31 July 2003 at the laboratory (5:00 – 9:00 PM).

Those of you interested in attending should contact Mrs. Beth Howell for details: 307 742 6638.

Press release from WSVL 18 June 2003

EXPERTS DEBUNK SUGGESTED LINK BETWEEN WEST NILE VIRUS VACCINE AND BIRTH PROBLEMS IN HORSES

Despite recent media reports, Wyoming, Colorado and U.S. Department of Agriculture (USDA) veterinary experts say that there is currently no scientific proof to link the West Nile virus vaccine to aborted, stillborn or deformed foals and that horse owners should continue to vaccinate their animals to protect them from the deadly disease.

Claims by a Denver newspaper that some pregnant mares may have been adversely affected by the popular vaccine are unfounded, according to spokespersons from the University of Wyoming (UW) and Colorado State University (CSU) veterinary diagnostic laboratories and the Wyoming and Colorado state veterinary offices, because none of the horses in question has been scientifically tested.

"These are rumors. None of the veterinary diagnostic laboratories in the U.S. are seeing this syndrome or associating it with West Nile virus," said UW Professor Donal O'Toole, head of the Department of Veterinary Sciences and of the Wyoming State Veterinary Laboratory. "We have absolutely no evidence that the West Nile virus vaccine is associated with birth defects."

O'Toole encourages horse owners to continue to vaccinate since he says approximately one in three horses that show clinical signs of West Nile virus will likely die. "We know the virus is a lethal one. It would be irresponsible for horse owners to ignore this in favor of rumors," he added.

Barb Powers, director of the CSU Veterinary Diagnostic Laboratory, supports O'Toole's statements. "We have not seen any scientific proof that the vaccine is causing a problem, and we recommend that people vaccinate their horses for West Nile virus," she said. "If people have any concerns with pregnant horses, they should contact their veterinarian, their state veterinary laboratory or their state veterinarian."

West Nile virus is a mosquito-borne disease that causes inflammation or swelling of the brain and spinal cord. Since its discovery in New York in 1999, the incidence of equine West Nile virus nationwide has more than doubled. A total of 96 horses and 22 birds in Wyoming tested positive for the virus in 2002. So far in 2003, four horses and one bird have been diagnosed in the state. Colorado, which reported 378 horse cases last year, has one confirmed case to date in 2003.

Millions of doses of the "killed virus" vaccine manufactured by Fort Dodge Animal Health of Kansas and given conditional approval by USDA in 2002 and full license in February of 2003 have been administered to horses throughout the country.

What Colorado State Veterinarian Wayne Cunningham refers to as "anecdotal reports" of vaccinated mares delivering stillborn or deformed foals apparently led to media reports questioning the vaccine.

"None of the horses that are referred to in the article has actually been tested. There's no scientific proof that the vaccine is causing this problem in horses," confirmed Terry

Creekmore, vector-borne disease surveillance coordinator for the Wyoming Department of Health. Creekmore said he has spoken with USDA officials for the past several days and that the national agency “has not received any information from people who have tested these horses.” The Web site for the USDA Center for Veterinary Biologics currently states that the center “has had no reports of adverse events” regarding the West Nile killed virus vaccine.

Creekmore added, “People should not stop vaccinating their horses based on incomplete information that has no scientific validation.”

A spokesman for the USDA Animal and Plant Health Inspection Service (APHIS) noted that none of the kinds of material that would normally be required for veterinary diagnosis has been submitted. Many of the concerns raised have apparently come from horse owners who have buried their animals. Experts from APHIS and other national and state programs first encouraged the development of the West Nile virus vaccine.

Of 1,574 confirmed cases of West Nile virus in horses in 2002 in Wyoming, Colorado and Nebraska, only 13 were in fully vaccinated horses, according to UW’s O’Toole. “Of these 13, the fate of 12 animals is known – all 12 horses survived. The estimated case fatality rate is 28.6 percent in horses that show clinical signs. Vaccinated horses that become sick are more likely to survive than unvaccinated horses,” he said.

Todd Cornish, UW assistant professor in the veterinary sciences department, encourages horse owners who have aborted or stillborn fetuses or deformed or dead foals to submit these animals to a diagnostic laboratory for necropsy so that if any problems do surface in relation to the vaccine, experts will have a better chance of detecting it.

“We ask them what evidence they have. They say they vaccinated on day X and the fetus aborted on day Y. That’s not evidence. Without evidence, nobody really knows what’s going on,” Cornish explained. “There are a number of other causes of abortion and reproductive loss in horses, and it is important that a necropsy examination and full range of tests are performed on these cases to reach the appropriate diagnosis,” Cornish added.

“Anytime you have a pregnancy there’s a risk of abortion, stillbirth or an abnormal fetus for a number of reasons,” said Wyoming State Veterinarian Jim Logan. “I have not seen anything from any laboratory or any clinical data to substantiate pointing a finger at the West Nile virus vaccine. Nor have I seen anything from any other parts of the country that would make me concerned,” he said.

“I recommend that horse owners vaccinate their horses. If they have pregnant mares, they can consult with their private veterinarians to weigh the risks of not vaccinating with the possible risks of vaccinating,” Logan said, noting that foals from unvaccinated mares would be unprotected.

Cunningham, Logan’s counterpart in Colorado, recommends that USDA and Fort Dodge investigate the rumored reports of fetal problems but has clarified a comment attributed to him in a Denver newspaper by saying that “at this time there is no scientific justification to warrant a cautionary statement on the (vaccine) label.” The

newspaper had quoted him as saying that a warning statement should be immediately attached to the label.

According to a June 17 press report from Cunningham’s office, “In a study released this year by the University of Nebraska and Colorado State University, the Fort Dodge Animal Health vaccine was shown to be effective in preventing or minimizing the symptoms of West Nile virus. The study showed that fully vaccinated horses either did not become ill, or if they did fall ill, they recovered. In none of the cases studied did a vaccinated horse die as a result of the disease...Vaccinations combined with measures that control mosquito populations are the best ways to protect your horses, other susceptible animals, and yourself.”

For further information on West Nile virus in horses, contact Cornish at (307) 742-6681, Ext. 191, or the Wyoming State Veterinary Laboratory at (307) 742-6638.

MONKEYPOX INFECTIONS IN ANIMALS

CDC GUIDANCE FOR VETERINARIANS

The Centers for Disease Control and Prevention (CDC) is working closely with state and local health departments, the U.S. Department of Agriculture, the Food and Drug Administration, and other partners to investigate cases of monkeypox virus infections among humans (including veterinarians and staff at veterinary hospitals) who had direct or close contact with ill prairie dogs. The first exposure to ill animals was reported after April 15, 2003.

Human monkeypox is a rare zoonotic viral disease that occurs primarily in the rain forest countries of central and west Africa. In humans, the illness produces a vesicular and pustular rash similar to that of smallpox. The incubation period from exposure to fever onset is about 12 days. In humans, case-fatality ratios in Africa have ranged from 1% to 10% (additional information: www.cdc.gov/ncidod/eid/vol7no3/hutinG1.htm).

Infection in humans may be acquired through contact or respiratory droplets, the nasopharyngeal, oropharyngeal, or cutaneous route. Most of the human cases in this outbreak appear to have been transmitted through the cutaneous route. The route of transmission in animals is less clear. The virus might be transmitted to animals through the nasopharynx or oropharynx route, through skin abrasions, or through the ingestion of infected animal tissue.

In this outbreak, most human cases of monkeypox have been associated with close contact with prairie dogs (including bites, handling, household contact, or handling of cages/bedding). CDC is currently investigating how the prairie dogs may have become infected. The current working hypothesis is that prairie dogs may have been infected by an imported species of exotic mammals, possibly Gambian giant rats, kept in close proximity.

In Africa, serologic evidence of monkeypox infection has been found in a wide variety of nonhuman primates, rodents, and squirrels; monkeypox virus has been isolated from a species of squirrel in Zaire, but the role of any particular species as a reservoir has not been established. Some species of primates, rodents, and lagomorphs are known to be susceptible. Although no infections have been previously reported in dogs or cats, these species may also be susceptible to monkeypox. Because the types of animals that may become ill with monkeypox are currently unknown, all mammals should be considered susceptible as a precaution.

In the current outbreak, illness in prairie dogs was reported to include fever, cough, conjunctivitis, and lymphadenopathy, followed by a nodular rash. Some prairie dogs died, whereas others apparently recovered. Preliminary information suggests the Gambian giant rat under investigation reportedly experienced a much milder illness than that observed in prairie dogs, with no respiratory signs and possibly limited dermatologic involvement.



Gambian giant-pouched rat (*C. gambianus*)

Veterinarians should be suspicious of monkeypox in ill prairie dogs or Gambian rats, or any animal presenting with a history of fever, conjunctivitis, respiratory signs, and nodular rash. In some states, health departments are recommending that animals with suspected monkeypox not be transported to veterinary clinics due to public health risks. Veterinarians should check with state and local health officials for recommendations in their state.

Veterinarians who decide to examine or treat animals with suspected monkeypox should use infection control precautions to protect the health of themselves, staff, and clients, as well as other animal patients in the clinic. Clients who have alerted the clinic in advance that they are bringing an animal with suspected monkeypox should **NOT** be allowed to enter through the waiting area of the clinic. Veterinarians should isolate the animal and wear personal protective equipment (PPE) during the examination. The animal should not be taken to a common treatment room, and all treatments and diagnostics should be performed in the examination room. The number of staff allowed in the exam room and that come in contact with the animal should be limited to as few persons as possible. **Veterinarians who do not wish to examine an animal with suspected monkeypox should advise the animal's owner to contact the local or**

state health department for further guidance. The most common route for transmission of monkeypox from animals to humans appears to be direct contact with infected animals; however, the possibility of airborne transmission cannot be excluded. When examining animals with suspected monkeypox, veterinarians and staff should use the following precautions:

1. Hand hygiene after all contact with a sick animal and contaminated surfaces.
2. Use of gown and gloves for any contact with the sick animal and contaminated surfaces.
3. Eye protection (e.g., tight-fitting goggles or face shield) if splash or spray of body fluids is likely.
4. Respiratory protection, including a NIOSH-certified N95 filtering disposable respirator (or other respirator offering comparable levels of respiratory protection), for entering the exam room or patient care area. Most veterinary clinics will not have N95 respirators. If N95 or comparable respirators are not available for veterinary personnel, then surgical masks should be worn to protect against transmission through contact or large droplets.
5. Contain and dispose of contaminated waste after consultation with state or local health officials. Do not dispose of waste in landfills or dumps.
6. Handle used patient-care equipment in a manner that prevents contamination of skin and clothing. Ensure that used equipment has been cleaned and reprocessed appropriately.
7. Ensure that procedures are in place for cleaning and disinfecting contaminated environmental surfaces. Any EPA-registered hospital detergent-disinfectant currently used by health-care facilities for environmental sanitation may be used. Manufacturer's recommendations for dilution (i.e., concentration), contact time, and care in handling should be followed.
8. Laundry (e.g., towels, clothing) may be washed in a standard washing machine with hot water and detergent. The use of chlorine bleach during hot-water washing can provide an added measure of safety. Care should be used when handling soiled laundry to avoid direct contact with contaminated material. Soiled laundry should not be shaken or otherwise handled in a manner that may aerosolize infectious particles.
9. Contaminated surfaces should be cleaned and disinfected. Standard household cleaners or disinfectants may be used in accordance with the manufacturer's instructions. The animal's bedding,

cage, toys, or food and water bowls should not be disposed of with the clinic trash or at a dump or landfill because this material may be potentially infectious; contact the state or local health department for further instructions. Items that cannot be disposed of should be disinfected as contaminated surfaces.

Veterinarians that suspect monkeypox in an animal (i.e., an animal with a clinically compatible illness or is asymptomatic but is associated with human illness) should contact the state health department for information on specimen submission. CDC will not accept any specimens that are not sent through state health department laboratories. CDC recommends that practicing veterinarians not perform necropsies or biopsies to collect samples for diagnosis because of the risk for infection to the veterinarian. Samples that may be obtained by minimally invasive procedures, such as serum or conjunctival swabs, should be collected only by personnel wearing PPE. If the animal is deceased, double bag the carcass and place it in a freezer pending a decision for shipment.

All animals with suspected monkeypox infection should be humanely euthanized to prevent further spread of the disease. Disposal of the carcass should not include burial in a landfill or backyard setting. CDC recommends incineration of the carcass. If the animal is associated with a human case, it should be tested for monkeypox. Do not perform necropsies on animals with suspected monkeypox. Rather, whole carcasses should be double bagged and frozen. Consultation with the state epidemiologist and state health laboratory is necessary to obtain submission instructions before sending specimens to CDC.

Exposed asymptomatic animals should be quarantined in the home and not allowed to come into contact with other animals or people. They should be observed for development of symptoms compatible with monkeypox for at least 1 month following the last date of exposure. Should such symptoms develop, the animal should then be evaluated and euthanized if indicated, in consultation with state or local health officials.

Veterinarians and staff who have come in contact with animals with monkeypox should be alert for signs of illness for 21 days following the date of last exposure. Although restriction of day-to-day activities is not recommended for healthy, asymptomatic persons, individuals who develop a fever, respiratory symptoms, or unusual skin lesions within 21 days of contact with the animal should immediately limit activities outside the home and contact their physician.

Veterinarians who are diagnosed with monkeypox should not examine animals during their illness because they may pose a risk of disease transmission to animals, and should isolate themselves at home to minimize contact with other persons and animals. Veterinarians may resume job

duties when their physician and state and local health officials have determined that they are no longer infectious.

At the present time, CDC is recommending smallpox vaccination for persons who are within 4 days of initial direct physical contact with sick prairie dogs acquired since April 15 within the affected areas. Vaccination should also be considered for persons with such contact within the past 2 weeks. In addition, vaccination can be considered for persons who have, within the past 2 weeks, had close contact likely to have resulted in exposure to this environmentally hardy virus in respiratory secretions or through fomites on contaminated surfaces.

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