Colorado State University Veterinary Diagnostic Laboratories

LABLINES

Volume 8, Number 1 **Spring 2003**

Letter from the Director

Springtime in the Rockies is always a memorable event! With our heavy snowfall in March that closed the University and the Laboratory for two days, we narrowly escaped a roof collapse of our modular unit office as it was stressed by a couple of feet of heavy, wet snow. But Colorado needed the moisture and the quick response of pathologists and facilities personnel saved our office, so all is well. In January, we had an excellent meeting with our external advisory committee that has been very helpful to us on a number of issues. Inside is a listing of these individuals who willingly volunteer their time to help us make our laboratory better and allow us to meet your needs more efficiently. Please contact them or us directly if you have any comments regarding our laboratory service.

In February, we received re-accreditation for five years--all three laboratories--from the American Association of Veterinary Laboratory Diagnosticians. In their report, the main concerns were lack of space (emphasizing our need for a new building), and lack of adequate staffing for the volume of work we do. Unfortunately, in these difficult economic times, neither of these problems can be readily solved. In fact, along with the rest of the University, we have had to manage serious budget cuts from the State. Regardless of these difficulties, we are ready to meet the needs of emergency diseases by working with the State Veterinarian's Office to offer surveillance for Exotic Newcastle's disease and testing for West Nile (see inside for details). We will continue to offer all our other tests as we have in the past and to implement new tests that are more efficient, accurate and cost-effective.

It was very good to see many of you in January at the CSU Annual Conference/CVMA Leadership Conference. As CVMA President-Elect, I will soon (or may have already!) be meeting with you on district visits. I look forward to our visits and to seeing you in September at the CVMA Annual Conference.

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UREA TOXICITY

—Dwayne Hamar and Cathy Bedwell

Non-protein nitrogen (NPN) is a collective term used to represent several compounds that can provide economical protein equivalents to ruminant animals. Urea is the most common of these compounds. Other compounds used in the livestock industry include biuret, ammonium phosphate, and ammonium sulfate. Urea is rapidly hydrolyzed to ammonia and carbon dioxide in the rumen. The ammonia is incorporated in the carbon skeleton of readily fermented carbohydrates; the major carbohydrate in ruminant diets is starch from grain. Naturally occurring proteins contain about 16% nitrogen, whereas urea contains 46.7%.

NPN may be added to ruminant rations as a part of the mineral/protein pellet in a total mixed ration (TMR), a range block, or 'lick-tank' (molasses, urea, etc). There are three recommendations for determining the amount of urea that can be safely added to the ration of ruminant animals. Add urea at a rate of approximately 3% of the grain ration or about 1% of the total ration, with NPN representing less than two-thirds of the total nitrogen in the ration. Urea is poorly-utilized in all-roughage diets, thereby increasing its toxicity.

Urea toxicosis usually results from improper mixing or formulation, feeding urea to starved animals or animals unaccustomed to NPN, adding urea to a ration low in energy and protein but high in fiber, or mixing urea into a palatable feed that is fed free choice. For the last two winters, we have had suspected cases of urea toxicity in cattle grazing cornstalks where lick-tanks containing urea were available to the animals. Urea usually is lethal to ruminants at 1-1.5g/kg of body weight. Approximately 4g/kg is lethal to horses. Urea is not very toxic to monogastic animals.

Onset of clinical signs occurs rapidly, ranging from 10 minutes to four hours after consuming a toxic dose of urea. These signs may include grinding of the teeth, kicking at the abdomen (indications of abdominal pain), frothy salivation, polyuria, incoordination, weakness, forced rapid breathing, bloat, violent struggling, and bellowing. Death usually occurs rapidly. There are no characteristic lesions of urea toxicity; however, pulmonary edema, congestion, and petechial hemorrhages commonly are observed.

Confirming urea toxicity presents somewhat of a diagnostic dilemma. Elevated rumen ammonia (>800ppm) is indicative of urea toxicity, but rumen contents must be sampled from an animal that just recently has died and been frozen immediately to arrest biological activity and eliminate loss of ammonia. Elevated blood ammonia (>1mg/dl) also is associated with urea toxicity; however, the sample must be placed on ice and analyzed within one hour of sampling. Normally, analyzing the feed for urea and/or analyzing properly sampled and stored rumen contents for ammonia, coupled with clinical signs and history, is the best diagnostic methodology available.

Since urea toxicity has a very rapid onset, treatment often is not possible. If the opportunity does arise, 5% acetic acid or vinegar should be given by ruminal infusion. Give from 2 to 6 liters for cattle, but no more than one liter for sheep and goats. Acetic acid lowers the pH of the rumen, decreasing the absorption of ammonia. Large volumes of cold water also

may be given. Cold water lowers the temperature in the rumen and decreases the rate of conversion of urea to ammonia.

Urea Analysis: Submit 1-2 cups pellets/TMR or liquid supplement, or fist-sized chunks of solid supplements. Fee=$12.00

Ammonia Analysis: Submit 4-5 cups FROZEN rumen contents (we currently do not provide blood ammonia analysis). Fee=$12.00

SALMONELLA NEWPORT UPDATE

—Doreene R. Hyatt

Reported cases of *Salmonella* serotype Newport infections are on the rise in the United States. This increase, seen during the past three years in both humans and animals, has resulted in the Centers for Disease Control holding meetings dedicated to the topic. The main reason for the intense interest in this organism is that it is resistant to many different antimicrobials. In addition, this resistance is encoded for on a plasmid. This plasmid appears to be very effective in spreading resistance and acquiring new resistance genes. In fact, the USDA has reported that the organism has increased its resistance from five to nine antibiotics during the last four years. The most common resistance profile is the same as that found in *Salmonella typhimurium* DT105; ampicillin, chloramphenicol, sulfonamides, streptomycin, tetracycline. Other isolates have been found with resistance to cephalothin, cefoxitin, ceftiofur, kanamycin, and trimethoprim/ sulfamethoxazole, and some with decreased susceptibility to ceftriaxone.

The National Antimicrobial Resistance Monitoring System (NARMS) has reported a rise in the number of human MDR *S.* Newport cases as a percentage of all Salmonella isolates over the past few years. From 1997 to 1999, *S.* Newport accounted for 5.3% (225/4,266) of human Salmonella isolates tested at NARMS. In 2002, *S.* Newport accounted for 9% (124/1,378) of human Salmonella isolates tested at NARMS. The proportion of *S.* Newport isolates identified as resistant to two or more antimicrobials increased from none of the 48 isolates in 1997 to 1.3% (2/78) in 1998, 17.2% (17/99) in 1999, and 22.6% (28/124) in 2000.

For the same three-year period (1997 to 1999), 1% (56/5,340) of animal Salmonella isolates collected at slaughter or processing facilities were *S.* Newport. There were no *S.* Newport isolates reported in 1997. The proportion of *S.* Newport isolates with the same MDR pattern as the human outbreak strain increased from 8.3% (1/12) in 1998 to 27.3% (12/44) in 1999. In 1999, all 12 Newport isolates with the multidrug-resistance pattern were taken from ground beef.

S. Newport was the third most common serotype identified at the National Veterinary Services Laboratory (NVSL) between July 2000 and June 2001. This was the first time this serotype was included among the 10 most common serotypes. It was the second most common serotype isolated from cattle in cases of clinical disease (51%). *S.* Newport also was the second most common serotype isolated from horses with clinical disease (18%). During that time period, the organism also was identified from bison, cats, chickens, deer, dogs, elk, feed, goats, reptiles, sheep, swine, turkeys, and others. From July 2001 to January 2002, 915 isolates were submitted to NVSL of which 53% were from cattle and 10% from horses.

In diary cattle, *S.* Newport infections most often are seen in adult cows with clinical signs including watery diarrhea, rapid drop in milk production, and high fever (104-106°F). These cattle are unresponsive to therapeutics used to treat enteric Salmonellosis, including anti-inflammatory agents and antibiotics. As with other Salmonella infections, it is more common for fresh cows and heifers to be affected than animals in other stages of lactation. Animals infected with *S.* Newport remain clinically ill for longer periods than animals with Salmonella infections from other serotypes, probably because of the poor response to therapy. Often, animals infected with *S.* Newport do not return to good milk production, and many are culled because of their poor production and decreased condition. In some areas, a significant occurrence of the disease also has been reported in calves.

Salmonella Newport isolations at the National Veterinary Services Laboratories and at the CSU-VDL

Year	# of Submissions to NVSL	# of Submissions to CSU-VDL
Jul 97–Jun 98	169	Unknown
Jul 98–Jun 99	315	7
Jul 99–Jun 2000	405	25
Jul 2000–Jun 01	978	148
Jul 01–Jan 02*	915	44
Feb 02–Jun 02*		10
Jul 02–Jan 03*		82

*Last date that statistics were available.

So, why should we in Colorado be worried about this organism? As you can see from the above table, we have been isolating more and more of this organism from submitted animal samples. From July 2002 until January 2003, we isolated *S.* Newport 82 times. If this isolation rate continues, we will surpass our previous yearly isolation rate of 148 (in 2000/2001).

Given the public health consequences, it is vital that veterinarians be aware of this organism and how they can help halt the spread throughout both animal and human populations. For more information, the USDA has put out a four-page bulletin (http:www.aphis.usda.gov/vs/ceah/cahm/ What's New/Snewport.PDF) entitled "What Veterinarians and Producers Should Know About Multi-Drug Resistant *Salmonella* Newport." Much of the information in this newsletter article was taken from this information sheet.

UPDATE ON WEST NILE VIRUS TESTING

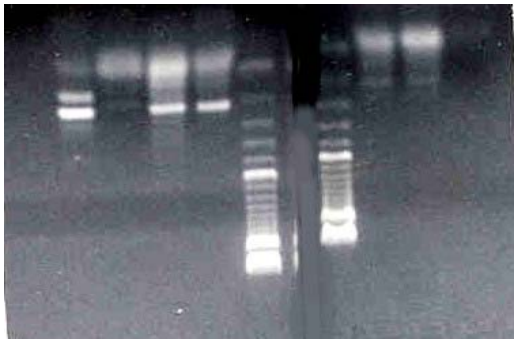
—Hana Van Campen

Last August, West Nile virus (WNV) arrived in Colorado. During the rest of 2002, 380 horses, 13 humans, and 138 WNV-infected wild birds were detected in the state. More equine, avian, and human cases are anticipated during the 2003 season. To aid veterinarians in the diagnosis of West Nile virus infections, we offer the following tests:

Serology

Horses only—Naturally infected horses develop WNV-specific IgM antibodies 7 to 10 days following infection and IgM antibodies usually are detectable by the time horses develop clinical signs of encephalitis. Horses vaccinated with the inactivated whole virus vaccine (Fort Dodge) do not develop WNV-specific antibodies of the IgM subclass. Therefore, WNV IgM capture ELISA differentiates naturally-infected and vaccinated horses. The WNV IgM capture ELISA is offered at the CSU Veterinary Diagnostic Laboratories at Fort Collins, Rocky Ford, and Grand Junction. Cost is $7.00/single serum sample or $5.00 for two or more samples per owner. This test also is available at the Rocky Mountain Regional Animal Health Laboratory in Denver.

All Other Species—Serology to detect WNV-specific antibodies in species other than horses should be sent to the Animal Health Diagnostic Laboratory, Cornell University, Ithaca, New York 14853. Serum neutralization (SN) is $25.00, turnaround is 7 to 10 days; avian WNV IFA/other species IFA is $20.00, turnaround is three days.



PCR gel of West Nile Virus and controls

Virus Detection

All Species—A polymerase chain reaction (PCR) test detects WNV genetic material (RNA) in fresh tissue samples. The WNV PCR has detected WNV in the brains of WNV-infected horses and one sheep with encephalitis, and is currently available at the Fort Collins Diagnostic Laboratory. Fresh tissues should be collected aseptically and shipped overnight on ice. Cost = $30.00/sample

All Species—Immunohistochemistry (WNV-IHC) to detect WNV proteins in formalin-fixed brain and other tissues currently is available at the Fort Collins Veterinary Diagnostic Laboratory. High amounts of WNV antigen can be detected readily in the brain and heart of many avian species by WNV-IHC. In contrast, WNV antigens occur in low amounts in the brains of horses with WNV encephalitis, meaning that the WNV-IHC is a relatively insensitive test in this species. Cost = $15.00/slide

All Species—Samples for virus isolation (WNV-VI) should be sent to Animal Health Diagnostic Laboratory, Cornell University, Ithaca, New York 14853. Cost = $50.00, turnaround is 21 to 30 days

Dead Birds (Wild Species)—CDPHE will continue to accept oral swabs from corvids (bluejays, crows) through 2003. Testing for WNV is free. For more information, contact the Colorado Department of Public Health and Environment, ATTN: Virus Laboratory, 8100 Lowry Blvd, Denver, CO 80230-6928. Telephone 303-692-2700. Complete information on how to submit samples is available on the CDPHE website (www.cdphe.state.co.us/dc/zoonosis/wnv/wnvhom.html).

DEVELOPING A BIOSECURITY PROGRAM, PART II

—James Kennedy/Rocky Ford

Part I in our previous LabLines dealt with an overview of a biosecurity program. Now, it's time to delve more deeply into the components of a biosecurity program starting with the selection and use of disinfectants.

Most producers, and far too many veterinarians, fail to understand the importance of selecting the proper disinfectant for their use. The most common mistake is the failure to understand that cleaning must proceed disinfection. Organic debris deactivates most disinfectants or, at a minimum, lengthens the time needed for that disinfecting agent to act. Prior to disinfecting any surface or object, that object should be thoroughly cleaned. Walking through a pan of disinfectant without removing the manure from your boots accomplishes only one thing—it places enough organic debris in the pan to render the remaining disinfectant ineffective for the next pair of boots. The importance of cleaning prior to disinfecting cannot be overstated, whether dealing with boots, instruments, equipment or vehicles. If it is not clean, you cannot expect any disinfectant to eliminate pathogens. Another issue of concern is selecting the disinfectant to do the desired job. You need to know what pathogens you are concerned about and if there are special considerations concerning that pathogen. Below is a table adapted from the Nebraska Extension Publication, "Selection and Use of Disinfectants" (Kennedy, Bek and Griffin), that provides some useful information when selecting a disinfectant.

Disinfectant Selection

	Chlorine 0.01-5%	Iodine Iodophor 0.5-5%	Chlor-hexidine 0.05-0.5%	Alcohol 70-95%	Oxidizing 0.2-3%	Phenol 0.2-3%	Quaternary Ammonium 0.1-2%	Aldehyde 1-2%
Examples	Clorox	Tincture/ Provodine	VikronS	Alcohol	Novalsan	Lysol	Roccal-D	Wavicide
Bactericidal	Good	Good	Very Good	Good	Good	Good	Good	Very Good
Virucidal	Very Good	Good	Very Good	Good	Good	Fair	Fair	Very Good
Envelope Viruses	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Non-Envelope Viruses	Yes	Yes	No	No	Yes	No	No	Yes
Bacterial Spores	Fair	Fair	Poor	Fair	Fair-Good	Poor	Poor	Good
Fungicidal	Good	Good	Fair to Good	Fair	Fair	Good	Fair	Good
Protozoal Parasites	Fair Strong Conc	Poor	Poor	Poor	Poor	Poor	Fair (Ammonia)	Good
Effective in Organic Matter	Poor	Fair	Fair	Fair	Poor	Good	Poor	Good
Inactivated by Soap	No	No and Yes	No	No	No	No	Yes	No
Effective in Hard Water	Yes	No	Yes	Yes	Yes	Yes	No	Yes
Contact Time (min)	5-30	10-30	5-10	10-30	10-30	10-30	10-30	10-600
Residual Activity	Poor	Poor	Good	Fair	Poor	Poor	Fair	Fair

As a side note, most of us need a reminder on specific viral characteristics, specifically which characteristics are enveloped and which are not. The scare in 2001 concerning the possible outbreak of foot and mouth disease in the United States, resulted in a misdirected attempt at disinfection without knowing the characteristics of the virus. Below is a table showing some common bovine viruses and their characteristics.

Envelope Characteristics of Some Common Bovine Viruses

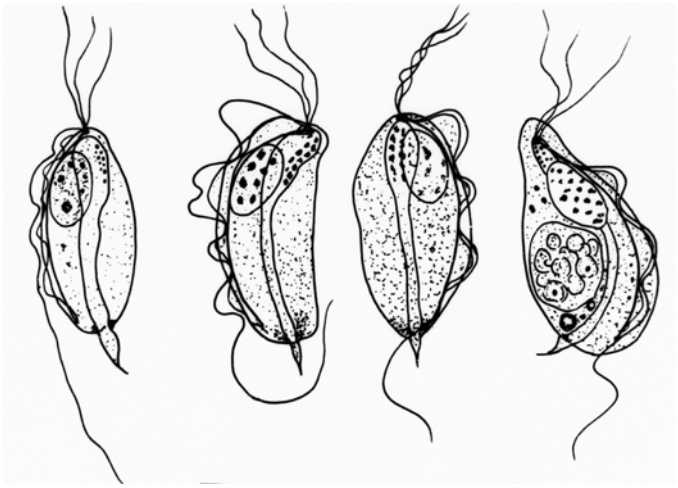
Virus	Envelope	Virus	Envelope	Virus	Envelope
Bluetongue	No	Malignant Catarrhal Fever	Yes	PI-3	Yes
Rotavirus	No	Enteric Coronavirus	Yes	Rabies	Yes
Papillomatosis	No	Respiratory Coronavirus	Yes	Herpes mammillitis	Yes
Leukemia	Yes	BVD	Yes	Cowpox	Yes
Papular stomatitis	Yes	BRSV	Yes	Pseudocowpox	Yes
Vesicular stomatitis	Yes	IBR/IPV	Yes	Lumpy Skin disease	Yes
Foot and Mouth	No				

We hope you find the above information helpful to understanding the importance of disinfection when developing a biosecurity program.

NEW TRICHOMONAS REGULATIONS IN COLORADO/TRAINING AND UPDATE

—John Cheney

The new Colorado regulations for Trichomoniasis went into effect in October 2002. As part of these regulations, veterinarians collecting Trichomonas samples from bulls are required to attend a training session on how these samples should be collected and handled. These training sessions were conducted on various ranches throughout the state where bulls were made available. Training was conducted in the following areas – Steamboat Springs, Fort Collins, Wellington, Rocky Ford, Platteville, LaGerita, Gunnison, Mancos, Silt, LaJunta, Montrose, and Fort Morgan. To-date, almost 200 veterinarians have received this training. The training was conducted by veterinarians from the State Veterinarian's Office, the State Cooperative Extension Veterinarian (Dr. Kimberling), and the Colorado State University Veterinary Diagnostic Laboratory (Dr. Cheney) in Fort Collins.



Trichomonas organism

Samples collected from these bulls can only be tested by one of the CSU Veterinary Diagnostic Laboratories in either Fort Collins, Grand Junction, or Rocky Ford, or the Rocky Mountain Animal Health Laboratory (RMAHL) in Denver. Samples which test positive in one of these laboratories for a "trichmonad organism" are sent to the RMAHL in Denver for PCR testing. This test will confirm if these are true *Trichomonas foetus* organisms or a non-pathogenic trichmonad organism. Bulls testing positive by the PCR test for *T. foetus* must then be tagged and can only be sent to slaughter. Bulls that test negative for the *T. foetus* organism are considered to have the non-pathogenic organism and can be cleared for sale and breeding.

If anyone has questions regarding the Trichomonas regulations or this testing, please contact the State Veterinarian's Office at 303-239-4161 or one of the Colorado State University Veterinary Diagnostic Laboratories.

Trichomonas culture: Submit a preputial scraping or vaginal wash. Fee=$8 for 1-50; $7 for 51+

EXOTIC NEWCASTLE DISEASE VIRUS OUTBREAK

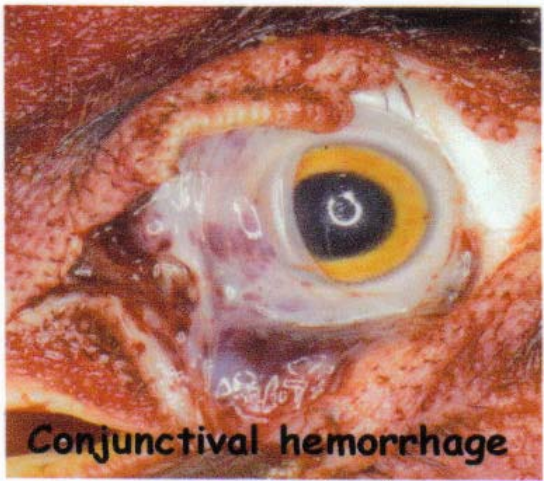
—Hana Van Campen

In October 2002, an exotic Newcastle disease virus (ENDV) was diagnosed in backyard chickens and game fowl in southern California. The virus quickly spread to San Bernardino, Los Angeles, and Riverside counties. The outbreak in California has claimed over 2 million chickens and includes eight large commercial premises. By the end of January 2003, infected chickens were detected in Clark County (Las Vegas), Nevada, and in La Paz County, Arizona , and in El Paso, Texas in April 2003. The rapid spread of ENDV has largely been attributed to traffic of infected game fowl. Exotic Newcastle disease is a reportable disease with severe economic consequences for the poultry industry. A USDA task force of 1500 people has been assembled and State Veterinarian Wayne Cunningham has initiated a surveillance program for END in Colorado. Suspect END cases should be reported to the State Veterinarian's office at 303-239-4161.

Exotic Newcastle disease virus is one of a large number of paramyxoviruses that infect a wide range of avian species. Newcastle disease viruses vary in virulence from lentogenic (low pathogenicity) strains used to vaccinate poultry, to velogenic (high pathogenicity) strains like the present END virus that cause 90% mortality in poultry. While some velogenic strains primarily cause neurologic signs such as ataxia, and paralysis of wings or legs, the California END virus is viscerotropic. Clinical signs include anorexia, depression, nasal discharge, dyspnea and diarrhea. Frequently, premonitory signs are not observed and owners may only note sudden death. Lesions include diphtheritic plaques in the oropharynx and esophagus, fibrinonecrotic tracheitis, necrosis and hemorrhage of cecal tonsils, and hemorrhages in conjunctivae, proventriculus, and cloacal mucosa. The virus is secreted in large quantities in oral-nasal secretions and feces. Spread of the virus occurs by transport of and exposure to infected birds, fecal-contaminated shoes, vehicles and other fomites, but also may be aerosolized. While the virus is readily inactivated by a variety of disinfectants including quaternary ammonium, bleach, and Lysol, it survives for weeks in organic material and carcasses especially in warm, humid conditions. Printed information on END is available through Colorado State University's Extension Service, the CVMA, or the State Veterinarian's office.

Diagnosis of END is by gross lesions observed at necropsy and confirmed by virus isolation from tracheal, oropharyngeal and cloacal swabs, or tissue samples (trachea, lung, spleen,

cecal tonsil). Any viruses isolated are further characterized using antiserum to NDV and PCR tests to distinguish END from avian influenza or lentogenic NDV. In Colorado, birds may be tested for END by sending tracheal and cloacal swabs in virus transport media (VTM) for virus isolation to the CSU Veterinary Diagnostic Laboratory in Fort Collins. Swabs and VTM tubes are available through the Diagnostic Laboratory. Swabs should be mailed overnight on ice/chill-packs. Serum samples can be tested for antibodies to NDV using an ELISA available at Fort Collins, Grand Junction, and Rocky Ford Veterinary Diagnostic Laboratories. Serology is most suitable for surveillance of commercial poultry with known vaccination histories. Finally, birds may be submitted for necropsy. For further information on submitting samples for END surveillance, please call us at 970-491-1281 in Fort Collins, 719-254-6382 in Rocky Ford, or 970-243-0673 in Grand Junction.



Newcastle disease virus
(San Bernardino Laboratory/CAHFSL)

FUMONISIN TOXICOSIS IN HORSES, MOLDY CORN POISONING REVISITED

—Jim Kennedy/Rocky Ford

A southeast Colorado equine owner called his veterinarian to request examination of a horse demonstrating neurological symptoms. The horse appeared depressed with some degree of incoordination that later progressed to lateral recumbency and death. The course of the apparent illness was approximately 48 hours from initial onset of clinical signs. Other horses stabled at the same facility did not demonstrate simultaneous clinical signs but, over the course of 10 days, five more horses developed similar signs and either died or were humanely euthanized. Tests for encephalitis and equine protozoal myelopathy were negative. Histological examination of brain tissue was suggestive of mild edema and an encephalomalacia of uncertain origin. Toxicological/chemical evaluations of feed and water samples were performed with a high level of sulfate, >3400ppm, found in the water supply. Nitrate levels in hay samples, although at levels toxic to cattle, were found to be insignificant when dealing with the equine digestive system. A second source of available feed, corn screenings, also was examined for the presence of toxic principles and found to contain 12ppm fumonisin. Levels above 5ppm fumonisin are considered toxic to horses and when found above 10ppm may produce an acute toxicosis. Clinical signs of fumonisin toxicity resemble those demonstrated by the horses and histological evidence of a leukoencephalomalacia supported the diagnosis of fumonisin poisoning.

Fumonisin toxicosis was previously referred to as moldy corn poisoning but with the ability to accurately identify the toxic principle, it is more correct to refer to toxicosis from moldy corn by the specific toxicological agent involved. It also is important to note that testing for aflatoxins differs from testing for mycotoxins or more specifically fumonisins. Fumonisins are produced by molds of the genus *Fusarium*, and at least seven individual *Fusarium* toxins have been identified. Furthermore, complicating this moldy issue is that the toxin associated with leukoencephalomalacia in horses is somewhat uncertain even though the specific *Fusarium* species concerned, *Fusarium moniliforme*, has been identified. Treatment of fumonisin toxicosis consists of removing the contaminated feed stuff, supportive therapy and, as with any neurological condition, ensuring the client is adequately informed that the chance of a full or even partial recovery is extremely low.

Retrospectively, this past year has been an ideal year for any of a variety of mold poisonings. The drought conditions significantly lowered grain yields and grains that had previously been in storage were placed on the market to meet demands. Improperly stored grains are frequently moldy and may contain any of a variety of mycotoxins that may be detrimental to livestock. Despite the existence of regulations in some states concerning the level of mycotoxins that are permissible in marketed grains, we frequently purchase our grain locally without the influence of state regulations, thereby leaving the door open for mycotoxins poisoning.

AN OUTBREAK OF TYPE C BOTULISM IN HORSES IN COLORADO

—Kristy Pabilonia

In October 2002, a horse farm in Colorado experienced a sudden outbreak of illness in their horses. The first horse affected presented acutely down and unable to rise. The horse became progressively non-responsive and died within 24 hours after the onset of clinical signs. One week later, another horse suffered the same fate. Post-mortem examination of the second horse revealed a hemorrhagic enteritis. Three days later, two horses from the same paddock presented acutely down and unable to rise. Both horses had a flaccid tongue and evidence of dysphagia. Paddling and muscle fasiculations increased in severity in the horses over time. Signs of shock

became evident just before death. The horses died within 15 minutes of each other, only 12 hours after the onset of clinical signs. Post-mortem examination of these horses also revealed a hemorrhagic enteritis. During the next three weeks, four more horses died. In total, eight of the 13 horses on the premises died. In addition, one of three horses from a neighboring horse farm died with similar clinical signs. Rigorous supportive care was administered to all of the horses but little improvement was noted. The last five horses were euthanized for humane reasons. Mild hemorrhagic enteritis or catarrhal enteritis was noted in three of the last five horses. The owners decided to administer multivalent antitoxin to the remaining five horses and remove them from the premises. To-date, these horses have remained healthy.

Samples from every horse were collected and submitted for a variety of tests, including aerobic and anaerobic culture, West Nile virus IgM ELISA and PCR, virus isolation, equine herpesvirus serology, CSF analysis, biochemistry panels and complete blood cell counts, organophosphate analysis, Western equine encephalitis IgM ELISA, Potomac horse fever PCR and lead, arsenic, and strychnine analysis. In addition, feed samples were tested for the presence of ionophores. Negative results from these tests ruled out these differential diagnoses.

Multiple samples from each horse, samples of feed, and organic material from the feed troughs were sent to the National Botulism Laboratory at the University of Pennsylvania for botulism testing. The mouse bioassay was performed on each of the samples. Two samples (stomach contents and small intestinal contents) from the same horse tested positive for Type C *Clostridium botulinum* preformed toxin and spores. All other samples were negative. Because the affected horses exhibited clinical signs of botulism and other differential diagnoses were ruled out, and the survival of horses given antitoxin, the one positive test result was considered sufficient to confirm the diagnosis of botulism.

Clostridium botulinum is a gram-positive, spore-forming, obligate anaerobe. The clinical syndrome of botulism is the result of exposure, usually via ingestion, to the neurotoxin produced by *C. botulinum*. The toxin binds irreversibly to peripheral cholinergic synapses, which blocks the release of acetylcholine. This results in the impairment of neuromuscular and autonomic transmission. Seven toxin types (types A through G) have been identified. In the United States, cases of Type A, B and C botulism have been reported in horses. Type B toxin in the most common (>85% of cases) form of botulism affecting horses, with Type C occasionally affecting horses. Type B botulism is the result of the production of toxin in decaying vegetable matter. Type C botulism is usually associated with feed materials that are contaminated by decomposing carcasses. Type A is extremely rare.

Botulinum toxin is the most potent biologic toxin known to man. Horses are extremely susceptible to botulinum toxin, especially when compared to other species such as dogs, cattle, or humans, which are relatively resistant. The onset of clinical signs occurs between 12 hours and 10 days following exposure. Exposure to large amounts of toxin can result in rapid progression of clinical signs and death within 12 hours. The most common clinical sign of botulism in horses is symmetrical motor paresis or paralysis. Other clinical signs that may be observed include mydriasis, ptosis, decreased tongue tone, slow tongue retraction, decreased tail tone, muscle fasciculations, and dysphagia. Death is typically the result of paralysis of the respiratory musculature.

Type-specific or multivalent antitoxin and rigorous supportive care can be used to treat botulism. Botulism is usually fatal, unless the horse is treated early. Once the horse is recumbent and unable to rise, treatment is ineffective and the prognosis is grave.

The circulating amount of botulinum toxin is typically so small in horses it is undetectable by available testing methods. As a result, a diagnosis of botulism is usually made after ruling out other possible diagnoses. The most sensitive test for detecting botulinum toxin is the mouse bioassay. This test can be performed on serum, feces, stomach contents, and intestinal contents. To detect preformed toxin, an extract of the sample is injected into two mice. If clinical signs of botulism develop, the injections are repeated in multiple mice. Half of these mice also are given antitoxin. If a specific antitoxin neutralizes the botulinum toxin and confers protection, then a conclusive diagnosis of botulism can be made. Detection of *C. botulinum* spores is a similar process. It is important to remember that an absence of preformed toxin and spores in mouse bioassay testing does not rule out a diagnosis of botulism. Typically, multiple samples from multiple horses (in the case of an outbreak) must be tested before preformed toxin or spores are found. An ELISA test is available for the detection of Types C and D toxin, but this test is not as sensitive or specific as the mouse bioassay.

The source of the *Clostridium botulinum* in this outbreak was never identified, despite attempts to locate an animal carcass in the feed or in the environment. The horse farm and

neighboring farm were large (more than 300 combined acres) and contained streams and marshy areas. These factors made surveying the property for an animal carcass difficult. No commonalities between the horses were identified except the location. The horses were many different breeds and ages. The horses were housed on various sites throughout the property. The horses had different feed sources (some ate baled hay/alfalfa and a variety of commercial grain feeds while some ate only pasture grass), and two different water sources. The neighboring farm also had different feed and water sources. The property was examined for poisonous plants but none were found that could have caused these clinical signs. The relationship between the hemorrhagic enteritis that was found in many of the horses during post-mortem examination and botulism has not been determined. Previous reports of Type C botulism in horses do not describe horses having this type of lesion. A report of an outbreak of Type C botulism in 12 horses and one mule in Arizona discusses the possibility of birds being a mechanical vector of botulism toxin. In that case, birds carried botulism toxin from a horse burial site to five horse facilities in the area. The owners of the horse farm in Colorado described an unusually large population of birds living on their property just prior to and at the start of the botulism outbreak. It is possible that birds acted as a mechanical vector during this outbreak.

EQUINE INFECTIOUS PNEUMONIA (EIA) TEST UPDATE/NEW APHIS RULES

—Anita Schiebel

New APHIS rules regarding EIA testing has resulted in some procedural changes that we must implement to meet these rules. These are summarized as follows:

For the Coggins tests (EIA AGID) only

- New cutoff time for sample submission—12:00 Noon
- Reported by 3:00pm on the following day except weekends
- No test can be read earlier than 24 hours after setup
- Submit serum (red-topped tubes) only

Coggins forms must be filled out completely

- We cannot run the test until the completed and signed form is submitted
- We will no longer be able to fax an unsigned form back to the veterinarian for signature. The veterinarian will either have to come to the laboratory and sign the form, or the form will have to be mailed back to the veterinarian for his/her signature.
- Failure to submit the completed form will delay receipt of results.

Only the USDA/APHIS "Equine Infectious Anemia Laboratory Test" (VS Form 10-11) can be accepted.

- Forms that list more than one animal cannot be accepted
- An individual form with complete description must accompany each animal's sample

Samples still can be tested as a STAT or as a regular EIA ELISA request if the sample is received by 3:00pm

- Please place a note on the form or notify the office staff if the sample is to be run as a STAT or ELISA request
- If not indicated otherwise, the sample will be tested by the AGID.

Federal Regulations: Title 9. Code of Federal Regulations (9CFR), section 75.49 – "Equine Infectious Anemia: Uniform Methods and Rules, Effective March 1, 2002" APHIS 91-55-064 can be downloaded from http://www/aphis.usda.gov/oa/pubs/umr.html

EIA testing: Submit serum as described above. Fee: EIA AGID=$7.50, EIA ELISA=$12.

UPDATE--DIAGNOSTIC SPECIMEN SHIPPING REGULATIONS (reprinted with permission from Dr. J. Andrews/University of Illinois)

Effective February 14, 2003, a new rule by the US Department of Transportation (DOT) went into effect changing the way diagnostic specimens are defined, classified, packaged, and transported.

Diagnostic specimens now are listed as Hazardous Materials: "Diagnostic Specimens," previously exempt from regulation now will be listed in the Hazardous Material Tables of Title 49 CFR and will be subject to the new rules summarized below. These rules relate only to specimens that are potentially infectious. Formalin-fixed tissues, for example, are exempted but should still be packaged in leak-proof containers with adequate absorbent material.

This article is only a summary and the complete regulation should be examined to ensure accurate interpretation at: www.epa.gov/fedrgstr/EPA-IMPACT/2001/January/Day-22/i92.htm

Definition of Diagnostic Specimens—A Diagnostic Specimen is defined as "any human or animal material including excreta, secreta, blood and its components, tissue, and tissue fluids being transported for diagnostic or investigational purposes, but excluding live infected humans or animals."

NOTE: Plates or cultures of bacterial or viral organisms are NOT included in the definition of "Diagnostic Specimen" and may only be shipped under much more stringent requirements and regulations.

NOTE: Specimens from suspected cases of **foreign animal diseases** (FADs) and other very highly infectious and virulent diseases do not fall within the guidelines in this document. Contact your State Veterinarian or the Federal Veterinarian-in-Charge if an FAD is suspected.

Packaging Required for Shipment of Diagnostic Specimens—Unless Diagnostic Specimens are transported by "ground-based private or contract carriers using dedicated vehicles," these materials must conform to the standards listed below.

NOTE: These regulations APPLY to FedEx and other commercial shipping companies. The stringent parcel size limitation in the section **"Shipments by Air of Diagnostic Specimens"** below is important for any company that routinely ships by air. Ask your carrier for a copy of the document "Packing Instructions 650" which includes additional requirements.

NOTE: The US Postal Service also has additional regulations that may be found at www.usps.gov or http://www.usps.com/cpim/ftp/pubs/pub52.pdf.

Diagnostic Specimens must be packaged in triple packaging consisting of:

A primary receptacle. Primary receptacles must be packed in secondary packaging in such a way that under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging.

Leak-proof secondary packaging. Secondary packaging(s) must be secured in outer packaging(s) with suitable cushioning material such that any leakage of the contents will not impair the protective properties of the cushioning material or the outer packaging. If several fragile primary receptacles are placed in a single secondary packaging, they must be individually wrapped or separated to prevent contact between them.

Outer packaging. Outer packaging must be clearly and durably marked with the words "**Diagnostic Specimens.**" The completed package must be capable of successfully passing the **drop test** at a drop height of at least 1.2m (3.9ft). Shipping papers are not required under the 49 CFR rules, but may be required by some shippers

Liquid Diagnostic Specimens must be packaged where the primary receptacle is leak-proof with a volumetric capacity of not more than 500ml (16.9 ounces). Absorbent material of sufficient quantity to absorb the entire contents of the primary receptacle(s) must be placed between the primary receptacle and secondary packaging. Multiple fragile primary receptacles placed in a single secondary package must be individually wrapped or separated as to prevent contact between them. Secondary packaging must be leak-proof.

Shipments by Air of Diagnostic Specimens additionally require that the primary receptacle or the secondary container is capable of withstanding without leakage an internal pressure differential of 95 kPa (14psi). Also required is outer packaging that does not exceed 4L (1 gallon) capacity. NOTE: This volume limitation does not apply to parcels containing animal body parts, whole organs, or whole bodies even if known to contain or suspected of containing an "infectious substance." However, the outer package must be labeled to indicate that the **"Contents are subject to special provision 49CFR A82."**

Training Requirements. Although no specific training is required, persons preparing or transporting diagnostic specimens "must know about and be able to apply the requirements of Sec. 173.199 (Title 49 CFR) to specific shipments."

Fines and Penalties. According to 49 CFR Sect. 171.1(c) "Any person who knowingly violates a requirement of the Federal hazardous material transportation law. . .is liable for a civil penalty of not more than. . .$27,500. . .and not less than $250 for each violation. . . .and shall be fined under Title 18, United States code, or imprisoned for not more than five years, or both."

BEWARE THE PIGEON BREAST

—Doreene R. Hyatt

As an update to the last issue of LabLines, there has been a steady submission of samples that have *Corynebacterium pseudotuberculosis* isolated. This increase has included not only horses with pigeon fever (pigeon breast), but also positive submissions from cattle. From January 2002 up to March 14, 2003, we have isolated 98 *C. pseudotuberculosis* from equine samples, 16 from bovine, and four each from caprine and ovine samples. This is an increase in the number of positive bovine samples as compared to previous years. We want you to be aware of the possibility of seeing more of these cases if you are doing any bovine work at your practice. Additionally, we have not seen the break in cases that we have seen in previous years during the winter months. We have isolated *C. pseudotuberculosis* at least once every month during the past year.

For more information on *C. pseudotuberculosis*, please see our previous LabLine articles on our website – http://www.cvmbs.colostate.edu/dlab and click on the newsletter link.

Fee: Aerobic culture—Submit tissue, swab or fluid. Fee=$12

GET TO KNOW YOUR LABORATORY

Wade Clemons has worked as a Laboratory Technician in Necropsy for 1.5 years. He assists Laboratory Coordinator Dennis Madden in teaching necropsy techniques to Senior students. He also procures anatomical specimens for student laboratories and performs other related laboratory duties. Wade is the "main man" for running our Tissue Digestor. He is currently working on our new dehydration system to control odors and speed the dehydration process, by redesigning the configuration between the digestor and dehydrator to make them work together more efficiently.

Wade enjoys working in the Necropsy area and looks forward to completing his MBA/TM degree in 2004. He plans to start Veterinary School in 2005.

Alkaline Hydrolysis Tissue Digestor

CHRONIC WASTING DISEASE (CWD) TESTING FOR THE FALL 2003 HUNTING SEASON

The Colorado Division of Wildlife (CDOW) will continue service to the hunters of Colorado by working with us and the Colorado Veterinary Medical Foundation Hunter Assistance Program (HAP) to provide testing for CWD for this Fall 2003 hunting season. Hunters can bring their harvested animals to CDOW collection sites or to veterinarians participating in the HAP program. The medial retropharyngeal lymph nodes are the tissue of choice for testing both deer and elk (obex if lymph nodes not available). Samples are tested for CWD using the rapid ELISA test, available at Fort Collins, Grand Junction, and Rocky Ford laboratories. Positive ELISA tests are confirmed with immunohistochemistry (IHC). The ELISA test is 99.6% accurate compared to IHC. Last hunting season we tested nearly 27,000 and elk with an approximately 1% rate of positive cases. The CDOW is planning for as many as 40,000 requests this fall and is implementing a new data system to more rapidly collect and release results to hunters. Veterinarians interested in participating in HAP should contact the CVMA office (303-318-0447) or Barb Powers (970-491-1281).

****HISTOMAILER SERVICE****

In an effort to enhance the histopath mailer service, new upgrades are being implemented into the system. This will include a computer tracking system and simplified shipping procedures.

The visible changes that you will encounter are no individual clinic addresses. It is critical that all clinic information is filled in on the submission forms since the address will not be on the postage label. We will have no cross-reference available. The other visible change is that absorbent pads will be included inside the mailer in the event of any leakage. These pads need to be included when specimens are returned to us.

Screened cassettes can also be supplied to you for endoscopic and needle biopsies. These cassettes are helpful in eliminating excessive handling and giving the pathologist a quality slide to read. If screened cassettes are used, site identifications can be written on the side of the cassette.

We strive to handle requests in a timely manner and our goal is to always improve our services. If there is a need to place a request or there are questions, please call Lee direct at 970/297-4505 or Email her at ldebuse@colostate.edu.

WHAT'S IN THIS ISSUE OF LABLINES

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