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Hanlon named head of KSVDL rabies laboratory

Dr. Cathleen Hanlon has joined the K-State Veterinary Diagnostic Laboratory (KSVDL) as director of the Rabies Laboratory. Dr. Hanlon earned V.M.D. and Ph.D. degrees from the University of Pennsylvania in 1987 and 1994, respectively, and is a diplomate of the American College of Veterinary Preventative Medicine. Her Ph.D. research involved evaluation of vaccinia virus to produce recombinant rabies vaccines for use in wildlife vaccination.



Dr. Cathleen Hanlon

Since then Dr. Hanlon has gained extensive research experience concerning rabies pathogenesis and prevention. She participated in the development of

an immunohistochemical staining test for rabies that can be performed at room temperature and examined with a routine light microscope. Dr. Hanlon came to K-State from the Rabies Section of the Centers for Disease Control (CDC) in Atlanta, Georgia, where she had been employed since 1996 as a visiting researcher, senior staff fellow, veterinary medical officer, and most recently as the acting head of the Rabies Section. In addition to supervision of the CDC Rabies Section, Dr. Hanlon was responsible for designing, conducting, analyzing, and communicating experimental and applied findings concerning rabies virus.

Her recent efforts at the CDC were primarily directed in three areas: 1) developing alternatives to the scarce and expensive human anti-rabies globulin used in treatment of humans exposed to rabies virus; 2) investigating human rabies cases

and unusual animal rabies cases — such as cases where large numbers of people are exposed — and using that information to improve post-exposure prophylaxis of humans and to develop recommendations for vaccination and control of rabies in humans and animals in the United States; 3) advancing rabies control in the United States by evaluating novel oral rabies vaccines for use in wild and feral animals.

Dr. Hanlon has participated in epidemiologic field investigations for and as a consultant to the CDC, the World Health Organization, the Pan American Health Organization, the United States Department of Agriculture, and Brazil. Dr. Hanlon also has extensive experience training and working with graduate and post-doctoral students and veterinary students studying at the CDC, and in classroom teaching of virology and epidemiology to veterinary and medical students.

Tritrichomonas foetus diagnostic capability to include real-time PCR

K-State Veterinary Diagnostic Laboratory

K-State's Veterinary Diagnostic Laboratory has expanded its *Tritrichomonas foetus* diagnostic capability to include both culture and real-time PCR in a combined assay.

The real-time PCR assay of McMillen and Lew [Veterinary Parasitology 141 (2006) 204-215] complements the InPouch™TF culture system in the diagnosis of *Tritrichomonas foetus*. Samples for both tests should be submitted in the same InPouch (one pouch per bull) follow-

ing directions included with the pouches. Preputial samples should be inoculated in the InPouch™TF culture system immediately and must be received by the lab within 48 hours. Overnight or one-day delivery is the best, or you can personally deliver samples to KSVDL Receiving. Call 785-532-4619 in advance to make arrangements for after-hours or Saturday delivery.

The combined assay consisting of the InPouch™TF culture and real-time PCR costs \$32 dollars per sample (\$7 for each

culture test plus \$25 for each real-time PCR test). The culture InPouch™TF will still be available as a separate assay for \$7 per sample. Pouches can be ordered in bulk from BioMed Diagnostics (1-800-964-6466) or by the pouch from KSVDL (1-866-512-5650).

Information about the InPouch™TF culture system, how to collect specimens, and how to inoculate the InPouch™TF bags can be found on the Web at: <http://www.biomeddiagnostics.com/docs/INSERT-TF.pdf>

High corn prices? Watch for moldy corn poisoning in horses

Larry C. Hollis, D.V.M., M.Ag., K-State Research and Extension, and Jerome C. Nietfeld, D.V.M., Ph.D., ACVP, K-State Veterinary Diagnostic Laboratory

With high grain prices causing the price of animal feeds to skyrocket, some livestock producers have already started looking for creative, low-cost ways to meet their animals' nutrient requirements. One solution is to purchase or clean up less than ideal grain – such as corn screenings, floor sweepings, or corn containing damaged kernels. While these grain sources can meet the nutrient demands of the animals, they are a potential source of various mycotoxins, including aflatoxins. Horse owners, in particular, should be careful to avoid a less well-known mycotoxin that often occurs in these grain sources and causes horse deaths every year. This fungus, *Fusarium moniliforme*, produces a group of toxins known collectively as fumonisins, which are extremely toxic to horses. They can cause tremendous damage to the brain or liver, with partial liquefaction of major portions of the brain being most common (Figure 1). Often the problem hits a group of horses being fed from the same grain source. Once lesions occur they are irreversible and usually lead to death, or at best, a dummy horse.

Ideal conditions for fumonisin production on grains occur in years when there are drought conditions during the summer months, followed by wet weather during harvest. However, fumonisins can grow in any environment where the *Fusarium moniliforme* mold has access to

cracked or otherwise damaged grain. This would include improperly dried or stored grain, insect-damaged grain, or grain that has been exposed to the external environment for any length of time. Horse owners should always be suspicious of grain that is visibly moldy, but it is not always possible to visually detect fumonisin-contaminated grain.

For details on leukoencephalomalacia, review the clinical case report and summary article written by Ragsdale and Debey and previously published in the Summer 2003 issue of *Kansas Veterinary Quarterly* at www.asi.ksu.edu/DesktopModules/ViewDocument.aspx?DocumentID=1870.

Because the only completely successful intervention is disease prevention, horse owners are advised to think twice before feeding less than ideal corn to their horses. The history of the grain source is often the first sign of potential problems. However, if a horse owner indicates they have found some corn that does not look moldy, it can always be tested by sending it to the Kansas Veterinary Diagnostic Lab. Unless cheap corn is known to be free of fumonisin, owners should be warned that this low-cost feed may come at a price.

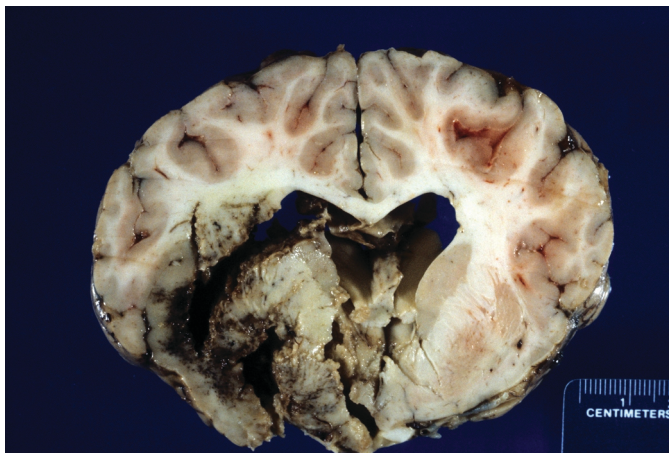


Figure 1

Correction: Osteopetrosis in Calves

Jerome Nietfeld, D.V.M., Ph.D.
K-State Veterinary Diagnostic Laboratory

The article “Watch for Osteopetrosis in Calves,” that appeared on page 3 of the Winter 2008, *Veterinary Quarterly* contained the following erroneous statement:

“This is the third consecutive year in which we have identified osteopetrosis in a red Angus calf, with each of the three cases from a separate herd.”

In fact, KSVDL diagnosed osteopetrosis in one red Angus calf in 2006 and in two red Angus calves in 2007. Each calf was from a different owner, but the dam of the third calf was purchased as a bred heifer from the owner of the second herd, and the sire of both 2007 calves was the same.

There have not been any confirmed cases of osteopetrosis at K-State thus far in 2008. The osteopetrosis in the red Angus calves was identical to the descriptions of inherited osteopetrosis that have been identified in several cattle breeds and best studied in the Angus breed (Thompson Keith, *Osteopetrosis*. In: *Pathology of Domestic Animals*, M. Grant Maxie, editor, 5th edition, 2007, volume 1, pp. 38-40).

Red Angus calves were negative for BVD virus, which has been associated with the occurrence of osteopetrosis-like lesions in calves. There are differences between the inherited form and the osteopetrosis-like lesions described in BVD virus-infected calves. The lesions in the three red Angus calves have been identical to those described in the inherited form. But as with any birth defect, breeding trials or — if enough calves are affected — scrutiny of the genealogy of affected calves is required to be certain that these are inherited rather than idiopathic.

Hardware disease associated with tires used as feeders

Larry C. Hollis, D.V.M., M.Ag.
K-State Research and Extension

A case report published in the proceedings of the 2007 South Dakota Veterinary Medical Association Annual Meeting and referenced in the SDSU *Animal Health Matters* veterinary newsletter¹, raises the possibility of underdiagnosed cases of hardware disease associated with cattle being fed from inverted large tire feeders. The observations presented in this report provide Kansas practitioners with another practical solution for unexplained deaths that may have occurred sporadically when cattle were being fed from feeders made from these old tires. It also makes the case for conducting necropsies when unexplained deaths occur. The case report follows.

During late winter 2006-07, seven cows from a herd of 330 crossbred beef cows had died over a period of a few weeks. Several others had shown a variety of vague clinical signs including fever, lethargy, anorexia, excessive salivation, abortion, and agonal recumbence. On March 15, 2007, the owner delivered two dead cows to the SDSU ADRDL. These cows were the fourth and fifth to die during the week.

Cow 1: Necropsy revealed this five-year-old cow to be in good body condition. A fetid rumen-like odor was noted when the right thoracic cage was removed. The pericardial sac was markedly distended and thickened. Approximately 3 gallons of brown, fetid fluid readily poured from the pericardial sac following puncture. The inner lining of the pericardium and the epicardial surface were diffusely covered by a thick (0.25 inches) layer of yellow/tan exudate. A wire (Figure 1) was observed extending from the reticulum, across the diaphragm and through the pericardial sac. The wire was 4 inches long and composed of thin, round, smooth metal. There were no other significant gross findings.

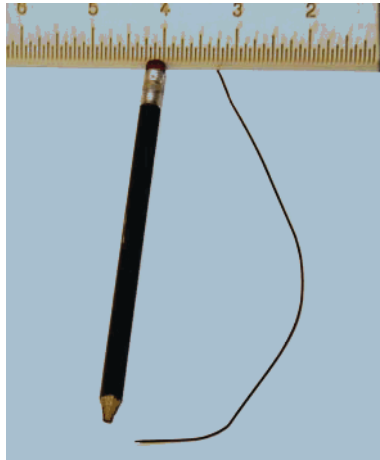


Figure 1. Wire retrieved from hardware case at necropsy.

Cow 2: Necropsy revealed this nearly toothless older cow to be thin. The owner reported that this cow died rather suddenly compared to the others. Upon exposing the thoracic cavity there was no odor as described in cow 1. However, again the pericardial sac was markedly distended and appeared somewhat dark. The pericardial sac was opened and found to be distended and filled with a large blood clot. Further dissection revealed a thin smooth round wire extending from the reticulum into the pericardial sac as described in the previous cow. Unfortunately for this cow, the wire then precisely struck and punctured a large myocardial artery causing acute and severe hemorrhage into the pericardial sac (hemopericardium). The immediate cause of death was attributed to the cardiac tamponade syndrome. During cardiac tamponade, the heart has difficulty performing due to constrictive pressures of the pericardial blood clot on the softer portions of the heart such as the atria and vena cava. Consequently, the flow of blood through the chambers is severely reduced or stopped and death ensues due to circulatory collapse.

The diagnostic summary was death due to classic Hardware Disease. The owner was sent home with the wire and instructed to look for the source. It was discovered that a large tire (large enough to encircle a round-bale) was being used as a hay-feeding station (Figure 2). The tire had been split in half and contained steel belts formed by a myriad of thin small wires. Over time, as the cattle reached over the tire to feed on the hay bale, their necks tended to wear away the rubber and expose the wires, which would eventually break free and drop into the hay.

During the summer when activities are low on most cow/calf operations, inspection and replacement of tires used as feeders is an activity that could pay dividends for cattle producers.



Figure 2. Old steel-belted tire used as hay feeder. Steel belts are fraying at the inside edge, exposing wires that eventually break off and fall into the feed (circle).

¹D.H. Zeman and J. Poindexter-Runge, Hardware Disease Associated with Tires Used as Hay Feeding Stations: A New Source of Hardware for an Old Disease. As reported in SDSU *Animal Health Matters*. February 2008.

Sample collection for rabies and BSE testing

Jerome Nietfeld, D.V.M., Ph.D.

K-State Veterinary Diagnostic Laboratory

Several years ago the Centers for Disease Control (CDC) changed sample requirements for fluorescent antibody (FA) testing of animal brains for rabies virus. Since then the KSVDL rabies laboratory has periodically received samples that do not meet the revised requirements for a valid test. Although the number of unsatisfactory samples has decreased, there is still some misunderstanding about what samples are required. Changes in sample requirements were not widely publicized, which has led to confusion.

This article is intended to help explain requirements for the FA test for rabies virus, and for the transmissible spongiform encephalopathies (TSE) tests — bovine spongiform encephalopathy (BSE), scrapie, and chronic wasting disease (CWD). The rabies information is from a paper titled "Protocol for Postmortem Diagnosis of Rabies in Animals by Direct Fluorescent Antibody Testing," which is available on the CDC's Web site at <http://www.cdc.gov/rabies/diagnosis.html>.

The FA test requires fresh tissue and testing of specific portions of the brain for a sample to be called negative. Formalin-fixed brain cannot be used for the rabies FA test. All brain samples received by KSVDL are tested, and if there is specific staining for rabies virus the sample is reported as positive, regardless of whether the required portions of the brain were examined. If there is no specific staining and the required portions of the brain were received, the sample is reported as negative. If there is no specific staining but the required parts of the brain are not available, the sample is reported as unsuitable, which in cases of human exposure usually means that someone has to undergo rabies prophylaxis.

Probably the most common causes of unsatisfactory samples are submission of half of the brain fresh and half formalin-fixed, and submission of the brain in multiple pieces (sometimes 10 or more). Twenty years ago practitioners were taught to saw the head in half, remove the brain, and submit half fresh and half fixed in formalin. That is no longer acceptable. In cases where the brain is submitted

in many pieces, it can be impossible to correctly identify portions of the brain, especially the brainstem. The personnel in the rabies laboratory make an effort to identify and sample the correct portions, and if they cannot identify one or more of the required areas, they have one of the pathologists examine the brain. If it is not certain that all of the required portions are present, the sample is tested and the deficiencies noted.

The most important sample is a complete cross section of brain stem. According to the CDC, the brain stem is "the tissue most reliably found to contain viral antigen" and "a negative finding for rabies can be made only if a complete cross-section of the brain stem is examined." "Examination may be made at the level of the pons, medulla, or midbrain." The CDC has found that on rare occasion rabies virus is located only in the right or left half of the brainstem, thus their insistence that both halves be tested. This means that if a brain is split lengthwise on the midline and half fixed in formalin and half submitted fresh, the rabies lab cannot call the FA test negative. In addition to the brain stem, either the cerebellum or both hippocampuses must be tested, and the cerebellum is preferred.

Cerebellar tissue also should be included. The FA test should include the vermis and both lateral hemispheres. If there is no cerebellar tissue, the hippocampus can be tested instead, but the cerebellum is preferred by the CDC and both hippocampuses must be tested. Figure 1 shows a horse brain with lines demonstrating where to cut to obtain the sample most preferred by the CDC.

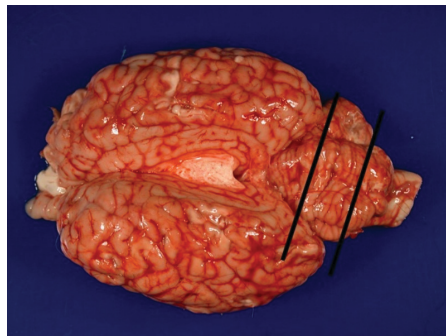


Figure 1

In the case of cattle, the USDA has a program to test rabies-negative animals for bovine spongiform encephalopathy (BSE). For this the obex is required. The obex is also the portion of the brain collected for scrapie and chronic wasting disease. A cross-section of cerebellum and brain stem can still be taken for rabies, but make sure that the most caudal cut through the brainstem is made anterior to the obex. Figure 2 demonstrates the location of obex in relation to where the cuts can be made for the rabies sample.

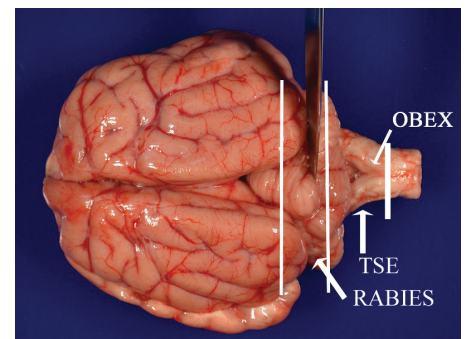


Figure 2

Making the cuts as illustrated in Figure 2 leaves you with the sample most preferred for the rabies FA and the brain sample required for BSE testing, which are illustrated in Figure 3. (It should be noted that there are tests for scrapie and chronic wasting disease for which lymphoid tissue can be used, but for BSE the obex is the only sample that can be tested). The remainder of the brain can be used for other tests, if so desired.

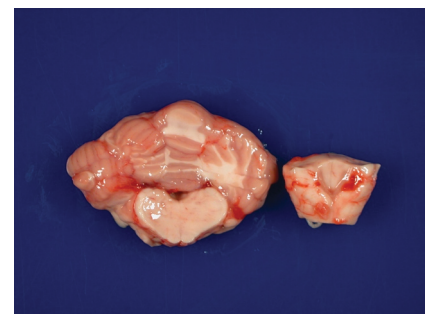


Figure 3

It is also acceptable to split the head longitudinally and cut the brain in half. Simply cut the required areas from each half of brain, as shown in Figure 4, and submit separately unfixed.

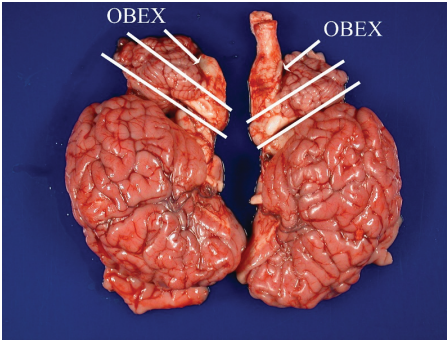


Figure 4

Another option is to submit the entire head. KSVDL technicians will gladly remove the brain and collect the proper

samples. However, in cases where this would require overnight shipment of a cow or horse head, you can save \$50 to \$100 by removing the brain and submitting it to the personnel in the rabies lab who will collect the proper samples. If you desire additional tests on rabies-negative samples, the rabies lab personnel will pass the brain not used for rabies or BSE tests on to the pathologist on duty. Keep in mind that if you desire histopathology on rabies negative samples that the tissue morphology will be better if you remove the samples for rabies and BSE and fix the brain before shipment than if we fix it after a day or two in the mail.

Notes from the rabies diagnostic lab

Rolan Davis M.S., C.P.M., Diagnostic Supervisor; Cathleen A. Hanlon, V.M.D., Ph.D., DiplACVPM, Director

Shipping samples during warm weather

As warm weather approaches, it is a good time to review shipping protocols to assure that samples arrive in suitable condition for testing.

The ideal decapitation process is to separate the head from the body at the atlanto-occipital joint, which provides direct access to the foramen magnum and avoids the creation of bone shards. Decapitated heads or whole brains removed from the calvarium of large animals should be shipped according to regulations for diagnostic specimens, which can be found at http://www.vet.ksu.edu/depts/dmp/service/pdf/VDL_Shipping.pdf.

An overnight shipping method is ideal to facilitate prompt delivery to the laboratory. Please note that shipping first-class or priority via U.S. Postal Service doesn't guarantee overnight delivery. A plentiful supply of frozen gel packs should be included with the sample to keep the contents cool during shipping.

CDC posts new guidelines for human rabies prevention

The updated Recommendations of the Advisory Committee on Immunization Practices — Human Rabies Prevention, United States, 2008 is now available at <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr57e507a1.htm>

10-day observation period OK for healthy animals

Please note that the Compendium of Animal Rabies Prevention and Control, 2008 (<http://www.nasphv.org/Documents/RabiesCompendium.pdf>) recommends that healthy dogs, cats, and ferrets (regardless of vaccine status) may be observed for 10 days rather than immediately euthanized and tested. If you have questions about this recommendation, please contact the laboratory at 785-532-4483.

Rabies cases reported

Current rabies cases report by the laboratory as of May 22, 2008

Kansas	Nebraska
31 skunks	9 skunks
3 felines	3 bovines
1 equine	2 equines
	1 red fox

PVR confirmed in Michigan sport pigs

In May 2008, the Michigan Department of Agriculture (MDA) confirmed that they had identified pseudorabies virus (PRV) infection in 19 sport pigs on a privately owned cervid facility in Saginaw County, Michigan.

"Michigan achieved PRV-free status in 2000, and the ability of this disease to be spread by feral hogs to other animals could be a risk to the swine industry," said Don Koivisto, MDA director. "We must protect Michigan's \$230 million swine industry."

All swine on the infected cervid facility are to be captured and destroyed. The USDA Wildlife Service is attempting to capture, euthanize, and test feral swine in the vicinity of the infected premise. The MDA is encouraging Michigan residents to shoot feral swine and notify the MDA so that the pigs can be tested for pseudorabies virus. Dr. Steven Halstead, the Michigan State Veterinarian, said that they are contacting other states and notifying them of the pseudorabies case. They are anticipating that other states will impose restrictions on importation of live swine from Michigan until tests confirm that this is an isolated case. Therefore, Michigan will be working to confirm as quickly as possible that the virus has not spread to other areas or to commercial farms. Michigan has banned the importation of swine intended for sport, hunting, or shooting. All facilities with commercial or sport pigs in a 5 mile radius of the infected cervid facility will be quarantined, and the pigs tested.

As long as the PRV outbreak remains confined to feral swine, Michigan's PRV-free status in commercial swine will remain intact.

Editor's note: It has been just over a year since pseudorabies was identified in two swine herds in Wisconsin, which were the first cases of PRV in U. S. commercial swine since 2003. In the past year, no new cases of PRV have been identified in Wisconsin.

Foreign Animal Diseases, 7th Edition

Foreign Animal Diseases is a reference book that has been published and updated by the United States Animal Health Association (USAHA) for more than 50 years. The book provides up-to-date information concerning foreign animal diseases including color photographs of pathologic lesions of most diseases. It also includes chapters on collecting samples, how to ship the samples, how to interpret test results, disinfectants, and other useful topics. The following Web address will take you to the USAHA Web site where you can purchase a copy of Foreign Animal Diseases, 7th edition, revised 2008, for \$25.00, or you can download the book free of charge. You can find it at <http://www.usaha.org/pubs/index.shtml#FAD>.

Continuing Education

August 14 and 15

2nd Annual Veterinary Conference for Care of Llamas and Alpacas (for veterinarians and veterinary technicians)

August 22

Calving Clinic for Producers and Ranch Hands

For the most complete, up-to-date conference information visit our Web site at: www.vet.ksu.edu and click on Continuing Education, or contact: Linda M. Johnson, Ph.D., at 785-532-5696 or johnson@vet.ksu.edu

September 13

16th Emergency Medicine Conference

Upcoming Events

July 11 – 12

Kansas Swine Classic – Manhattan

August 7 – 8

K-State Beef Conference – Manhattan

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