From the Director
Stephen B. Hooser

Since the spring newsletter, two disease have made the headlines: TB and pandemic H1N1 2009 influenza virus. Fortunately, at this time, TB has only been diagnosed in captive cervids in Indiana, and Indiana’s TB free status for cattle and bison remains unaffected. Definitive diagnosis of TB in the U.S. is made by the USDA National Veterinary Services Laboratory (NVSL) in Ames IA. However, in some cases, the ADDL does assist the Indiana Board of Animal Health with necropsies and sample collection in cases dealing with TB suspect animals, and its pathologists are constantly on the watch for lesions in domestic animals and wildlife that could be due to Mycobacterium sp. Of course, when performing evaluations of TB suspect animals, the necropsies are most secure, and offer the least amount of risk to personnel, if performed in a BSL3 laboratory environment. If built, such a laboratory would provide a higher level of biosecurity for TB and other zoonotic diseases. The other disease which has received a great deal of air time is pandemic H1N1 2009 influenza which early on was misnamed by the press, much to the detriment of the swine industry. As a full-service diagnostic laboratory, the Virology, Serology, and Molecular Diagnostics sections of the ADDL have various capabilities and levels of detail for the diagnosis of influenza in swine. At the most basic level, diagnosis of respiratory disease in swine as influenza only confirms that the animal has type A influenza, usually either H1 or H3. Although the ADDL has the capability to isolate the influenza virus, determine if it is an H1N1, and perform DNA sequencing to determine if it is the same as the human pandemic H1N1 2009 virus, this will not be done without the express permission of the submitting veterinarian or owner. We can only hope that Indiana swine do not catch pandemic H1N1 2009 from the people with whom they have contact.

Focus on… Virology

Under the supervision of Dr. Roman Pogranichniy, the staff in the Virology section utilize various procedures, including virus isolation, fluorescent antibody, antigen capture ELISA, sequencing analysis of isolates and electron microscopy to identify the presence of viral agents in submitted samples.

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From left to right:
Roman Pogranichniy, Virology/Serology Section Head
Phyllis Lockard, EM Technician, 9 years at ADDL
Jeff Wiese, Current Laboratory Supervisor, 2 years at ADDL
Crystal McFarland, Laboratory Technician, 4 years at ADDL
Mary Woodruff, Retired Laboratory Supervisor, 41 years at ADDL
Tammy Crowell, Laboratory Technician, 23 years at ADDL
Katrina Miller, Laboratory Technician, 4 years at ADDL
Information on Charges – repeated from Spring 2009 Diagnostic Forum

- The standard $7.00 accessioning fee (to cover processing, IT, etc) is now being added to all cases submitted to the ADDL, including Serology cases.
- Sequencing of influenza A virus hemagglutinin region and partial gene analysis $100.00/isolate
- Sequencing of influenza A virus full length analysis of the hemagglutinin gene $200.00/isolate
- EIA $3.00 + $7.00 accessioning fee  $10.00/animal
  (cost of EIA test reduced to partially offset the addition of accession fee)
- Encephalitozoon real time PCR $25.00/sample
- Decalcification of samples submitted for histopathology additional $17.00
- Evaluation of painted margins additional $25.00

Mary Woodruff, long time Supervisor of the Virology Laboratory at the ADDL, was presented the Outstanding Staff Award from the Purdue School of Veterinary Medicine in May. Mary is pictured with Dr. Willie Reed, Dean of the Purdue SVM. Mary retired from ADDL on May 29, 2009, after nearly 41 years of service to ADDL and the state of Indiana. Please join us in wishing her a happy and healthy retirement.

Dr. Bill Wigle joined the ADDL faculty as a mammalian veterinary diagnostician in April, 2009. Dr. Wigle completed his B.S. in Poultry Science at the University of Maryland in 1974, his M.S. in Poultry Science from Oregon State University in 1976 and his DVM from Purdue SVM in 1984. Postgraduate training was completed in 1990 at the Purdue ADDL. He received an MBA in 2005 from Sam Houston State University in Huntsville, TX.

He was most recently a veterinary pathologist at the Texas Veterinary Medical Diagnostic Lab in College Station, Texas.

Our congratulations to Megan Schnur, freshman student in the Biochemistry Department at Purdue University who was recently awarded first place in the Undergraduate Research and Poster Symposium at Purdue. Megan was a Merck fellowship research recipient during the summer of 2008 and, under the supervision of Dr. Roman Pogranichniy, ADDL Head of Virology/Serology, conducted a project on BVD, “Isolation and Genetic Analysis of Bovine Viral Diarrhea Virus from Infected Indiana Cattle.”

ADDL Schedule

Purdue ADDL and Heeke ADDL will be closed on the following University holidays in 2009

- September 7.............................. Labor Day
- November 26-27.......................... Thanksgiving
- December 24-25.......................... Christmas
- December 31, 2009 - Jan 1, 2010....New Year
Laboratory Diagnosis of Bovine Abortion

Fetal loss has a major impact on the dairy and beef industries. An estimated 6-10% of pregnancies terminate in abortion, stillbirth or perinatal death at a projected cost up to $900 per case.

Prompt submission of the entire fetus with placenta in every abortion case is ideal. When this is impossible or impractical, please contact the Purdue ADDL or Heeke ADDL at SIPAC to discuss specimen selection and submission. Necropsy in abortion cases is performed according to a standard procedure. Macroscopic examination is used to detect malformations or gross lesions. Selected formalin-fixed tissues (placenta, brain, heart, lung, liver, kidney, spleen, digestive tract, and skeletal muscle) are examined histologically to detect microorganisms, specific lesions, or inflammation as a clue to infectious disease, or degenerative changes that might incriminate a toxic or nutritional disease. Ancillary tests are applied as indicated by history or lesions. Microbiologic tests are applied as routine abortion screens for those cases in which neither the history nor pathologic examination incriminates a specific cause. Placenta, lung, liver and abomasal content are appropriate specimens for bacterial culture; for virologic testing, placenta, lung and spleen. Fetal pericardial or thoracic fluid can be evaluated serologically to detect antibodies to known abortifacient agents. Selenium concentrations can be measured in fetal liver.

In 2008, an infectious cause was suspected in 21 of 47 cases of bovine abortion, stillbirth, or perinatal death that were examined by necropsy. Twelve of these cases were attributed to various bacterial pathogens; 6 were considered viral abortions; 3 cases were attributed to Neosporum caninum. Four of the six viral abortions were diagnosed as Infectious Bovine Rhinotracheitis (IBR); immunohistochemistry provides an excellent means to detect IBR herpesviral antigen within the necrotic foci that are the diagnostic lesion in this disease. Twinning and dystocia were noninfectious factors that contributed to several perinatal deaths. Disappointingly, no cause for abortion, stillbirth, or perinatal death was determined in 17 cases (36%). Incomplete submission (particularly omission of the placenta) and post mortem decomposition of the fetus may account for failure to determine the cause for abortion or stillbirth in many cases.

In cases of bovine abortion, stillbirth or perinatal death, rapid and accurate diagnosis is essential for management and reduction of fetal loss. Continued surveillance and improved diagnostic testing are our goals. The ADDL and Heeke ADDL at SIPAC hope to increase diagnostic success in bovine abortion cases by increasing the number and quality of submissions.

Please try to deliver the entire fetus with placenta to the diagnostic laboratory in each case of bovine abortion.

-by Dr. Peg Miller, ADDL Pathologist

References:

The FY 2008 annual report of the Indiana Animal Disease Diagnostic Laboratory at Purdue University -is now available on our web page.

www.addl.purdue.edu

Print copies are available. Please contact Linda at 765-494-7448 or yankovil@purdue.edu if you prefer a printed copy.
Equine Viral Arteritis

Equine viral arteritis (EVA) is caused by an arterivirus within the family Arteriviridae and is seen worldwide. It is not extremely prevalent in the United States but, when it does occur, it is usually as an outbreak in a location where animals come and go frequently, such as a racetrack or breeding farm. It is important to know the clinical signs of EVA so that it will be on the differential list and proper testing can be implemented to confirm diagnosis. This will allow an outbreak to be brought under control quickly and effectively, minimizing morbidity and mortality.

Transmission of the virus occurs by either sexual contact or inhalation. The virus is shed in the semen of carrier stallions, which are the reservoir for EVA in the equine population. The carrier state can last months to years. Fresh, chilled, and frozen semen from a carrier stallion can infect mares bred by artificial insemination. Infected horses also transmit the virus through aerosolization and subsequent inhalation by a susceptible horse.

EVA can be a diagnostic challenge because the clinical signs are variable and non-specific. Some horses are very mildly affected while others are so severely affected that the disease is fatal. Interestingly, there is breed-specific variation in EVA seroprevalence. Standardbred seroprevalence has been reported to be as high as 80%, although no breed-specific disease susceptibility has been discerned. There are differences based upon the signalment of the animal in question as well. A racehorse may manifest EVA as one or more of the following signs: pyrexia (up to 105°F), anorexia, depression, nasal and ocular discharge, coughing, skin rashes, and/or edema, with dependent and periorbital edema being the most common. Some of the less common clinical signs include ataxia, papules on mucosal surfaces, severe respiratory distress, and lymphadenopathy. Abortion may be the only clinical sign of EVA infection in a pregnant mare, although they can also have the aforementioned clinical signs followed by abortion. A neonatal foal infected with EVA will usually show severe respiratory distress and die within 24 hours. All of the clinical signs seen are related to the panvasculitis that occurs when the horse is infected with EVA. The virus replicates within macrophages, mesothelium, and endothelium.

If there is suspicion of Equine Viral Arteritis, standard blood work will not provide much helpful information. The only parameter that may be useful is the evidence of leukopenia seen on the CBC. The next best step would be to submit paired serology samples, one acute and the other convalescent. A fourfold increase in the EVA titer would be highly suggestive of infection. A single serology test for EVA titer is of limited usefulness as horses can be exposed and seroconvert without becoming clinically ill. In an outbreak situation, the best samples to collect from exposed horses that may be early in the disease process are nasopharyngeal swabs or washes, conjunctival swabs, and blood samples collected in EDTA tubes. In mares that abort, the virus can sometimes be isolated from the placenta or fetal tissues. Additionally, the mare can be evaluated for seroconversion and an endometrial biopsy can be tested by Polymerase Chain Reaction (PCR) and virus isolation. At necropsy, immunohistochemistry and virus isolation are useful diagnostic tools. There has also been a report of an antemortem diagnosis of EVA using immunohistochemistry on skin biopsy samples although, to date, this method is not widely used.

Prevention of Equine Viral Arteritis can be accomplished through proper testing and vaccination protocols. There are both modified live and killed vaccines available. The modified live vaccine only protects against clinical signs of EVA, and may only be partially effective. The killed vaccine protects against infection. The immune response produced by the vaccines may be protective for up to two years, although annual boosters are recommended at least 21 days before the start of the breeding season. All breeding stallions should be tested for carrier status. If the stallion is a known carrier, the mares to which he is bred should be seropositive before coitus or artificial insemination, either via natural infection or vaccination. If the stallion is EVA negative, this should be documented; the stallion should then be vaccinated annually 28 days prior to the start of each breeding season. Young colts that may be used as breeding stallions in the future should be vaccinated after the maternal antibodies have declined, typically between two and six months of age. To be certain that there will be no maternal antibody interference, the colts are usually given the first vaccine between 6-12 months of age and then annually thereafter.

It is worthwhile to note that animals that are vaccinated may not be eligible for export to certain countries because it is not possible to differentiate natural infection from vaccine seroconversion.

Although Equine Viral Arteritis is not a widely prevalent disease, it can cause significant problems on breeding farms and racetracks in the event of an outbreak. It is important that this disease remains on the differential list, and that equine practitioners are aware of the best samples to submit for EVA testing. Proper testing and vaccination strategies could effectively eliminate this disease from the equine population.

-by Amber Boring, Class of 2009
-edited by Dr. Ryan Jennings, ADDL Graduate Student

References
Final Diagnosis: Encephalitic Listeriosis in a Goats

In the summer of 2008, Hecke ADDL was presented with a one-year old female Boer goat with a two day history of anorexia, “drunken” staggering, weakness, glass-eyed appearance, and lateral recumbency. Five out of 20 goats in this herd had died with similar clinical signs. The goats had been purchased four months prior to the onset of illness, had been treated with anthelmintic 2 weeks prior to submission, and anthelmintic treatment was repeated on the day prior to submission. The diet consisted of grass hay and a grain supplement (Goat Chow). No silage was fed.

At submission, the goat was alive but moribund in lateral recumbency. The animal was in good nutritional condition with adequate muscling and body fat. Fecal flotation revealed high numbers of *Hemonchus contortus* ova and high numbers of coccidial oocysts. The blood had a packed cell volume of 26%. Following physical examination, the goat was humanely euthanized and necropsied.

Grossly, the goat had mild meningoencephalitis, mild bronchopneumonia, and mild abomasal hemochromatosis. The meningoencephalitis was characterized by slight reddening, unusual wetness, and slight cloudiness of the meninges overlying the cerebellum and the caudal brain stem. The bronchopneumonia affected only the tip of the right cranial lung lobe which was red-gray and firmly consolidated. The abomasum contained a small amount of red-brown watery fluid, and low to moderate numbers of *Hemonchus*-like nematodes. Grossly, the goat did not appear anemic. The rumen was filled with finely ground grassy forage and a few fragments of broadleaves. Intestines were unremarkable and the rectum contained normal firm fecal pellets. Cytologic impression smears of brain stem meninges had increased numbers of neutrophils, consistent with mild suppurative meningitis.

Histologically, the medulla oblongata, cerebellar peduncle, and midbrain had multifocal suppurative encephalitis. Scattered mild neutrophilic infiltrates were also present in the meninges. No histologic lesions were present in the thalamus and cerebral cortex.

Bacterial cultures isolated *Listeria monocytogenes* from the brain stem and *Mannheimia haemolytica* from the lung. Serologic tests were negative for caprine arthritis encephalitis (CAE).

Although the clinical history in this case initially suggested parasitism, the final diagnosis was primary encephalitis listeriosis, complicated by concurrent abomasal hemochromatosis and pneumatic pasteurellosis. Because of similar clinical presentation, it was assumed by the owner that all six of his dead goats had died of listeriosis, but the cause of death in the remaining animals was never confirmed and we cannot exclude the possibility that parasitism contributed to some of those deaths. The persistence of *Hemonchus* in the face of repeated worm medication is indicative of anthelmintic resistance.

Listeria is a common cause of encephalitis in all ruminants, and most large animal practitioners are familiar with it in cattle. This case illustrates a number of observations about caprine listeriosis that differ slightly from bovine listeriosis, and these differences are further examined in an epidemiologic review of ADDL records below.

ADDL pathology records for the past 5 1/2 years (2004-2009) reveal that caprine encephalitic listeriosis has been diagnosed 42 times (25 at the West Lafayette lab, 17 at Hecke lab). In comparison, bovine encephalitic listeriosis has been diagnosed only 29 times (16 at West Lafayette, 13 at Hecke lab). Since our overall cattle submissions outnumber goat submissions, the higher incidence in goats suggest that goats are more susceptible to listeriosis. Increased susceptibility in goats is also suggested by the epidemiologic data within the submitting herds. Available submission forms and case records for the Hecke lab submissions (17 caprine, 12 bovine) were pulled and tabulated (see tables at end of article). Of the 17 caprine cases, four (24%) involved outbreaks of three or more animals in the flock, five (29%) involved two animals, and eight (47%) involved only one animal at the time of submission. In contrast, of the 12 bovine cases, none (0%) involved three or more animals, three (25%) involved two animals, and nine (75%) involved only one animal at the time of submission. Thus, goat herds appear more likely to suffer listeriosis outbreaks that involve multiple animals.

Bovine listeriosis is often associated with the feeding of silage, presumably spoiled silage. As in the case reported here, almost all cases of caprine listeriosis that we have seen do not involve the feeding of silage. Dietary information was provided for eight of the Hecke lab goat submissions and, in all eight cases, silage was not a part of the diet (0% feeding silage). In contrast, dietary information provided for 10 of the bovine cases suggested that seven (70%) of those cases were associated with silage feeding. The source of infection in the goat cases is undetermined, but is presumed to be spoiled hay or feed that has been contaminated with dirt or feces, as the natural reservoir for *Listeria monocytogenes* appears to be soil and mammalian GI tracts. One previous study found that listeriosis was more prevalent in goats that browsed heavily, as compared to goats consuming hay or pasture (Johnson, 1996). Our data provided no information about browse patterns. Another study has suggested that venereal transmission may occur in goats (Wiedmann).

Meningitis was grossly visible in this case and, in my experience, is much more likely to be present in cases of caprine listeriosis. In contrast, meningoencephalitis is seldom noticeable in cases of bovine listeriosis. Gross meningitis...
was reported in six (35%) of the caprine cases, but it was reported in only one (8%) of the bovine cases. Although meningitis was more frequently seen in goats, it is still not a reliable diagnostic finding. Interestingly, grossly visible encephalitis (focal hemorrhage and necrosis of the brain stem) was more frequently reported in the bovine cases. Four (31%) of the bovine cases reported grossly visible encephalitis, whereas none (0%) of the goat cases had grossly visible encephalitis.

It has been my impression that Listeria monocytogenes is more easily isolated from the brains of affected goats, and a previous study suggests this may be true (Johnson, 1995). Our records show a similar tendency, though less dramatically. Listerial cultures were attempted in 14 of the goat cases and 10 bovine cases, and it was successfully isolated from four (29%) of the affected goat brains, and from two (20%) of the affected bovine brains. In a fifth goat case, we isolated an unidentified Corynebacterium-like species. Although this isolate differed biochemically from the usual strains of Listeria monocytogenes, positive immunohistochemistry results suggested that it was antigenically related to Listeria. In one bovine case, we failed to isolate Listeria, but Brevibacillus borstelensis was isolated from the brain. The histologic lesion in this case was typical of listeriosis, but it is possible that Brevibacillus was the actual cause of the lesion. In both species, bacterial culture continues to be a relatively unreliable diagnostic procedure for encephalitis listeriosis. The poor rate of isolation may be due to prior treatment with antibiotics, as most of these animals had reportedly been treated prior to death.

In our laboratory, most cases of listeriosis are diagnosed by histopathology and, of the traditional diagnostic procedures, histopathology continues to be the most reliable method of diagnosis for encephalitic listeriosis. Formalin fixed sections of brain stem are the preferred sample to submit. (Please remember that Listeria lesions occur only in the brain stem, and submission of cerebral cortex may provide false negative results). Several newer and more sensitive diagnostic procedures are also available at ADDL. As mentioned above, we can diagnosis listeriosis by immunohistochemistry (IHC) and the preferred sample again is a formalin-fixed section of brain stem. A previous study found that IHC was much more sensitive than bacterial culture (Johnson, 1995). In addition, our molecular diagnostics lab can now diagnose listeriosis by PCR analysis. For this, the preferred sample would be a segment of fresh (unfixed) brain stem.

Our data provided no information about treatment of listeriosis, but treatment is often unrewarding due to the difficulty of getting therapeutic levels to the brain. Nevertheless, one previous study reported that, of nine animals treated with a combination of gentamicin and ampicillin, six survived (Braun).

-by Dr. Duane Murphy, ADDL Pathologist
Heeke ADDL

References
Diagnostic Forum is a quarterly newsletter of current services, regulations and research projects involving the ADDL which may be of interest to Indiana veterinarians and animal owners. It is our intention that the information provided will serve you. Please send your comments, suggestions, requests and questions to: Diagnostic Forum Editor, Purdue ADDL, 406 S. University St., West Lafayette, IN 47907 or email to addl@purdue.edu.

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