**APPENDIX D**
**SAES-422**
**Format for Multistate Research Activity**
**Accomplishments Report**

***Note:*** *This report is submitted each year of an activity's duration and is due 60 calendar days following the annual meeting. The SAES-422 is submitted electronically by AAs into NIMSS. Annual Reports for MRF projects are available to CRIS and CSREES through NIMSS.*

**Project/Activity Number:** NC1024

**Project/Activity Title:** Domestic Surveillance, Diagnosis, and Therapy of Transmissible Spongiform Encephalopathies

**Period Covered:** June 2007-June 2008

**Annual Meeting Date(s):** June 2008

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Regrets sent by:
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Mo Salman – Colorado State University
Michael Samuel - University of Wisconsin.
J. Bradley Thurston – North American Deer Farmer's Association.

**Brief summary of minutes of annual meeting**:
**Idaho:** Marie Bulgin began by describing the Idaho chronically infected sheep flock, held at the CAINE center at the University of Idaho. She detailed the extensive records kept of all births, infections, and genotypes, and the current methods used to bank tissues from each animal. In general, they hold about 100 sheep total with about 74% prevalence of scrapie in the flock. As a result, they have determined highly susceptible and weakly susceptible genotypes that will be useful in breeding programs. Specifically, the presence of the Valine allele at amino acid position 136 overcomes the presence of a heterozygous Arginine allele at position 171 , such

that so-called "QR" animals become susceptible to scrapie. In addition, she described a study with various potential antemortem tests and susceptibility, concluding that no test currently has sufficient results for 100% specificity and sensitivity. The most promising tissue for antemortem biopsy and diagnostics appears to be the retropharyngeal lymph node, however even the brain is not 100% positive for $PrP^{Sc}$ at death in infected animals which clearly indicates that precise disease markers continue to be elusive. In a USDA validation study, the IDEXX RAMALT rectal biopsy assay showed good results, with the provision that the difficulty lies in accurate and repeatable biopsy of sufficient lymphoid follicles for analysis. Lastly, a small study demonstrated no evidence for fence line transmission of scrapie between uninfected and infected animals, indicating a general lack of casual horizontal transmission. At the conclusion of the meeting, the group determined that maintenance of the Idaho flock is a high priority for providing access to affected tissues for continued study.

**Colorado:** Ed Hoover described CWD studies at Colorado State University, specifically addressing the issue of infectious material in body fluids and the patency of the species barrier for CWD transmission. Firstly, he described a study examining the infectivity of deer blood, saliva, feces, and urine using both a deer bioassay and transgenic mouse bioassay. In this study, blood and saliva transmitted disease to transgenic mice, but urine and feces did not. In an attempt to examine the nature of the transmissible agent in blood, whole blood, blood cells, platelets, and plasma were collected from infected animals and reinjected into deer to test for presence of the disease agent. Whole blood, white blood cells, and platelets all appeared to transmit disease, whereas plasma did not. Current studies are underway to examine the ability of unique cell subsets to transmit disease. In a final series of studies, Dr. Hoover described the use of PMCA to identify $PrP^{CWD}$ in affected animals. In these studies, PMCA demonstrated 82.6% sensitivity and 62.5% specificity compared to immunohistochemistry. At the conclusion of the meeting, the group agreed that maintenance of the CSU deer facilities should be a major goal for NC1024 to provide continued access to research animals and materials.

**South Dakota:** Alan Young described collaborative efforts to determine the role of the immune system in the pathogenesis and transmission of prion disease, and development of a small cervid model to study CWD pathogenesis in conventional animal facilities. In the first series of experiments, delivery and targeting of $PrP^{Sc}$ to lymph nodes was examined. Specifically, $PrP^{Sc}$-containing brain homogenate was injected into the lymphatic drainage area of a subcutaneous lymph node, and the delivery of $PrP^{Sc}$ to the regional node was monitored directly in the draining lymph and indirectly through timed histology of the draining node. $PrP^{Sc}$ was detected associated with migratory leukocytes in the draining lymph within 24 hours of injection, but was never observed in the cell-free lymph plasma. In contrast, kinetic experiments revealed that $PrP^{Sc}$ was actually targeted to the regional lymph node germinal centers as early as 15 minutes following injection, independent of the susceptibility genotype of the tested animal. Notably, $PrP^{Sc}$ was retained within the germinal centers of scrapie-susceptible animals at least 2 months following injection, whereas it was cleared from the germinal centers of resistant animals 1-2 weeks following injection. The mechanism of this clearance was under investigation, but the data implied that the earliest infection of germinal centers occurs independently of scrapie susceptibility genotype, whereas maintenance is dependent upon susceptibility. In a second series of experiments, muntjac deer were investigated as a potential small cervid species to study the pathogenesis of CWD in traditional laboratory environments. These deer are roughly the size

of a mid-sized dog, and can be housed in conventional animal facilities. Following either intracerebral or oral infection with CWD, $PrP^{CWD}$ could be detected in lymph nodes and brain of affected animals. Specific disease symptoms were observed 17-20 months following inoculation, when the animals succumbed to disease. This clearly demonstrates that this exotic animal species, found in many zoos across the US, is also susceptible to CWD. More importantly, it provides an additional, natural cervid model to study the pathogenesis of CWD.

**Kansas:** Juergen Richt described studies performed on PrP Knockout cattle to determine susceptibility of these animals to cattle-adapted TME. Normal cattle inoculated with cattle-adapted TME developed disease 18 months following injection. In contrast, PrP-knockout cattle never developed disease. However, some of the knockout cattle demonstrated reactivity to the mental status tests prior to beginning of the studies, and several of the test cattle were developing Purkinje cell degeneration at 32 months of age. In general, this appears to confirm the importance of PrP in TSE susceptibility in cattle, and may give clues to the normal role of the PrP protein in cattle.

**Nebraska:** Jason Bartz described studies examining strain interference in a hamster model. This model uses two strains of hamster transmissible mink encephalopathy differing in incubation period: DY, which induces disease in about 217 days in hamsters, and HY which induces disease at about 78 days in hamsters. In order to examine the role of strain infection in blocking further infection, DY was injected into animals on day 0, followed by an additional injection of HY at 60, 90, or 120 days post infection. When injected at least 90 or 120 days post infection, DY appears to interfere with the ability of HY to cause disease. There are several potential mechanisms that may explain this phenomenon, which are currently under examination. Firstly, DY could cause the death of the ventral motor neurons, but this does not appear to be supported by pathology. Secondly, there is the potential that the first strain effectively interferes with the replication of the second. This is partially supported by PMCA studies that are currently underway. In a final possibility, one strain could be directly blocking the ability of the second to replicate.

**Louisiana:** Frank Bastian described his studies on the similarities between Spirplasma infection and TSEs. He found that antibodies to scrapie-associated fibrils cross-reacted with Spriplasma sp., suggesting some antigenic similarity which could lead to difficulties in test interpretation. Furthermore, he described the identification of 16S rDNA in natural cases of CWD, scrapie and CJD, which may indicate co-infection associated with the clinical diagnosis of these TSEs. When deer were inoculated with spiroplasma, they developed a wasting and neurological disease similar to CWD 3.5 months post-IC inoculation, with apparent vacuolization in the brain similar to that observed with TSEs. Similar experiments in goats demonstrated a TSE-like disease at 11 months. Work is now progressing to test for further cross-identification of spiroplasma in TSEs.

**Minnesota:** Sri Sreevatsan described proteomics approaches to the identification of Protein Biomarkers of TSEs. Mass Spectrometry did not provide relevant results, so instead they attempted the iTRAQ technique (Isobaric Tags for Relative Quantification of Proteins). Using this technique, they observed about 8 proteins that routinely differed between scrapie and normal animals across multiple time points. About 32 total proteins differed at various specific time points following incubation. In additional studies, aptamers were created with the specific

ability to bind to multiple species and strains of $PrP^{res}$-including CWD, scrapie, and CJD. These aptamers were being investigated as a novel approach to diagnostics. Pam Skinner described a DNA microarray approach to identify genes altered during scrapie pathogenesis. In order to target specific changes, she focused on a number of regulatory genes that were altered in scrapie-infected but not normal mice. A number of altered genes were discussed, and additional collaborations were sought to examine changes in naturally susceptible species (ie deer, sheep) infected with TSEs.

**Montana:** Richard Bessen described four TSE projects, many involving NC1024 participants including 1) TSE infection of the oral and nasal mucosa in sheep, deer, and cattle (in collaboration with J. Richt), 2) the progression of chronic wasting disease infection in muntjac deer in order to establish a small cervid model for CWD (in collaboration with A.Young), 3) the role of the immune system in TSE neuroinvasion from the oral and nasal mucosa (in collaboration with J. Bartz), and 4) experimental TSE infection of the olfactory system and evidence for TSE shedding in nasal secretions. In this latter model, it was demonstrated that the TSE agent could spread from the brain to the nasal mucosa along the olfactory nerve. In the mucosa, TSE infection was found in the olfactory receptor neurons and in the nerve cell dendrites at the edge of the mucus layer. TSE infectivity was demonstrated in nasal lavages using animal bioassay and cell-free amplification methods. These studies suggest that release of TSEs into nasal secretions may be a mechanism of TSE dissemination between ruminants. The role of the oral and nasal mucosa as sites of TSE entry and exit will continued to be explored in experimental and natural hosts.

**Corporate Partner:** Howard Urnovitz (Chronix Biomedical) described a surrogate test under development by Chronix Biomedical that could define TSE-affected cattle by representation of nucleic acid sequences in serum. In this study on 15 BSE infected cattle and 95 normal animals, nearly 25,000 sequences were isolated defining the health status of the cattle. By comparing the differences between normal and health cattle, they identified a series of "groups" whereby animals infected with BSE could be identified. At 40 months post infection, all 15 BSE infected cows showed similar patterns when compared to the profile from healthy animals. Specifically, unique small nucleotide polymorphisms (SNP's) on repetitive genomic sequences defined BSE-infected animals. These sequences are being investigated as a surrogate marker-based diagnostic test for BSE.

**Group Discussion:** The group was commended by Richard Isaacson for the degree of collaboration already existing within the group. Current funding for further support from the USDA appears to be somewhat unclear, but it is anticipated that the transition of CSREES into the new National Institute will not result in a loss of opportunities. A number of funding options for collaborative projects within the group, including center or program grants for support of infrastructure through the NIH were discussed. It was generally agreed that the primary focus for the group should be to maintain existing resources, including the Idaho sheep flock, the CSU deer facility, and the muntjac deer program at SDSU, for continued access to research materials. These facilities are used to perform experiments in the natural hosts and to serve as a tissue bank for research purposes.

**Accomplishments:**

*Pathogenesis*

- Demonstration of CWD in saliva and blood of infected deer. This is significant since it indicates that 1) CWD could be shed and/or transmitted via saliva, and 2) blood or blood-derived products are potential sources for CWD infection.
- Demonstrate that CWD infection in muntjac deer is similar to infection in natural cerivds and, due to their small size, muntjac deer provide a significant advantage for investigating CWD compared to North American deer and elk.
- Demonstrated that the nasal and oral mucosa can serve as sites for experimental TSE entry, infection, and exit including the release of TSE infectivity into nasal secretions. This provides new information on potential routes of prion neuroinvasion following oral exposure and TSE replication at these mucosa also has implications for agent shedding and transmission.

Environmental/Transmission

- Demonstration that PrP null cattle are not susceptibly to experimental TSE infection indicating the livestock that are resistant to TSEs are a potential option for controlling and reducing TSE risk to animal health and food safety.
- Performed interspecies transmission of scrapie, CWD, and transmissible mink encephalopathy into sheep, cattle, and deer. These studies established the relative risk of each host to TSE infection from a TSE disease that they can potentially be exposed to under natural conditions. This information is important for risk assessment of domestic livestock and cervids to animal TSE diseases.

*Diagnostics*

- Comparative analysis of commercial TSE assay kits on different tissues from sheep with scrapie.
- Developed methodology to amplify CWD agent in vitro by protein misfolding cyclic amplification. This assay has the potential to detect very low levels of the TSE agent in blood and to form the basis of a TSE diagnostic assay.
- Identified a TSE-specific pattern of gene expression during the progression of experimental scrapie using DNA microarrays and genomics approaches. These findings can provide important clues to the basis of molecular and cellular basis of TSE neurodegeneration and also identify early markers of disease that are useful in TSE diagnosis.
- Utilized RNA aptamer technology to identify aptamers with high affinity for the prion protein among different host species. The advantage of an aptamer based TSE diagnostic assay is that a single reagent could be used for universal detection of prion proteins.
- In collaboration with our industry partner we have develop a novel in vitro diagnostic assay for rapid identification of TSEs from blood using repetitive DNA sequences as a surrogate marker for BSE infection.
- Determined that endocrine metabolites can serve as surrogate markers of TSE infection in serum of rodents and ruminants during preclinical infection. These biomarkers could form the basis for an ante-mortem blood test for TSEs in livestock.

**Impacts:**

1. Acquisition of multiple-investigator, collaborative grants between NC1024 members.
2. Publication of peer-reviewed manuscripts that include NC1024 members.
3. Definition of a small cervid model of prion disease.
4. Investigation of multiple ante-mortem diagnostic technologies with the potential to streamline TSE Surveillance.
5. Initiation of new research projects among committee members.
6. Extensive representation at National and International TSE Meetings.
7. Increased understanding of the early pathogenesis of prion diseases at the molecular, cellular, and host levels.
8. Increased understanding of TSE pathogenesis and transmission.
9. Increased collaboration between TSE researchers through the shared use of core resources.

**Publications:**

Sigurdson CJ, Mathiason CK, Perrott MR, Eliason GA, Spraker TR, Glatzel M, Manco G, **Bartz JC**, Miller MW, **Hoover EA**. 2008. Experimental chronic wasting disease (CWD) in the ferret. J Comp Pathol. 138(4):189-96.

Schutt CR & **Bartz JC**. 2008. Prion interference with multiple prion isolates. Prion 2:61-3.

**Bartz JC**, Kramer ML, Sheehan MH, Hutter JA, Ayers JI, **Bessen RA**, Kincaid AE. 2007. Prion interference is due to a reduction in strain-specific PrPSc levels. J Virol. 81:689-97.

Kurt TD, Perrott MR, Wilusz CJ, Wilusz J, Supattapone S, Telling GC, Zabel MD, **Hoover EA**. 2007. Efficient in vitro amplification of chronic wasting disease PrPRES. J Virol. 81:9605-8.

Green KM, Browning SR, Seward TS, Jewell JE, Ross DL, Green MA, Williams ES, **Hoover EA**, Telling GC. 2008. The elk PRNP codon 132 polymorphism controls cervid and scrapie prion propagation. J Gen Virol. 89:598-608.

Baron T, Bencsik A, Biacabe AG, Morignat E, **Bessen RA**. 2007. Phenotypic similarity of transmissible mink encephalopathy in cattle and L-type bovine spongiform encephalopathy in a mouse model. Emerg Infect Dis. 13:1887-94.

Bailey JD, Berardinelli JG, Rocke TE, **Bessen RA**. 2008. Prominent pancreatic endocrinopathy and altered control of food intake disrupt energy homeostasis in prion diseases. J Endocrinol. 197:251-63.

Clawson ML, Heaton MP, Keele JW, Smith TP, Harhay GP, **Richt JA**, Laegreid WW. A sequencing strategy for identifying variation throughout the prion gene of BSE-affected cattle. BMC Res Notes. 2008 Jun 23;1:32.

Hamir AN, **Richt JA**, Miller JM, Kunkle RA, Hall SM, Nicholson EM, O'Rourke KI, Greenlee JJ, Williams ES. Experimental transmission of chronic wasting disease (CWD) of elk (Cervus elaphus nelsoni), white-tailed deer (Odocoileus virginianus), and mule deer (Odocoileus

hemionus hemionus) to white-tailed deer by intracerebral route. Vet Pathol. 2008 May;45(3):297-306.

Clawson ML, **Richt JA**, Baron T, Biacabe AG, Czub S, Heaton MP, Smith TP, Laegreid WW. Association of a bovine prion gene haplotype with atypical BSE. PLoS ONE. 2008 Mar 19;3(3):e1830.

Hamir AN, Kunkle RA, Nicholson EM, Miller JM, Hall SM, Schoenenbruecher H, Brunelle BW, **Richt JA**. Preliminary observations on the experimental transmission of chronic wasting disease (CWD) from elk and white-tailed deer to fallow deer. J Comp Pathol. 2008 Feb-Apr;138(2-3):121-30.

Hamir AN, Kunkle RA, **Bulgin MS**, Rohwer RG, Gregori L, **Richt JA**. Experimental transmission of scrapie agent to susceptible sheep by intralingual or intracerebral inoculation. Can J Vet Res. 2008 Jan;72(1):63-7.

Hamir AN, Kunkle RA, **Richt JA**, Miller JM, Greenlee JJ. Experimental transmission of US scrapie agent by nasal, peritoneal, and conjunctival routes to genetically susceptible sheep. Vet Pathol. 2008 Jan;45(1):7-11.

Gavier-Wid√©n D, N√∂remark M, Langeveld JP, Stack M, Biacabe AG, Vulin J, Chaplin M, **Richt JA**, Jacobs J, Ac√≠n C, Monle√≥n E, Renstr√∂m L, Klingeborn B, Baron TG. Bovine spongiform encephalopathy in Sweden: an H-type variant. J Vet Diagn Invest. 2008 Jan;20(1):2-10.

Hamir AN, Kunkle RA, Miller JM, Cutlip RC, **Richt JA**, Kehrli ME Jr, Williams ES. Age-related lesions in laboratory-confined raccoons (Procyon lotor) inoculated with the agent of chronic wasting disease of mule deer. J Vet Diagn Invest. 2007 Nov;19(6):680-6.

Brunelle BW, Hamir AN, Baron T, Biacabe AG, **Richt JA**, Kunkle RA, Cutlip RC, Miller JM, Nicholson EM. Polymorphisms of the prion gene promoter region that influence classical bovine spongiform encephalopathy susceptibility are not applicable to other transmissible spongiform encephalopathies in cattle. J Anim Sci. 2007 Dec;85(12):3142-7.

Hamir AN, Miller JM, Kunkle RA, Hall SM, **Richt JA**. Susceptibility of cattle to first-passage intracerebral inoculation with chronic wasting disease agent from white-tailed deer. Vet Pathol. 2007 Jul;44(4):487-93.

Nicholson EM, **Richt JA**, Rasmussen MA, Hamir AN, Lebepe-Mazur S, Horst RL. Exposure of sheep scrapie brain homogenate to rumen-simulating conditions does not result in a reduction of PrP(Sc) levels. Lett Appl Microbiol. 2007 Jun;44(6):631-6.

Sorensen G, Medina S, Parchaliuk D, Phillipson C, Robertson C, **Booth SA**. Comprehensive transcriptional profiling of prion infection in mouse models reveals networks of responsive genes. BMC Genomics. 2008 Mar 3;9:114.

Blanchong JA, **Samuel MD**, Scribner KT, Weckworth BV, Langenberg JA, Filcek KB. Landscape genetics and the spatial distribution of chronic wasting disease. Biol Lett. 2008 Feb 23;4(1):130-3.

Choi CJ, Anantharam V, Saetveit NJ, Houk RS, Kanthasamy A, **Kanthasamy AG**. Normal cellular prion protein protects against manganese-induced oxidative stress and apoptotic cell death. Toxicol Sci. 2007 Aug;98(2):495-509.

Triantis J, Dennis MM, **Salman MD**, Gould DH. Effect of time and temperature on PrPCWD immunoreactivity as evidenced by Western blot. J Vet Diagn Invest. 2007 Jul;19(4):389-91.