# Pathobiology, Genetics and Detection of Transmissible Spongiform Encephalopathies

- Prion Research conducted at the Nation's premier Chronic Wasting Disease research center, National Animal Disease Center, Ames Iowa.
- Found Genetic selection of elk has the potential to reduce the impact of Chronic Wasting Disease.
- Also found that two metal ions (copper and manganese) were found to destabilize the prion protein and that these same metals also decrease the tendency for the protein to misfold.
- Asterisk draws attention to points of particular interest.

**Research Project:** Pathobiology, Genetics, and Detection of Transmissible Spongiform Encephalopathies

**Location: Virus and Prion Research**

**2018 Annual Report**

## Objectives

Objective 1: Investigate the mechanisms of protein misfolding in prion disease, including the genetic determinants of misfolding of the prion protein and the environmental influences on protein misfolding as it relates to prion diseases. Subobjective 1.A: Investigate the differences in the unfolded state of wild-type and disease associated prion proteins to better understand the mechanism of misfolding in genetic prion disease. Subobjective 1.B: Investigate the influence of metal ions on the misfolding of the prion protein in vitro to determine if environmental exposure to metal ions may alter disease progression. Objective 2: Investigate the pathobiology of prion strains in natural hosts, including the influence of prion source genotype on interspecies transmission and the pathobiology of atypical transmissible spongiform encephalopathies (TSEs). Subobjective 2.A: Investigate the pathobiology of atypical TSEs. Subobjective 2.B: Investigate the influence of prion source genotype on interspecies transmission. Objective 3: Investigate sampling methodologies for antemortem detection of prion disease, including the utility of blood sampling as a means to assess prion disease status of affected animals and the utility of environmental sampling for monitoring herd prion disease status. Subobjective 3.A: Investigate the utility of blood sampling as a means to assess prion disease status of affected animals. Subobjective 3.B: Investigate the utility of environmental sampling for monitoring herd prion disease status.

## Approach

The studies will focus on three animal transmissible spongiform encephalopathy (TSE) agents found in the United States: bovine spongiform encephalopathy (BSE); scrapie of sheep and goats; and chronic wasting disease (CWD) of deer, elk, and moose. The research will address sites of protein folding and misfolding as it relates to prion disease, accumulation of misfolded protein in the host, routes of infection, and ante mortem diagnostics with an emphasis on controlled conditions and natural routes of infection. Techniques used will include spectroscopic monitoring of protein folding/misfolding, clinical exams, histopathology,

immunohistochemistry, and biochemical analysis of proteins. The enhanced knowledge gained from this work will help understand the underlying mechanisms of prion disease and mitigate the potential for unrecognized epidemic expansions of these diseases in populations of animals that could either directly or indirectly affect food animals.

**Progress Report**

All 8 project plan milestones for FY18 were fully met. Research efforts directed toward meeting Objective 1 of our project plan center around the production of recombinant prion protein from either bacteria or mammalian tissue culture systems and collection of thermodynamic data on the folding of the recombinant prion protein produced. Both bacterial and mammalian expression systems have been established. Thermodynamic data addressing the denatured state of wild-type and a disease associated variant of bovine prion protein has been collected and a manuscript has been submitted. A manuscript published this year reports our initial finding on the binding of metal ions to recombinant prion protein. In research pertaining to Objective 2, all studies have been initiated and animals are under observation for the development of clinical signs. The animal studies for this objective are long term and will continue until onset of clinical signs. In vitro studies planned in parallel to the animals studies have similarly been initiated and are ongoing. Objective 3 of the project plan focuses on the detection of disease associated prion protein in body fluids and feces collected from a time course study of chronic wasting disease inoculated animals. At this time samples are being collected as planned and methods for analysis are under development.

**Accomplishments**

1. An amplification based approach to prion disease detection identifies bovine spongiform encephalopathy (BSE) in cattle prior to the onset of clinical disease. The bovine spongiform encephalopathy (BSE) disease process involves conversion of the normal cellular prion protein PrP to a pathogenic misfolded conformation. This conversion process can be conducted in the lab using a misfolding amplification process known as real-time quaking induced conversion (RT-QuIC). RT-QuIC uses recombinant prion protein to detect minute amounts of the abnormal infectious form of the prion protein. ARS researchers in Ames, Iowa evaluated whether prions from cattle inoculated with the agent of BSE could be detected with recombinant bovine prion protein using RT-QuIC prior to the onset of clinical signs. The results showed that RT-QuIC detects BSE in cattle prior to onset of clinical signs despite being undetectable by traditional methods of diagnosis. As the origin of classical, feedborne BSE remains unknown and low numbers of BSE are diagnosed worldwide each year, parties with interest in the cattle and beef industries and regulatory officials responsible for safe feeding practices of cattle will be interested in this work.

* 2. Genetic selection of elk has the potential to reduce the impact of chronic wasting disease (CWD). Chronic wasting disease (CWD) is a fatal disease of deer and elk that causes damaging changes in the brain. The infectious agent is an abnormal protein called a prion that has misfolded from its normal state. Whether or not an elk will get CWD is affected by their

genetics. ARS researchers at Ames, Iowa evaluated pathologic features and properties of abnormal prion protein from elk of 3 different genotypes that were infected with CWD. The genetic differences tested were at position 132 in the elk prion protein, which is homologous to the human codon 129 that is associated with susceptibility to human prion diseases. The study results indicate that there are differences in incubation periods, patterns of abnormal prion accumulation in the brain, and fibril stability features in these different genotypes of elk. While no genotype is completely resistant to CWD, elk expressing the L132 allele of the prion protein do have greatly prolonged incubation periods. These long incubation periods are associated with more stable prion fibrils. These findings suggest that genetic selection for the L132 allele has the potential to reduce (but probably not eliminate) the impact of CWD in captive and free-ranging elk populations. This information is useful to wildlife managers and captive wildlife owners that are selectively breeding animals and has implications for future regulations for the control of CWD in the United States.

* 3. Metal ions in the environment influence misfolding of the prion protein. Misfolding of the normally folded prion protein in mammals results in chronic wasting disease (CWD) in deer and elk. The binding of divalent metal ions is known to influence some aspects of prion folding but has not been well characterized for the elk prion protein. ARS researchers at Ames, Iowa assessed the binding of divalent metal ions to elk prion protein and evaluated changes in the folding, stability, and misfolding of the protein. Two metal ions (copper and manganese) were found to destabilize the prion protein and that these same metals also decrease the tendency for the protein to misfold. This report demonstrates the complex importance of metal ions on the folding, stability, and misfolding of the prion protein with the potential to impact disease. Given the different environmental sources and levels to which deer and elk may be exposed, this has the potential to influence CWD control measures implemented by owners and regulatory officials.

4. Characterization of a small experimentally bred herd of cattle bearing a mutation that results in genetic bovine spongiform encephalopathy (BSE). Until recently, no examples of inherited, disease-causing mutations were known in livestock. In 2006, a case of bovine spongiform encephalopathy (BSE) was identified in an older beef cow which carried a change in its prion gene corresponding to the human E200K gene (E211K in cattle). E211K cattle can serve as an experimental model to increase our understanding of the link between this genetic change and disease pathology in humans. Their relatively long life span and the fact that cattle are a natural host for prion diseases, with a relatively low transmission barrier to humans, make

cattle a superior model system to mouse models. ARS researchers at Ames, Iowa reported the properties of a small experimentally-bred herd of cattle bearing this genetic mutation, including a characterization of the molecular genetics of these cattle and a molecular

validation of these cattle as a model system. This work aids in understanding the progression of inherited prion disease and provides a characterization of a system in which to further study the disease.

**Review Publications**

*Vrentas, C.E., Greenlee, J.J., Foster, G.H., West, J., Jahnke, M.M., Schmidt, M.T., Nicholson, E.M. 2017. Effects of a naturally occurring amino acid substitution in bovine PrP: a model for inherited prion disease in a natural host species. BMC Research Notes. 10:759. https://doi.org/10.1186/s13104-017-3085-8.*

*Moore, S.J., West Greenlee, M.H., Kondru, N., Manne, S., Smith, J.D., Kunkle, R.A., Kanthasamy, A., Greenlee, J.J. 2017. Experimental transmission of the chronic wasting disease agent to swine after oral or intracranial inoculation. Journal of Virology. 91(19):e00926-17. https://doi.org/10.1128/JVI.00926-17.*

*Moore, S.J., Vrentas, C.E., Hwang, S., West Greenlee, M.H., Nicholson, E.M., Greenlee, J.J. 2018. Pathologic and biochemical characterization of PrPSc from elk with PRNP polymorphisms at codon 132 after experimental infection with the chronic wasting disease agent. BMC Veterinary Research. 14(1):80. https://doi.org/10.1186/s12917-018-1400-9.*

*Hwang, S., West Greenlee, M.H., Balkema-Buschmann, A., Groschup, M.H., Nicholson, E.M., Greenlee, J.J. 2018. Real-time quaking-induced conversion detection of bovine spongiform encephalopathy prions in a subclinical steer. Frontiers in Veterinary Science. 4:242. https://doi.org/10.3389/fvets.2017.00242.*

*Samorodnitsky, D., Nicholson, E.M. 2018. Differential effects of divalent cations on elk prion protein fibril formation and stability. Prion. 12(1):63-71. https://doi.org/10.1080/19336896.2017.1423187.*