Towards Detection of PrP^{Sc} in Soil
by Optical Biosensing

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE), a fatal neurodegenerative disease found in deer and elk. TSEs are prion diseases that affect certain mammalian species (bovine spongiform encephalopathy, BSE, scrapie in sheep and goats) as well as humans, Creutzfeldt-Jakob disease (CJD) [1]. Public health concerns are rising as evidence suggests some of the prion diseases can cross species, which most alarmingly, includes humans [2,3]. Transmission occurs via the infection prion (PrP^{Sc}), which is an abnormal isoform of a normal component of the host, PrP^{C}. The two do not differ in amino acid sequence, rather only by a change in conformation [4]. Recently, Pedersen et al found these prions to adhere to soil minerals, which create a reservoir of the disease from decomposed infected carcasses in the wild. PrP^{Sc} was also found to remain infectious while attached to the soil particles [5].

We intend to create a sensor in which soil samples can be taken from various sites which could possibly be infected with the prion. Currently, detection methods for TSE analyze tissues of infected animals, or take relatively long time periods to get results, which many times are not very reliable. Current laboratory methods are immunohistochemistry (IHC), Western blotting technique (WB), and scrapie associate fibrils (SAF) examined by electron microscopy. Bio-Rad has developed an assay for a variety of species of animal suspected to carry their specific TSE, but this is also an assay for tissue, not soil [6].

This proposal has many sources of intellectual merit. It will advance the knowledge and understanding of CWD and where it has spread in the United States. Detecting this prion is crucial, as the disease targets animal health, wildlife population health, as well as human health. Joel A. Pedersen is an

expert in the environmental persistence of prion proteins, as well as other toxicants in aquatic, soil, and sediment systems.

This proposal has many broader impacts than simply testing the soil to detect for PrP^{Sc}. First, we propose to partner with Centennial High School, in Northampton County, to encourage the students to continue their education to college. Centennial High School as one of the lowest average test scores for the Pennsylvania state assessment tests. Many of the students there are members of hunting and/or farming families, in which CWD is a concern. We propose to partner with the 11^{th} graders in charting where the more recent confirmations of PrP^{Sc} has been found in soil across the country, and offer summer internships in our laboratory to two graduating seniors. We also propose to speak about our research at the Pennsylvania Envirothon, which is a high school competition that focuses natural resource and environmental sciences. Stewart and Pedersen will attend the 55^{th} and 56^{th} *Annual Meeting of Wildlife Disease Association and American Association of Wildlife Veterinarians* to present our findings. In order to disseminate our results effectively, we will submit data to the game commission of states in which we detect PrP^{Sc}-soil samples, as well as constantly update the EPA. Another proposed outreach is to submit articles to the general public via hunting, farming, and ranching magazines and newsletters, such as *Field and Stream, Hunting,* and *Deer & Deer Hunting.*

We propose to develop a device, in which soil samples can be inserted, that will register a positive or negative detection of PrP^{Sc}. An optical biosensor that uses an immunoglobulin (IgG) antibody to detect the presence of PrP^{Sc} by fluorescence will be investigated. The first year's work will entail running experiments in order to be sure only the PrP^{Sc} is being detected in the laboratory, and not PrP^{c}. The next step in the first year will be to add PrP^{Sc} adsorbed soil, fabricated in the lab, to the detection system. We realize this will be a great source of error, and most likely one of the biggest issues to overcome due to the fact that there are so many particles that compose soil, and the prion may only

be attached to certain soil minerals. However, with Pedersen's soil laboratory resources, it will be possible to separate out some of the minerals. Pedersen has found that the prion has a much higher affinity for clay minerals than quartz and some other minerals [5], making the separation of clay, such as monmorillonite (Mte), desirable. Another expected issue is the light scatter due to the addition of the soil particles. Particles such as mica and quartz would be expected to scatter light and be a source of false results, whether positive or negative. The soil will be washed and filtered to remove some excess matter. Pedersen has found that the binding of PrP^{Sc} to Mte is extremely strong, so much that he has found only one method of desorbing the prion. Thus, a gentler washing of the soil to remove some particles that will affect the fluorescence is not much of a concern to the condition of the prion. In the second year, we propose to begin to sample soils from around the country. Differences in soils will call for adjustments in the design. In the third year, soil sampling will continue and data will be submitted as stated above. For each detection, the experiment will be run several times to reduce false positives, and the results will be confirmed with IHC, which today is considered the most accurate method of detection for PrP^{Sc}.

In conclusion, the proposed method of developing an optical biosensor for the detection of PrP^{Sc} and thus CWD, will help not only with the control of the disease, but will impact the public by finding regions where the prion exists, thus reducing exposure to the public. The proposal also strives to involve high school students from a scientific field as well as a depressed area, to get involved in the scientific community and continue their education in the field.

References

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