

More work is needed to develop BSE tests

by Carol Hugunin

“Mad cow,” scrapie in sheep, Creutzfeldt-Jakob disease (CJD), and similar degenerative conditions in humans, are all forms of spongiform encephalopathies. The nature of the disease—malformations of the host’s own proteins—presents problems in developing tests for the disease. Currently, the only solid test is a post-mortem study of stained slides of the brain.

It is also possible to biopsy the brain, take a tissue sample, and if that tissue, when injected into the brain of a mouse, causes the mouse to develop spongiform encephalopathy in 200-500 days, then it can be concluded that that brain was infected with the agents called prions. However, this test in mice takes one to two years to get definitive results. In the medical realm, either waiting for the patient to die, or doing a biopsy, and then waiting one to two years for a mouse to develop spongiform encephalopathy, leaves a lot to be desired.

Surveillance and testing of cows, U.S.A.

Since BSE was first officially designated in 1986, the U.S. Animal Plant Health Inspection Service and the Food Safety Inspection Service have periodically monitored slaughterhouses, pulling out of the group of cows going to slaughter any with clinical signs of rabies or any other central nervous system disorder. These cows are strictly examined, including extensive studies of stained slides of the brain. Roughly 250-300 cows a year looked clinically suspicious; but none turned out to have BSE. Nonetheless, as BSE became a more heightened concern, on March 20, 1996, the USDA expanded this surveillance operation. On June 10-11 in Ames, Iowa, the USDA Agricultural Research Service’s annual conference, “USDA Scrapie/Bovine Spongiform Encephalopathy Consultant’s Group Meeting,” discussed research efforts to develop methods for testing.

There is need for increased, broad-based efforts to develop tests for animals, humans, and for food and medical products potentially involved in transmissibility of disease. Progress in two testing approaches is being reported but serious questions remain about where, when, and how to draw substances from the person, or animal, to be tested.

The most promising of these tests, so far, in the research phase, is being developed by Dr. Michael Harrington, at the California Institute of Technology in Pasadena, California,

and Dr. Clarence Gibbs at the National Institutes of Health, National Institute of Neurological Diseases and Stroke, in Bethesda, Maryland. They discovered that CJD in humans (or scrapie in sheep; or BSE in cows) leads to a form of brain damage in which two proteins normally found only in the brain (protein 130 and 131), are found also in spinal fluid. They developed an antibody test for 130 and 131 in the spinal fluid, which takes two days to get results, and is technically simple enough to be done in hospitals and clinics.

These two proteins can be found in patients who have herpes encephalitis, a virally induced disorder. Since herpes encephalitis is clinically a very different disorder from CJD, this testing method opens up the possibility of solid diagnosis of CJD well before the patient’s death—a necessity for any treatment to be developed; as well as the possibility of testing breeding stock, or even, possibly, whole animal herds.

There is also work on developing a test under way at the USDA’s National Animal Disease Center in Ames, Iowa, at the Respiratory and Neurologic Disease Research Unit. The work was begun by Dr. Mary Jo Schmerr, on sheep scrapie brain material. Since scrapie in sheep and goats is the prototype of transmissible spongiform encephalopathies found in other animals and humans, the test technique can have potentially wide applicability.

Dr. Schmerr and collaborators now have a means to identify the presence—using micro-level amounts of brain matter—of the abnormal disease protein (the prion). The way the test works is that some brain protein is denatured, and prepared into a monomer form (of prion protein) with a molecular mass of 27 kilodaltons. Then, peptides labeled with fluorescein are added, along with a specific antibody that is known to react with both prion and the peptide. At this point, a technique called “free capillary electrophoresis” can be used to evaluate the materials, which will differentiate themselves, based on “competitive” binding for the antibody. Depending on how much of the peptide is tied up, the scientists can tell whether there is any prion present or not.

The first phase of the test—preparing the material—takes a day and a half. The capillary electrophoresis test is fast. This method involves running current across a very small capillary, about 20 micrometers (or a nanoliter). Laser-induced fluorescence detection is done using an argon laser. What you then “see,” when the voltage is run across the material, is the differentiated migration of proteins. From reading the pattern of “peaks,” the presence of infective prion can be detected.

The test is still in what the developers call the validation stage, but technically, it does work well. A full report of the test will be published this year in the *Journal of Chromatography* (“Improvements in a Competition Assay to Detect Scrapie Prion Protein by Capillary Electrophoresis,” by Mary Jo Schmerr, Kathryn R. Goodwin, Randall C. Cutlip, Allen L. Jenny). (See interview with Dr. Randall C. Cutlip, research leader of the Ames USDA unit, in *EIR*, May 3, 1996.)