Bovine Spongiform Encephalopathy: Finding Practical Methods of Detection

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Some diseases are caused by neither virus, fungus, or bacteria, but rather caused by misfolded proteins, or prions. These prions can lead to a group of diseases known as transmissible spongiform encephalopathies (TSE). Among the most talked about TSE is bovine spongiform encephalopathy (BSE), also known as mad cow disease. It gained notoriety in the 1990’s when a large outbreak hit the European cattle industry and lead to a small number of the human population getting infected. The disease was traced to infected tissues throughout the central nervous system in cattle causing regulations and protocols to change throughout the industry. Initial tests could only be executed on deceased animals, so a need to detect BSE within the living cattle population arose. As the infection by the prion was being understood, more methods of detection were being tested.

Bovine spongiform encephalopathy is caused by a prion. The tissues within cattle contain normal proteins called cellular prion proteins (PrP\(^C\)) and in especially high numbers within the tissues of the central nervous system. PrP\(^C\) is thought to have a role in immune response, signal transduction, copper binding and synaptic transmission. Its structure consists of 209 amino acids (for human PrP\(^C\)), and each species’ length varies slightly. It has two notable regions: an asparagine terminal region which is unstructured and flexible due to repeating octapeptides, and a cysteine terminal region comprised of three alpha helical structures with two anti-parallel beta sheet structures. When infected, they fold into disease associated prion proteins (PrP\(^Sc\)). PrP\(^Sc\) comes into contact with PrP\(^C\) and acts as a template to facilitate the refolding. The alpha helical structures are folded into beta sheets on the soluble, protease sensitive PrP\(^C\) when converted to the insoluble, protease resistant PrP\(^Sc\). PrP\(^Sc\) shows an affinity to form aggregates and is responsible for neural degeneration.

The details for the mechanism of PrP\(^Sc\) folding is still under consideration. Recently, a group was able to develop a new cell line from Madin-Darby Bovine Kidney (MDBK) cells, that over expresses PrP\(^C\) and will allow the infection of BSE to be observed in vitro. This should provide a useful tool to facilitate the screening of anti-prion substances, the formation and inhibition of pathogenic prions leading to potential therapies and the diagnosis of BSE.
Through Western blot analysis, it has been discovered that there are two types of BSE in addition to the classical form: H-type and L-type. H-type BSE is characterized by a high molecular mass of the unglycosylated isoform of the PrP$^{Sc}$ and L-type is characterized by a low molecular mass. They are thought to have different incubation periods. L-type BSE is further characterized by PrP$^{Sc}$ positive amyloid plaque accumulations and granular accumulations at the synaptic cleft. The plaque accumulations from L-type BSE are more widespread throughout the brain than in the other types.

BSE causes degeneration of the nervous system. Holes will form in the brain tissue resulting in spongy architecture due to vacuole formation in neurons (spongiform changes in the brain) and neurons will die. There is thought to be an incubation period for the disease before symptoms are shown or recognized, from months to years. Cattle display changes in temperament, usually as nervousness or aggression, abnormal posture, lack of coordination and difficulty in standing, decreased milk production, or loss of body weight despite continued appetite. It is suggested that the symptoms of BSE aren’t necessarily caused by the PrP$^{Sc}$ plaque deposition alone, but rather the simultaneous loss of function by PrP$^{C}$ leading to the progressive degeneration of the nervous system.

There are no effective treatments to stop the protein misfolding. It has shown resistance to most forms of microbial inactivation. It has also been proven that there is no practical temperature at which to inactivate the prion. Infected tissues at different states (wet and dry) show drastically different inactivation temperatures. The prognosis for BSE and any other transmissible spongiform encephalopathy is eventual death.

BSE positive tissues can infect other species and manifest as other transmissible spongiform encephalopathies. There is a prion species barrier between certain species, such as mice and hamsters. However, some species can consume the infected tissues from the central nervous system of other species and get infected. The PrP$^{C}$ is thought to be of similar sequence and structure allowing infection between species. For example, if a human consumes infected tissues from a cow, they will contract BSE. In humans, the BSE prion causes a variant form of Creutzfeldt–Jakob disease, which is a degenerative neurological disease. However, atypical types (H/L) are unconfirmed in crossing the species barrier to humans. L-type BSE is thought not to cross to humans but it has been seen in another primate: macaques. L-type BSE is also found to cross from cows and mice.

Since 1990, the USDA has been running a program to detect BSE. Guidelines that enforce feed bans were implemented as cattle can get infected from as little as 1 mg of infected
The diet of a cow was previously supplemented with ruminant derived proteins. This was an effective way to spread BSE, so a ban was enforced. Any meat from the central nervous system was banned. The FDA also implemented product holding while screening takes place. They screen about 40,000 animals annually, which is above guidelines, to protect human consumers.

The need to establish the integrity of the cattle industry and protect its consumers led to attempts to establish various methods for detecting BSE. BSE isn’t diagnosed until clear symptoms arise and most tests are done post mortem.

In order to detect the circulation of BSE within ruminant populations, a highly sensitive assay called serial protein misfolding cyclic amplification assay (sPMCA), is used. It is used to detect trace amounts BSE in conjunction with other transmissible spongiform encephalopathies. Serial protein misfolding cyclic amplification assay (sPMCA) is similar to PMCA. PMCA is used to amplify the amount of misfolded prion proteins (PrP\textsuperscript{Sc}). As PrP\textsuperscript{Sc} infects normal proteins present, a chain of misfolded proteins grows. This is blasted apart by ultrasound to increase the number of infected chains available to further spread. This is repeated until the normal proteins are quickly converted to PrP\textsuperscript{Sc}. In this sPMCA, the product from a single round of PMCA was used to inoculate a new PMCA reaction by diluting the product with fresh substrate. This procedure makes it possible to detect down to a single infectious prion protein.

Retinal screening was tested as an antemortem method for detecting BSE. Tests for detecting BSE were performed on tissue samples extracted from the retina. The tests were done on eyes removed from infected cattle that were slaughtered for easier access. The retina is the most accessible part of the central nervous system where PrP\textsuperscript{Sc} is found. Removing an eye from a live cow is rather invasive, but the alternative is to kill the cow and remove the brain to test. Multiple tests can be performed on the retina once a homogenous solution has been made from it including electroretinography and fluorescence spectroscopy. Electroretinography was performed to detect abnormal function of the retina by measuring the electrical response of the ganglion cells attached to rod and cone cells. Retinal pathology from BSE is known to contain PrP\textsuperscript{Sc} aggregate build-up. Electroretinography was able to detect BSE up to 11 months before any other symptoms confirmed the diagnosis. Differences in the severity of detection were detected throughout the span of the trial.

Fluorescence spectroscopy was also performed on retinal tissue. The researchers observed that central nervous system tissues will fluoresce with up to ten times more intensity
than tissues found elsewhere because of the accumulation of lipofuscin, a highly fluorescent material produced when neurological disease caused by TSE damages the eye.

A sound alternative to retinal screening as an antemortem test is to screen the cattle’s urine for disease induced biomarkers. Urine is easy to collect and has a comparatively simple protein profile. A set of control proteins were created and observed over time via analysis of mass spectrometry and two dimensional differential gel electrophoresis (2D-DIGE). They were observed and compared to proteins found in BSE positive urine. A protein was identified that discriminated between BSE infected cattle and the control. This lays a promising foundation towards screening for BSE in cattle.

BSE is a prion induced disease for which there is no cure. It can cross the species barrier and infect humans with a grim prognosis. The need to sustain the population by raising livestock has lead to increased screening and detection regulations throughout the industry. The advancements in BSE detection on living cows show promise for further disease prevention and more advanced human safety while increasing the humane standards of the cattle industry.

References


