Prion Diseases

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1.0 CE/Contact Hour
Level: Beginning to Intermediate

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Prion Diseases

Objectives
After completion of this course the participant will be able to:
1. define Transmissible Spongiform Encephalopathies
2. discuss prions
3. describe animal TSE including Scrapie, Chronic Wasting Disease, and Bovine Spongiform Encephalopathy
4. describe human TSE including Kuru, Gerstmann-Sträussler-Scheinker Disease, Fatal Familial Insomnia, Alpers’ Disease, Creutzfeldt-Jakob Disease, and variant Creutzfeldt-Jakob Disease
5. describe sterilization and disinfection practices

Introduction
The headlines in Britain shouted messages such as “Bring Back the Beef,” and “Scientists baffled by mystery of new BSE cases.” In 1985 an epidemic of Mad Cow Disease began devastation in the beef industry in the United Kingdom that jolted the world. A disease among the cows was diagnosed as being caused by a transmissible spongiform encephalopathy (TSE), also known as a prion disease. TSEs are responsible for a number of animal and human conditions that were first thought to be caused by a virus. Research has indicated that the causative infectious agent does not contain a nucleic acid genome, therefore cannot be a virus. Others speculate that the agent is a virino, which is a small non-coding regulatory nucleic acid coated with a host-derived protective protein. Still others believe the agent is a prion. In both humans and animals this agent causes progressive brain damage and ultimately death.

Animal TSE can affect cows in the form of Bovine Spongiform Encephalopathy (BSE), also known as “Mad Cow Disease.” BSE is most prevalent in the United Kingdom and has caused the government to dispose of hundreds of thousands of infected cattle, causing a crisis in the beef industry. In 1995 a few cases of TSEs began appearing in young adults in England. These cases were a variant of Creutzfeldt-Jakob Disease and were ascribed to eating infected meat from BSE cattle. Other TSEs are found in animals as well as in humans. While these diseases, in humans, are rare, they are always fatal.

Transmissible spongiform encephalopathies (TSE)
TSEs are also known as prion diseases. The prion (proteinaceous infectious particle) is a normal protein that has a change in its three dimensional configuration. As the prion attacks the brain it causes large vacuoles in the cortex and cerebellum, resulting in the spongiform appearance. The normal protein (PrP\textsuperscript{c}) is a glycoprotein with secondary structures dominated by alpha helices, easily soluble and easily digested by proteases. The abnormal, disease-producing prion (PrP\textsuperscript{Sc}) is a glycoprotein with secondary structures dominated by beta helices, insoluble in all but strong solvents, and resistant to digestion by proteases. When PrP\textsuperscript{Sc} molecules come in contact with PrP\textsuperscript{c} molecules, the PrP\textsuperscript{c} molecule is converted into the PrP\textsuperscript{Sc} molecule. As this process occurs, the molecules form aggregates that might be the cause of the cell damage that results in vacuole formation.

Prion disease may be acquired through one of three routes. First, when the disease has no known apparent cause, it is called sporadic. Second, the disease may be inherited through an
autosomal dominant trait, and third, the disease may be acquired through infected food, homografts, or medical equipment. Prions appear to be resistant to enzymes, chemicals and heat that break down other proteins. Normal disinfection procedures do not eliminate prions either. In addition, they are extremely resistant to high doses of ionizing and ultra-violet irradiation with some residual activity remaining in the environment. In 2000, the World Health Organization developed recommendations on the safest and most unambiguous method for ensuring that there is no risk of residual infectivity on contaminated instruments. The recommendation is for incineration of all disposable instruments, material and wastes, and is the preferred method for all instruments exposed to highly infected tissues. For non-disposable instruments, pretreatment by immersion in sodium hydroxide followed by heating in a gravity displacement autoclave is required prior to routine sterilization. For surfaces and heat sensitive instruments, flood with 2N sodium hydroxide for 1 hour and then rinse. Dry goods can be immersed in sodium hydroxide followed by heating in a porous load autoclave.

All TSEs, human and animal, have long incubation times and do not induce an inflammatory response. They are not known to spread from human to human, but transmission can occur through exposure to infectious materials during invasive medical procedures. Exposure to human cadaveric-derived pituitary hormones, dural and cornea homografts, and contaminated neurosurgical instruments has been documented to cause infection. Formalin and glutaraldehyde-fixed TSE tissue retains infectivity for long periods, if not indefinitely, consequently the same precautions should be used with this type of tissue as with fresh material.

**Prion Diseases**

**Animal:**
- Scrapie
- Chronic Wasting Disease
- Bovine Spongiform Encephalitis
- Feline spongiform encephalitis
- Transmissible mink encephalitis

**Human:**
- Kuru
- Gerstmann-Sträussler-Scheinker Disease (GSS)
- Fatal Familial Insomnia (FFI)
- Alpers’ Disease
- Creutzfeldt-Jakob Disease (CJD)
- Variant Creutzfeldt-Jakob Disease (vCJD)

**Animal Diseases**

**Scrapie**

More than 250 years ago Scrapie was recognized as a disease in sheep and goats in Western Europe. In 1947, the first case was identified in the United States in a flock of British origin. Since then more than 1,000 flocks in the U.S. have been diagnosed with Scrapie. As with the other prion-caused diseases, Scrapie is a fatal, degenerative disease affecting the central nervous system. In the U.S. it has been reported in the Suffolk breed of sheep, along with over a dozen other breeds and some crossbreeds. As of October 2003, 2,350 cases in sheep and 127 cases in goats have been diagnosed.
Transmission is mainly from the ewe to her offspring through contact with the placenta and placental fluids. The incubation period is two to five years with the sheep living one to six months after symptoms appear. There is no evidence that Scrapie is transmittable to humans.

Early signs of Scrapie include slight changes in behavior or temperament, followed by scratching or rubbing against fixed objects. This rubbing phenomenon is how the disease was named Scrapie. Other symptoms include loss of coordination, weight loss, hopping like a rabbit and swaying of the rear end. Diagnosis is made based on the animal’s physical symptoms, the animal’s history and finally by exam of brain tissue. A diagnostic test is undergoing evaluation by USDA’s Animal and Plant Health Inspection Service for the detection of Scrapie in live animals.

Increased concern over this disease has caused packers and producers to have difficulty in disposing of sheep offal and dead sheep, causing increases in disposal costs. In addition, other countries are hesitant to purchase sheep products from the U.S. Control programs are focusing on developing a diagnostic test, investigating transmissibility, and providing effective cleanup strategies that are economic for packers and producers.

**Chronic Wasting Disease (CWD)**

North American deer and elk are the target of this TSE. It was first discovered in Colorado in the mule deer population and manifested itself as a “wasting” syndrome, resulting in severe weight loss and consequent death. CWD has spread outside the endemic zone of Colorado and Wyoming, including small areas in New York, West Virginia, and Wisconsin. The present range includes eleven states and two Canadian provinces and is expected to grow. The disease has also been diagnosed in farmed elk herds in South Dakota, Nebraska, Oklahoma, Montana, Kansas, and Colorado. These herds have undergone quarantine and no further disease has been identified. CWD has infected Rocky Mountain elk, mule deer, white-tailed deer, and moose. There is no evidence that this disease has been passed to other ruminant animals, such as cattle, sheep, and goats. CWD occurs mostly in adult animals. Symptoms include not only weight loss over time, but also decreased interaction with other animals, listlessness, and repetitive walking in a set pattern. Nervousness may also be exhibited in elk. A decreased appetite for hay and increased drinking have been observed.

Transmission of the disease is thought to be from animal to animal but may occur through birth. There is also evidence that it can be spread through exposure to prions in the environment. Currently researchers are in the process of developing a live-animal diagnostic test. As in other spongiform diseases, brain lesions occur and current diagnosis is made after the animal has died. Prevention of CWD is by elimination of infected animals and limiting the distribution of the disease to the endemic area for free-range animals and surveillance of farm-raised animals. Hunters should contact state wildlife officials to avoid endemic areas. Precautions to be taken when field-dressing these animals include using gloves, boning-out the meat from the animal, and minimizing handling of the brain and spinal cord.

**Bovine Spongiform Encephalopathy (BSE) or Mad Cow Disease**

In 1985, an epidemic began in England; before it was under control over 200,000 cattle were stricken with BSE in Britain and Europe, crippling the British livestock industry. “Mad Cow Disease” is aptly named due to the behavior exhibited by the cattle when they are infected. The origin of the disease appears to be cattle feed that contained Scrapie infected sheep brain tissue that had been treated in a new way that did not destroy the infectiousness of the Scrapie prions. Yes, the sheep Scrapie had crossed over into the cattle population. In addition, waste cattle (presumably contaminated) were also ground up for feed. In 1988, such food was banned,
but it took until 1993 for the epidemic to decline, due to the incubation period of three to eight years.

The epidemic in cattle peaked in January, 1993 at 1,000 new cases per week. British agricultural officials took a series of actions to eradicate BSE, making BSE a notifiable disease, prohibiting the inclusion of ruminant-derived proteins in ruminant feed, and preemptively destroying over four and a half million asymptomatic cattle over 30 months of age. As a result of these actions the epidemic markedly subsided and now few animals are diagnosed with the disease.

BSE had not been shown to exist in the United States. This was due to the banning of use of ruminant feed in 1997. However, in 2003 a cow infected with BSE was found in Washington State. This cow was traced back to an import from Canada. Since that time, two cases that appear to be endemic have been identified in Texas and Alabama.

**Human Diseases**

**Kuru**

Kuru is a prion disease that was discovered in the early 1900s in the people of New Guinea. The disease manifests itself as a neurodegenerative disorder starting with unsteadiness, deterioration of speech, and tremor. It then moves on to cause more severe tremors, shock-like muscle jerks, and uncontrolled bursts of laughter. In the final stage, all the symptoms become severe, and difficulty in swallowing and inability to feed oneself lead to starvation. The incubation period for Kuru was determined to be from 2 years to 23 years from exposure. The disease reached epidemic proportions in the 1960s after five decades of neurological disease and death, mostly in the female population. So, how did this group of natives acquire this devastating disease? In this part of New Guinea there was a ritual of mortuary cannibalism. The females would remove organs of the dead, which were then used as food sources, especially for children and the elderly. Fortunately for these natives of New Guinea, this practice has been eliminated from the culture. With the elimination of this practice, the disease has disappeared in New Guinea.

**Gerstmann-Sträussler-Scheinker Disease (GSS)**

GSS Disease is an inherited neurodegenerative disorder caused by an accumulation of a mutated prion protein amyloid. It is inherited as an autosomal dominant disease, which means that both sexes are affected and there are no carriers of the mutant gene. GSS slowly progresses with symptoms beginning between the ages of 30 and 70. Patients experience lack of muscle coordination and have difficulty walking. As the disease progresses, symptoms include slurring of speech, involuntary movements of the eyes, rigid muscle tone and eventually dementia, which is less common than in Creutzfeldt-Jakob Disease (CJD). In some cases the disease progresses rapidly and consequently cannot be distinguished from CJD. In GSS, spongiform changes in the brain tissue may or may not occur. Patients with GSS can live from 2 to 10 years with treatment aimed at alleviating symptoms. Currently there is no cure for this rare inherited disease. Current research is focused on the prion that causes the disease, attempting to characterize it, clarify the disease mechanism, and then developing ways to prevent, treat, and cure GSS disease.

**Fatal Familial Insomnia (FFI)**

FFI is a rare autosomal dominant hereditary disease caused by a prion that results in amyloid plaques that affect the thalamus, causing severe selective atrophy. The thalamus is a center in the brain that is responsible for regulation of sleep. As a result of the degradation of the thalamus, there is an interruption of the body’s circadian rhythms. Consequently, patients with
FFI lose sleep, can have hallucinations, and eventually go into coma, with death in about 18 months. The age of onset ranges from 30 to 60. The four stages of FFI are:

- Progressive insomnia, panic attacks, and bizarre phobias developing over a four-month period characterize the first stage.
- The second stage lasts about five months with symptoms including hallucinations, panic, agitation, and sweating.
- In stage three, total insomnia is paired with weight loss and lasts about three months.
- The final stage, which lasts six months, includes dementia, total insomnia, loss of hearing and sudden death.

New techniques such as DNA sequencing or molecular hybridization should be developed to make an early diagnosis, as the disease does not begin progression until after childbearing years. Currently there is no cure for this disease, but gene therapy could be promising to prevent FFI. In this case, the correct gene could be inserted to cause the correct protein to be developed, consequently allowing for the thalamus to function normally, thus preventing insomnia and subsequent deterioration.

**Alpers’ Disease**

Unfortunately, Alpers’ Disease affects infants and children. It is an autosomal recessive disorder that can be seen in siblings and is known also as Christensen’s disease or Christensen-Krabbe disease. Alfons Jakob first recognized it in the early 1900s and his students, Souza, Freedom, and Alpers further described cases. It is manifested by convulsions, developmental delay, mental retardation, and dementia. Only thirteen cases have been identified since 1931, but others may have been missed due to chronic liver dysfunction being present, which may mask diagnosis of Alpers’ Disease. Liver failure is usually the ultimate cause of death within the first two years of life. Final diagnosis is at autopsy when spongiform plaques are identified in the gray matter of the brain. There is no current treatment for the disease, only for the symptoms, such as anti-convulsants for the seizures.

**Creutzfeldt-Jakob Disease (CJD)**

CJD is also referred to as subacute spongiform encephalopathy due to the formation of microscopic vacuoles or holes in the neurons that appear “sponge-like.” The disease is named for Drs. Hans Creutzfeldt and Alfons Jakob who documented the first cases in the 1920s. This disease affects both men and women in the 50 to 75 year age range, with one case per million per year. Cases in persons under 30 years of age are extremely rare, with fewer than 5 cases per billion. A person can acquire CJD in one of three ways. Firstly, the disease can appear sporadically, without any apparent cause. Secondly, it can be inherited as an autosomal dominant pattern. This type of transmission occurs in about 10-15 % of the cases. Thirdly, an infectious agent can transmit the disease. Iatrogenic transmission is an unintended consequence of a medical procedure using instruments tainted by contaminated human growth hormone (about 100 cases), by corneal grafts from asymptomatic infected individuals, or by infected neural material. In 1976, more stringent sterilization procedures were put into place. Additionally, recombinant DNA technology is now used for producing human growth hormone. Because of these advances, no further documented cases of CJD have occurred from iatrogenic transmission. There are no known instances of transfusion-related CJD.

Symptoms begin with insomnia, depression, confusion and problems with memory, coordination, and sight. As the disease progresses, patients experience progressive dementia and involuntary jerking movements. In the final stages of the disease, patients lose all mental and physical functions, lapse into coma and die, usually from pneumonia due to the unconscious
state. CJD patients will succumb within one year of diagnosis. There are no known effective
treatments for CJD, so treatment focuses on easing symptoms.

CJD is difficult to diagnose, so the first step is to rule out other diseases that might have
similar symptoms. It may be mistaken for Alzheimer’s disease, Pick’s disease, Huntington’s
disease, cerebral hematomas and vascular irregularities. An EEG can detect a characteristic
abnormal brain pattern associated with the later stages of the disease, but cannot confirm a CJD
diagnosis. A new test to detect a specific protein (14-3-3) in cerebrospinal fluid (CSF) has been
developed, but again this does not give a definitive diagnosis. CJD can definitively be diagnosed
by performing a brain biopsy or autopsy. However, a brain biopsy can be a dangerous
procedure, can result in a false-negative result if the wrong area of the brain is chosen, and is
quite costly. In addition, there is a risk to healthcare workers if strict sterilization and infection
control precautions are not taken. When available, disposable equipment should be used in
suspected cases of CJD and then incinerated. If equipment is to be reused, steam sterilization or
cleaning with 1 N sodium hydroxide (followed by steam sterilization) can be utilized. If this
cannot be accomplished, the equipment must be disposed of by incineration. Contaminated skin
surfaces are to be washed with 1 N sodium hydroxide or 10% bleach followed by rinsing with
copious amounts of water. Splashes to the eyes may be treated using copious amounts of water
or saline. Contaminated dry waste or sharps waste should be autoclaved for 4.5 hours prior to
incineration.

**Variant Creutzfeldt-Jakob Disease (vCJD)**

In 1996, a disturbing fact emerged that showed a causal relationship between BSE and a
new disease called variant Creutzfeldt-Jakob Disease (now referred to simply as vCJD). Young
adults were dying after exhibiting clinical symptoms of CJD, including dementia and muscle
jERKS. Cases were predominately coming from Britain, but several cases were documented from
patients outside of Britain. These patients were found to have lived in the British Isles for at
least five years during the epidemic (1980-1995). As of June, 2007 there have been 161 deaths in
Britain from definite or probable vCJD with four probable cases still alive. Thirty-nine other
cases have occurred outside Britain, primarily in France and other European countries that
imported meat from Britain. Of the three documented cases in the U.S, two had lived in Britain
and one had lived in Saudi Arabia.

The incubation period for vCJD is still unknown. Documented patient incubation times
have been six or more years. The current risk of acquiring vCJD from eating beef cannot be
determined for travelers to Britain. However, the risk decreases by avoiding beef or beef
products or selecting beef or beef products that are solid muscle pieces (versus calf brains or
burgers or sausages). Public health preventive measures have been put into place including
enhancement of BSE surveillance, culling of sick animals, and using the “over thirty months
scheme.” This excludes animals over 30 months of age from both the human and animal food
chain.

In 2002, there was a case of person-to-person, blood-borne transmission of vCJD. This
occurred in a 69 year-old man who had received, six years previously, several units of blood.
One of those units came from a 24 year-old donor who developed vCJD three years after
donation. Taking into consideration all other factors, the conclusion was made that the recipient
indeed did contract vCJD from this donor. Due to the fact that vCJD can be easily detected in
lymphoid tissues and the existence of a possible blood phase had led researchers to believe that
blood-borne transmission of vCJD was possible.
To date there have been four cases of probable transmission of vCJD by blood transfusion in Britain. The donors developed CJD 17 months to 40 months after donation. The recipients developed vCJD six to eight years after receiving blood.

There is a donor deferral program in place in the U.S. Permanent deferrals are for anyone who has been diagnosed with CJD or vCJD or are relatives of anyone who has been diagnosed. An indefinite deferral is in place for anyone who has spent more than three months in the United Kingdom from 1980 to 1996, or anyone who has spent more than five years in Europe from 1980 to present. Indefinite deferrals also apply to anyone who received a blood transfusion in the United Kingdom from 1980 to present.

**Laboratory Testing**

The National Prion Disease Pathology Surveillance Center was established in 1997 to acquire tissue samples and clinical information from as many cases of human prion disease as possible. There are specific diagnostic activities available within the center. In CSF, they can search for the presence of 14-3-3 protein, a marker of Creutzfeldt-Jakob disease. In DNA extracted from blood, brain, or other tissues, they can search for the presence of a mutation of the prion protein gene and determine the polymorphism at codon 129. This polymorphism is an indicator of host susceptibility and the phenotypical disease expression of familial, iatrogenic or sporadic CJD. Unfixed brain tissue from biopsy or autopsy can be searched for the presence of the abnormal disease producing prion (PrP<sup>Sc</sup>). In fixed brain tissue they can exclude, confirm, and characterize the prion disease by microscopic examination. Only frozen brain tissue examination can confirm or exclude the diagnosis of prion disease.

**Conclusion**

Transmissible spongiform encephalopathies (TSE) are prevalent in both human and animal populations. Surveillance, along with advances in detection, and prevention are needed to eliminate these prion caused diseases. Research into how prions are formed and transmitted may be the key to unlocking the mystery. There is no current treatment for prion diseases. Once there is more information about how prions work, treatment modalities may be discovered.
References


REVIEW QUESTIONS
Course #DL-983
Choose the one best answer.

1. Kuru disappeared from the native population in New Guinea because
   a. immunization against the disease was developed
   b. cow meat for consumption was banned
   c. mortuary practices were discontinued
   d. the affected tribe died out

2. The causative agent of transmissible spongiform encephalitis is
   a. a proteinaceous infectious particle
   b. an abnormally folded nucleic acid
   c. a virino
   d. an amino acid substitution

3. Which of the following are human prion diseases?
   a. Scrapie, Kuru, BSE
   b. Kuru, GSS, FFI
   c. BSE, GSS, Kuru
   d. Scrapie, FFI, GSS

4. The main characteristic of vCJD is:
   a. vCJD attacks young adults
   b. vCJD attacks natives of New Guinea
   c. vCJD is inherited
   d. vCJD has not been identified in the US

5. The best method of dealing with instruments contaminated by prions is
   a. submerging in 1N sodium hydroxide
   b. autoclaving
   c. incinerating
   d. baking

6. Which prion disease is not inherited?
   A. Alpers’
   B. GSS
   C. FFI
   D. BSE
7. Chronic wasting disease is
   a. found in elk in California
   b. causes loss of weight in cattle
   c. was first identified in deer in Colorado
   d. is restricted to Wyoming and Colorado

8. Which of the following prion diseases is associated with BSE?
   a. FFI
   b. vCJD
   c. CJD
   d. GSS

9. Which prion disease affects sheep?
   a. Scrapie
   b. Chronic Wasting Disease
   c. Variant Creutzfeldt-Jakob disease
   d. Bovine spongiform encephalitis

10. The National Prion Disease Pathology Surveillance Center can test for
    a. prions in blood
    b. polymorphism in codon 129 for susceptibility to FFI
    c. PrP\textsuperscript{c} in brain tissue to identify vCJD
    d. 14-3-3 protein in CSF, a marker for CJD
    a.
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3. a b c d 8 a b c d
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